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## **Estrous Synchronization in Lactating Cows**

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### INTRODUCTION

The objective of any successful synchronization program is to manipulate the estrous cycle of normally cycling females so that a large percentage will exhibit estrus with normal fertility at a pre-determined time. Synchronization programs have typically been most successful with heifers due to a lack of interference from extraneous factors. Since minimum age and weight are the primary prerequisites for a heifer of a particular breed to reach puberty, a nutritional program can be designed to allow the majority of heifers to reach their appropriate target weight by 13-14 months of age. Provided there are no health problems, or extreme environmental effects, this allows for a large percentage of these heifer to be cycling by the time the manager wants to breed them. Consequently, synchronization programs utilizing prostaglandins, progestogens or combinations of the two have been relatively successful at the conclusion of appropriate heifer development programs.

Several factors complicate this process with the lactating cow. First, cows don't all calve at the same time. Therefore we have differences in the number of days postpartum. Lactation and the suckling stimulus tend to prolong the postpartum period to first estrus. Body condition and/or nutrition will play a role and often be confounded by the age of the female. In addition, winter environments in the northern Great Plains and intermountain range country, can have a major impact on condition and energy requirements.

It should be emphasized in these introductory comments that successful estrous synchronization, whether it be with heifers or postpartum cows, requires a high level of management in order to be successful. A total herd health program and absence of calving difficulty along with a minimum postpartum anestrous period would be additional factors required to identify appropriate candidates for a synchronization program.

The objective of synchronization efforts should also be considered when selecting the synchronization approach and the most appropriate system. Producers will want to meet their objectives with the most economically feasible system. In a majority of cases, synchronization is being utilized in order to enhance the genetic contribution through the use of superior sires by artificial insemination. In other situations a producer might merely be attempting to group breeding/calving dates to enhance management. In extensively managed operations where producers utilize public land grazing or have management restrictions necessitated by the grazing environment, the producer might simply want to get as many cows bred as possible prior to the time these restrictions are applied. In some cases this may mean utilizing natural service sires.

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### **Events of the Estrous Cycle**

It would seem appropriate to review the physiological changes that takes place during the

estrous cycle since this is the basis of synchronization schemes.

The 18-21 day period known as the estrous cycle is controlled by chemical compounds called hormones, which are secreted in minute quantities by glands or organs, are carried through the bloodstream and elicit responses from other glands or tissues. As an example of the extremely small quantities of these hormones and their power, the hormone progesterone is typically measured in nanograms. A nanogram is one billionth of a gram.

The estrous cycle is controlled by a cascade of hormones. The sequence is initiated by hormones from the hypothalamus. This small but very important portion of the brain secretes gonadotropin releasing hormone (GnRH), which travels via the bloodstream and elicits a response from the pituitary gland. The pituitary, in turn, releases a group of hormones called gonadotropins. Two of these gonadotropins are very important to the estrous cycle - follicle stimulating hormone (FSH) and luteinizing hormone (LH). These two hormones are in turn carried by the blood to the ovary where they affect its activity.

FSH stimulates growth of the follicles which contain the ovum or egg. Follicle maturation proceeds under the influence of FSH while the second gonadotropin, LH, acts upon the ovarian tissues at the site of ovulation, transforming them into a structure called the corpus luteum or CL.

Acting together, FSH and LH stimulate the maturing ovarian follicle to produce estrogen. LH also stimulates the CL to produce progesterone. These two ovarian hormones, estrogen and progesterone, play important roles in the regulation of the cycle.

Estrogen acts upon the central nervous system (the brain) to cause the cow to show heat (estrus; day 0 of the cycle) and mate. Estrogen also prepares the uterus for the events associated with mating, such as sperm transport, capacitation of the sperm and ova transport. LH stimulates the CL to produce progesterone (called a functional CL) by day 5 of the cycle. Progesterone acts upon the uterus to enhance the uterine environment for the possibility of developing an embryo and prevents estrus. Estrogen initiates the cascade of hormones by causing the hypothalamus to release GnRH. Conversely, progesterone stops the cascade and maintains pregnancy if conception has occurred. If pregnancy has not occurred, the uterus releases prostaglandins ( $\text{PGF}_2\alpha$ ) which causes the CL to regress beginning on about day 16 of the cycle. Secretion of progesterone stops and the cycle repeats itself. These hormonal relationships are extremely complex and only described in a very general sense in this paper. However, synchronization techniques are based on the manipulation of these interrelationships.

### **Management of Postpartum Anestrus**

Successful synchronization of estrus can only be accomplished if a large percentage of the females (essentially all of them) are cycling at the time the synchronization treatment is administered. In an excellent review of physiological mechanisms controlling anestrus and infertility in the postpartum beef cow, Short et al. (1990) suggested four causative factors: general infertility, lack of uterine involution, short cycles and anestrus. These authors suggested

that general infertility is common to any estrous cycle and might be expected to reduce potential fertility by 20 to 30%. Furthermore they suggested that incomplete involution may prevent fertilization in the first 20 days after parturition but is unrelated to anestrus. Short estrous cycles were suggested to reduce fertility during the first 40 days postpartum by allowing the cow to return to estrus prior to the recognition of pregnancy. Anestrus was identified as the major cause of postpartum infertility with the two major contributions coming from suckling and nutrition. Other minor contributions come from such items as parity, season, breed, dystocia, and lack of biostimulation from the presence of a bull. It was suggested that suckling and nutrition cause direct effects on anestrus but also interact with one or more additional factors to control postpartum anestrus. These authors suggested management practices to minimize the impact of postpartum anestrus/infertility which included restriction of the breeding season to  $\leq 45$  days; provide nutrition so body condition ranges from 5 to 7 prior to parturition; stimulate estrous activity by reducing dystocia; provide biostimulation with presence of a sterile bull; utilize estrous synchronization techniques; and finally, careful use of suckling management.

Suckling and lactation have been shown to have a prolonging effect on the postpartum period to first estrus. This is demonstrated research which suggests that when calves are weaned prior to ten days of age (Bellows et al. 1974) cows will resume their estrous cycle before 25 days postpartum. However, this enhanced cyclicity does not necessarily mean earlier conception. Short et al. (1972), mastectomized a group of cows during gestation, removed calves at birth from a second group and let a third group suckle normally. The length of the period to first estrus was 12, 25 and 65 days for the mastectomized, non-suckled and suckled cows, respectively. The mastectomized and non-suckled cows even lost weight during this period. However, services per conception were 3.0, 2.2, and 1.7 for the three respective groups. Therefore, earlier cycling did not enhance conception in this trial of relatively small numbers. Laster et al. (1973) found conception rates significantly higher in 2 and 3 year-old cows with calves weaned 8 days prior to the breeding season. Randel (1981) reported that once daily nursing for 30 minutes starting at 30 days postpartum and continuing until the Brahman X dam had exhibited estrus, decreased the length of postpartum anestrus significantly. Although calf gain was reduced during the once per day suckling period, weaning weights were not different from the normally suckled calves. Reeves and Gaskins (1981) reported a 20-day reduction in postpartum anestrus by suckling Angus cows for one 30-minute period daily, starting at 21 days postpartum. However, there was no difference in postpartum period to conception, 63 vs 68 days, for once-suckled and normally nursed cow, respectively. These researchers also indicated an increased incidence of short cycles in the once-a-day suckled cows. This short cycle problem was also reported in 80% of early-weaned cows (as compared to 1% in normally nursed cows) by Odde et al. (1980). Neither early weaning nor once-daily suckling may have much practical application in most extensively managed range operations.

Temporary calf removal, particularly in combination with estrous synchronization schemes, has shown some enhancement value and will be discussed later in this paper.

Biostimulation is a term used to describe the positive stimulatory effects of a male on estrus, ovulation or pregnancy (Chenoweth, 1983). Zalesky et al. (1984) reported an advancement of return to estrus by approximately 20 days when multiparous cows were exposed

to bulls during the first 53 days postpartum. Custer et al. (1990) and Fernandez et al. (1993) also showed these positive effects in first-calf females. Burns and Spitzer (1992) also reported a biostimulatory effect from testosterone-treated cows. Much of the literature suggests that biostimulation has its effect early in the postpartum period. Therefore, with a fixed breeding season, no effect was shown on interval to pregnancy.

Elimination of dystocia will also have a positive effect on the reduction of the postpartum interval. Available genetic indicators (i.e. EPDs for birth weight and calving ease) should be utilized in selecting sires to be used on first-calf heifers in particular, to reduce dystocia to as low a level as possible. It is obvious that management can influence postpartum anestrus. Cumulative effects of these various factors affecting the postpartum interval can have major impact on cycling activity. This could have positive economic implications, particularly as it affects young, nutritionally stressed and late calving cows. With the success of estrous synchronization systems requiring normally cycling females, a review of these factors was thought to be essential to understand why synchronization programs may not always be successful.

### **Methods of Synchronization: Prostaglandins and/or Progestogens**

Manipulation of estrous cycles by synchronization schemes generally utilizes one of two approaches or a combination of the two. Prostaglandin  $F_2\alpha$  ( $PGF_2\alpha$ ) and its analogues cause regression of the corpus luteum (CL) and return to estrus when given to cattle from day 5 to day 15 of the estrous cycle (day of estrus is defined as day 0). Prostaglandins are ineffective early in the cycle. Therefore systems such as two injections 11 days apart or observation/breeding for five days prior to prostaglandin injection have been developed. The latter system has the additional benefit of providing an estimate of cycling activity before the product is administered. There are three prostaglandin products currently available for synchronization in cattle: 1) prostaglandin  $F_2\alpha$ , marketed as Lutalyse by the Upjohn Co.; 2) cloprostenol, marketed as Estrumate by Mobay Corporation; and, 3) fenprostalene, marketed as Bovilene by Syntex Animal Health.

Progestogens are compounds that suppress estrus and ovulation in cattle. Melengestrol acetate (MGA), is marketed by the Upjohn Company and has traditionally been used as an oral progestogen to keep feedlot heifers out of heat and to enhance gain. There has been interest in the synchronization potential of this product since it is relatively cheap and does not require handling of the cattle. Conversely, inadequate consumption can reduce effectiveness. When MGA was fed for 14 days at a rate of .5 mg/hd/d, it was found to synchronize estrus (Zimbelman and Smith, 1966; Zimbelman et al., 1970). However, the estrus immediately following MGA treatment was found to be infertile (DeBois and Bierechral, 1970). The reduced fertility is confined to breeding at an estrus within 10 days after MGA withdrawal. Fourteen to 16-day MGA treatment has successfully been used to synchronize estrus by combining it with a  $PGF_2\alpha$  injection, 16 to 17 days after withdrawal of the MGA (Brown et al., 1988). Consistent, uniform consumption of the MGA may be the most limiting factor for using this system in the extensively managed, postpartum range beef cow.

A progestogen/estrogen combination is available on the market in the form of Syncro-Mate B (SMB). Based on research by Wiltbank and Gonzalez - Padilla (1975), this product is currently marketed by CEVA Laboratories, Inc. and consists of a 9-day norgestomet (a synthetic progesterone) implant plus an injection containing 3 mg of norgestomet and 5 mg estradiol valerate (an estrogen) at time of implant insertion.

As indicated earlier, temporary calf removal (sometime referred to as shanging) at time of implant removal and maintained until breeding (approximately 48 hours), is sometimes utilized to improve estrous response (Dowling et al., 1977) and synchronized pregnancy rate (Kiser et al., 1980). This appears to be more useful when breeding at a timed insemination. However, Pace and Sullivan (1980) and Brown et al. (1986) reported no benefit from calf removal in regard to pregnancy rate compared to SMB treatment without calf removal. However, the latter two studies were breeding on observation of estrus rather than at a timed insemination. In addition, Kiser et al. (1980) suggested that calf removal was less beneficial when cows were in adequate body condition.

### **Identified Areas of Concerns with Available Systems**

Both research projects and practical application have resulted in variable rates of success with all available products and synchronization schemes. These differences have resulted even when females were known to be healthy and cycling, in adequate body condition and consuming appropriate diets.

Stage of the estrous cycle when a synchronization treatment is administered appears to affect success of the synchronizing process. Estrous response, conception rate and interval to estrus after prostaglandin injection have all been shown to be affected by the stage of the cycle when the prostaglandin was administered. King et al. (1982), suggested that females in the late cycle (days 10 to 15) had a higher estrous response than females in the early cycle (days 6 to 9). This was also suggested by Tanabe and Hann (1984) and Watts and Fuguay (1985). The former also found a longer interval from treatment to onset of estrus in late cycle females.

Stage of estrous cycle at the beginning of treatment may also influence conception with SMB. Beal and Custer (1993) reviewed why 100% of cyclic females treated with SMB did not achieve synchronization. To be successful in the first 7 days of the cycle, the injected estrogen and norgestomet must cause luteolysis before the implant is removed. Miksch et al. (1978) indicated the 5 mg of estradiol valerate regressed CL in 80 to 86% of heifers beginning SMB treatment on day 1 through day 8. Pratt et al. (1991), reported CL regression in only 48% of cows treated on day 3 versus 100% when treatment started on day 9. Brink and Kiracofe (1988) reported a 47% conception rate for heifers that were administered on day 11 or less of the estrous cycle compared to 37% for those treated on day 12 or later. Odde (1990) suggested that this reduction in conception may be due to progestogen exposure (natural progesterone plus the norgestomet in the treatment) since long-term progestogen treatment has been shown to reduce conception rates. Luteal dysfunction has been suggested as a possible cause of this reduced fertility (King et al., 1986). This may be due to reduced LH production following implant removal (Hixon et al., 1981).

With enhanced fertility being suggested from reduced exposure to progestogens, Beal and Custer (1993), used ultrasound technology to allow sequential monitoring of the ovulatory follicle of animals treated with progestogens late in the estrous cycle. Treatment with MGA beginning on day-17 of the cycle delayed estrus and caused a large, estrogen-active, dominant follicle to persist on the ovary throughout a 7-day treatment period in 80% of the treated cows. In each case the persistent follicle ovulated after the synchronized estrus. The largest follicle present in cows beginning treatment on day 7 regressed during MGA feeding and another follicle developed and ovulated.

### **MGA/Prostaglandin Treatment in Cows**

Currently there is considerable research activity in the use of this synchronization system in the postpartum cow. The 14-day, .5 mg/hd/day administration of MGA followed by a prostaglandin injection on day 17 after MGA withdrawal, is very effective in synchronizing heifers. Theoretically, it should also be successful in cows. However, consistent consumption and varying lengths of time between calving and MGA administration make the system slightly less practical in cows. Since feeding of MGA starts 33 days before actual breeding, some cows in the herd may not have even calved by this time. King and Odde (1993) recommended that candidates for synchronization be separated and the 14-day MGA treatment be administered to only those cows at least 7 days postpartum at the start of the feeding period.

Yelich et al. (1988) used the traditional MGA/PGE<sub>2</sub> system in cows and found that 51.1% of the treated cows exhibited estrus within 5 days after treatment compared to 8.2% of controls. Synchronized conception rates for the treated cows was 80% versus 50% for the controls.

Although progestogen utilizing synchronization systems will always be more successful if the females are cycling prior to administration, there is evidence that progestogens such as MGA can initiate some cycling activity in the postpartum beef cow (Beal and Good, 1986; Boyd and Corah, 1986). This is not true of those systems using only prostaglandin injections which require a functional, progesterone-producing CL of the cycling female in order to be effective.

Yelich et al. (1989) completed an additional study using the MGA/prostaglandin system and included 48-hour calf removal beginning the second day after the MGA feeding. Serum progesterone levels were analyzed prior to and after the MGA treatment. Calf removal did not enhance estrous response, synchronized conception rates or pregnancy rates in the study. Both the traditional MGA system and the traditional system plus calf removal increased the percent of cows cycling (11 to 14%) compared to the controls.

Range beef cow producers often lose control of the cow herd during at least a portion of the breeding season. They may go onto public lands or be involved in grazing associations where they run in common with other producers. Kentucky researchers (Patterson et al., 1991) reported results of field demonstrations where heifers received MGA (.5 mg/hd/d) for 14 days in a grain supplement carrier. Fertile bulls were put with the heifers 15 to 18 days after withdrawal of the MGA, without any administration of prostaglandin. One bull was utilized per 15 to 20 heifers.

Based on rectal palpation within 100 days after the bulls were put with the heifers, 69% (413/601) became pregnant during the synchronized period and 83% (500/601) conceived within the first 30 days of the breeding season. The average total pregnancy rate at the end of the breeding season in these herds was 89%. Therefore, of the total number of heifers that became pregnant, only 10% failed to conceive within the first 30 days of the breeding season.

Currently a University of Wyoming study is evaluating the synchronizing effects of three systems on 200 head of postpartum cows. Cows were assigned to treatment based on date of parturition. One group received MGA plus PGF<sub>2</sub>α in the traditional system (breeding on observation of estrus after the PGF<sub>2</sub>α injection). Another group received MGA without the PGF<sub>2</sub>α (breeding on observation of second estrus after withdrawal of MGA). The third group received a double injection of Lutalyse scheduled so the second injection was given at the same time as the PGF<sub>2</sub>α injection to the MGA-treated cows. Cycling activity was monitored prior to the MGA administration and progesterone levels were determined on serum samples taken 7 and 17 days post-MGA administration, to determine presence of a functional CL in all cows. Although data collection is not complete at the time of this writing, it will be included in the oral presentation.

### **Summary**

Successful synchronization programs require high quality integrated management systems. Adequate body condition and balanced nutrition programs are essential to the success of synchronization efforts. Management factors can be applied to enhance the effectiveness of synchronization systems, particularly when cows are young and/or are in slightly less than optimum body condition. Research efforts will continue to fine-tune available synchronization systems for enhanced control and improved breeding efficiency.

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