Development of an estrus synchronization protocol for beef cattle with short-term feeding of melengestrol acetate: 7-11 synch

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ABSTRACT: An estrus synchronization protocol (7-11 Synch) was developed to synchronize the first follicular wave and timing of ovulation in postpartum beef cows. In Exp. 1, follicular development and timing of ovulation in response to the following protocol were evaluated. Beef heifers (n = 12) and cows (n = 6), at random stages of the estrous cycle, were fed melengestrol acetate (MGA; 0.5 mg·animal⁻¹·d⁻¹) for 7 d and injected with PGF₂α (PG; 25 mg) on the last day of MGA. A second injection of PG was administered 11 d after cessation of MGA. After the second injection of PG, estrus was synchronized in 6/12 heifers and 3/6 cows. The interval to estrus in heifers and cows was 54 and 64 h, respectively (P > .10). All animals exhibiting estrus ovulated first-wave follicles. Animals that failed to respond to the second injection of PG were in estrus later than 6 d after cessation of MGA and had corpora lutea that were unresponsive to the injection of PG. Based on the variation in interval to estrus following the first PG injection on the last day of MGA feeding in Exp. 1, an injection of GnRH (100 µg) was added to the protocol 4 d after the cessation of MGA to ensure ovulation or luteinization of dominant follicles and synchronization of first-wave follicular development. This revised protocol was termed “7-11 Synch.” In Exp. 2, two estrus synchronization protocols were compared. Multiparous beef cows were stratified by breed and postpartum interval and randomly assigned to the 7-11 Synch (n = 44) or Select Synch protocols (GnRH injection followed by PG injection 7 d later; n = 45). Timing of estrus after the last PG injection (0 h) ranged from 42 to 102 h in the 7-11 Synch group and 30 to 114 h in the Select Synch group. Eight cows (18%) in the Select Synch group exhibited estrus 30 h before to 18 h after PG. Synchronized estrus peaked between 42 and 66 h after the last PG injection, and a maximum number of cows were in estrus at 54 h for both treatment groups. Synchrony of estrus from 42 to 66 h was greater (P < .05) in 7-11 Synch (91%; 41/44) than in Select Synch cows (69%; 31/45). Artificial insemination pregnancy rate from 42 to 66 h was greater (P < .05) in the 7-11 Synch group (66%; 29/44) than in the Select Synch group (40%; 18/45). In summary, the 7-11 Synch protocol improved synchrony of estrus without reducing fertility. This protocol has potential future application for fixed-time AI in beef cattle production systems.

Key Words: Artificial Insemination, Beef Cows, Estrus, Synchronization


Introduction

Precise control of estrous cycles in cattle requires the synchronization of follicular growth and synchronized luteal regression. Feeding melengestrol acetate (MGA) for 14 d (0.5 mg·animal⁻¹·d⁻¹) followed by an injection of PGF₂α (PG) 17 d after MGA feeding (14/17-d MGA/PG protocol) is an effective method of estrous cycle control in heifers (Brown et al., 1988; Patterson and Corah, 1992). Recently, an increase in estrus response, synchronized conception, pregnancy rates, and fecundity in the postpartum cow was reported among cows treated with the 14/17-d MGA/PG protocol compared to PG alone (Patterson et al., 1995; Fralix et al., 1996). The advantages of using MGA for estrus synchronization include ease of administration and reasonable cost; however, length of the treatment protocol creates a need for increased management and, in some cases, extends the duration of the treatment period beyond practical limits. Short-term feeding of MGA (5 or 7 d) combined with an injection of PG has been shown to be effective in synchronizing estrus in a high percentage of cattle com-
Estrus synchronization in beef cattle

**Materials and Methods**

**Experiment 1**

*Experimental Protocol.* Crossbred beef heifers (n = 12) and nonlactating beef cows (n = 6), at random stages of the estrous cycle, were used to characterize changes that occur in cows and heifers that were treated with the new estrus synchronization protocol. Animals were fed MGA (.5 mg·animal⁻¹·d⁻¹) in a carrier pellet (Cattle Charge, MFA Inc., Columbia, MO) for 7 d and injected with PG (25 mg Lutalyse Sterile Solution, Pharmacia and Upjohn, Kalamazoo, MI) on the last day of MGA feeding. A second injection of PG was given 11 d after cessation of MGA feeding (Figure 1).

*Estrus Detection and Ultrasonography.* Heifers and cows were observed for signs of behavioral estrus every 6 h for 6 d following the last feeding of MGA and for 5 d following the second injection of PG. Development of first-wave follicles and corpora lutea (CL) was monitored by real-time ultrasonography (Aloka 500V, Corometrics Medical Systems, Wallingford, CT) with a 7.5-MHz linear array transducer. Ultrasonography was performed every other day beginning at the last feeding of MGA through the second injection of PG, and every 12 h thereafter for 5 d, to determine the timing of ovulation.

**Blood Collection and RIA.** Blood samples were collected via jugular venipuncture at 10 and 2 d before the start of MGA feeding. Additional blood samples were collected on the last day of MGA feeding, 5, 7, and 9 d after MGA feeding, and daily for 5 d following the second injection of PG. Samples were collected into EDTA-treated tubes and placed immediately on ice. Within 4 h of collection, plasma was harvested by centrifugation and stored at −20°C until concentrations of progesterone were quantified by RIA within a single assay (Kirby et al., 1997; Coat-A-Count, Diagnostic Products, Los Angeles, CA). Intrassay coefficient of variation was 6.9% and assay sensitivity was 2 ng/mL.

**Statistical Analysis.** Intervals to the onset of estrus after MGA feeding and after the last injection of PG, diameter of dominant follicles, and timing of ovulation were analyzed by ANOVA using General Linear Models procedures of SAS (1988). Duncan’s new multiple range test (Steel and Torrie, 1980) was used for mean separation.

**Experiment 2**

*Experimental Protocol.* Based on the results from Exp. 1, an injection of 100 μg GnRH (Cystorelin, Merial, Islin, NJ) was included in the treatment protocol 4 d following the last day of MGA feeding to ensure ovulation or luteinization of dominant follicles and synchronization of the first follicular wave. This revised protocol was termed “7-11 Synch” (Figure 1). In Exp. 2, 89 multiparous beef cows with calves were stratified by breed and postpartum interval (range 15 to 77 d) and were randomly assigned to two estrus synchronization treatments, 7-11 Synch (n = 44) or Select Synch (n = 45). The Select Synch protocol was chosen to compare with 7-11 Synch because of similarity in the treatment schedules for injections of GnRH and PG (Figure 1).

All cows were fed a carrier pellet as described in Exp. 1, starting 3 d prior to the initiation of MGA treatment. The first day of MGA feeding was defined as d 1 of the experiment (Figure 1). Cows in the Select Synch group continued to receive carrier pellets for an additional 7 d (from d 1 to 7). Cows in the 7-11 Synch group were fed MGA (.5 mg·cow⁻¹·d⁻¹) in the carrier pellet for 7 d (from d 1 to 7) and received an injection of PG on the last day of MGA feeding (d 7). Four days after the last MGA feeding (d 11), GnRH was administered and a second injection of PG was given 11 d after the last day MGA was fed (d 18). Cows in the Select Synch group received an injection of GnRH (d 11), and then an injection of PG 7 d later (d 18).
Estrus Detection and AI. Cows were observed three times a day (0600, 1200, and 1900) for signs of behavioral estrus after the GnRH injection and for 7 d following the last injection of PG (from d 11 to 25). Kamar heatmount detectors (Kamar, Steamboat Springs, CO) were used to aid in detection of estrus. Timing of behavioral estrus was recorded for each cow. Body condition scores (BCS; 1 to 9 scale, 1 = emaciated, and 9 = obese) were recorded on all cows before insemination. Cows were artificially inseminated 12 h after detection of behavioral estrus by one of two experienced technicians and semen from three bulls was used (Select Sires, Plain City, OH). The number of cows in each treatment group that were inseminated was determined by a single technician by natural service was determined by a single technician by natural service beginning on d 39. Conception rate to AI or natural service was determined by an individual technician was approximately equal. Cows were exposed for a 60-d natural service period beginning on d 39. Conception rate to AI or natural service was determined by a single technician by transrectal ultrasonography approximately 60 d after the last AI (approximately d 85). Pregnancy status was confirmed by rectal palpation approximately 120 d later and calving record.

Blood Collection and RIA. Blood samples were collected on d 1 and 7 from the 7-11 Synch group and on d 4 and 11 from the Select Synch group to determine postpartum status (cyclic or anestrous). The blood collection schedule is shown in Figure 1. Cows were considered cyclic when concentrations of progesterone were greater than 1 ng/mL in at least one sample collected 7 d apart. Additional samples were collected on the day of GnRH injection (d 11) for the 7-11 Synch group and on the day of second PG injections (d 18) for both groups to determine the presence of luteal activity. Sample collection was performed as described in Exp. 1. Concentrations of progesterone were quantified by RIA within a single assay as described in Exp. 1. Intra-assay coefficient of variation was 7.3% and assay sensitivity was .2 ng/mL.

Statistical Analysis. Synchrony of estrus, conception rate, and pregnancy rates during both the AI period (d 11 to 25) and peak response period were analyzed using chi-square analysis, CATMOD procedure, of SAS (1988) including the fixed effects of treatment, breed, AI technician, AI sire, cyclicity status, BCS, number of days postpartum, and the interactions of each with treatment. The Fisher’s exact test (Steel and Torrie, 1980) was used for mean separation. Maximum estrus response occurred at 54 h for both treatment groups; therefore, “peak response” period was defined as 12 h before (42 h) and 12 h after (66 h) the maximum estrus response (54 h). During the 24-h peak response period (42 to 66 h), 14, 73, and 5% of the cows in the 7-11 Synch group and 20, 36, and 13% of the cows in the Select Synch group exhibited estrus at 42, 54, and 66 h, respectively. Degree of synchrony was analyzed by ratio of variance (F-test) of mean time interval to onset of estrus. No breed × treatment interaction was observed (P > .10); therefore, all data from different breeds within a treatment group were pooled for analysis. No effect (P > .10) of AI technician, BCS, or number of days postpartum on pregnancy rates were observed, and those were removed from the model.

Three AI sires were used in this experiment and were assigned to equal numbers of cows for each estrus synchronization protocol. Overall AI pregnancy rates of three AI sires, regardless of treatment, were not different (75%, 52, and 76%; P > .10). Because no significant differences occurred in pregnancy rate among sires, AI sire was removed from the model.

Results

Experiment 1

The number of cattle exhibiting estrus within 7 d after MGA treatment and the interval to estrus were 10/12 and 96 ± 4.4 h (mean ± SE), respectively, in heifers and 4/6 and 84 ± 7.0 h, respectively, in cows. Timing of estrus did not differ (P > .10) between heifers and cows. After the second injection of PG, 6/12 heifers and 3/6 cows were synchronized. The interval to estrus was not different (P > .10) between heifers (54 ± 6.2 h) and cows (64 ± 4.0 h). All cattle exhibiting estrus after the second injection of PG ovulated first-wave follicles and timing of ovulation did not differ (P > .10) between heifers (80 ± 7.4 h) and cows (96 ± 4.0 h). Cattle that failed to respond to the second injection of PG had either a cystic follicle (n = 1) or early developing CL that were not responsive to PG (n = 8). Early developing CL were the result of delayed estrus and ovulation after the last feeding of MGA. Diameter of dominant follicles at the time of the second PG injection tended to be larger (P < .07) in cows that were successfully synchronized than in those that did not respond (16.0 ± 1.0 mm and 8.3 ± 2.8 mm, respectively). Among heifers, mean diameter of the dominant follicle did not differ (P > .10) between synchronized heifers and those that did not respond (14.1 ± 0.4 mm and 11.5 ± 2.2 mm, respectively).

Experiment 2

Average postpartum interval at the first PG injection on d 7 (7-11 Synch) or the GnRH injection on d 11 (Select Synch) and BCS before AI were not different (P > .10) between the 7-11 Synch (56 ± 2.4 d and 5.4 ± .04, respectively) and Select Synch groups (60 ± 2.3 d and 5.4 ± .05, respectively). The proportion of cows that were anestrous or cyclic at the GnRH injection was not different (P > .10) between the 7-11 Synch (34%; 15/44 and 66%; 29/44, respectively) and Select Synch groups (38%; 17/45 and 62%; 28/45, respectively).

Timing of estrus after the last PG injection (0 h) ranged from 42 to 102 h in the 7-11 Synch group (60-h period) and −30 to 114 h in the Select Synch group (144-h period; Figure 2). Synchrony of estrus during the 14-d AI period did not differ (P > .1) between 7-11 Synch (95%; 42/44) and Select Synch treated groups (96%; 43/45). Eight cows (18%) in the Select Synch group exhibited estrus from −30 h to 18 h after PG injection. Synchronized estrus peaked at 54 h after the last PG injection for both the 7-11 Synch (73%; 32/44) and the Select Synch groups.
Figure 2. Distribution of estrous response in cows treated with either the 7-11 Synch or Select Synch protocols (Hour 0 = time of prostaglandin F₂α, [PG] administration). The dashed-line box indicates the 24-h peak response period (42 to 66 h). Cows were observed three times a day (0600, 1200, and 1900) for behavioral estrus. As indicated by the lower variance for mean interval to estrus analyzed by F-test, degree of estrus synchrony was greater (\( P < .0001 \)) in the 7-11 Synch cows (111.6; df = 41) than in the Select Synch cows (768.7; df = 42).

Overall AI pregnancy rates during the 14-d AI period (after the GnRH injection and for a 7-d period following the last injection of PG: from d 11 to 25) and overall pregnancy rates during the breeding season (14-d AI period followed by 60-d natural service) did not differ \( (P > .10) \) between treatments (7-11 Synch: 70%: 31/44 and 89%; 39/44, respectively; Select Synch: 69%: 31/45 and 91%; 41/45, respectively).

Synchrony of estrus during the peak response period (42 to 66 h: 24-h period) was greater \( (P < .05) \) in 7-11 Synch (91%: 40/44) than in Select Synch cows (69%: 31/45; Figure 3). Consequently, AI pregnancy rate during the peak response period was greater \( (P < .05) \) in the 7-11 Synch group (68%: 30/44) than in the Select Synch group (47%: 21/45; Figure 4). During the peak response period, greater synchrony of estrus \( (P < .05) \) was observed in cyclic cows treated with the 7-11 Synch protocol than in those treated with the Select Synch protocol, resulting in greater \( (P < .05) \) AI pregnancy rates; anestrous cows responded similarly \( (P > .10) \) to these treatments (Figures 3 and 4).

At the time of the last PG injection, three observations were made based on blood samples for progesterone: 1) cows with concentrations of progesterone greater than 1 ng/mL, indicating presence of a functional CL; 2) concentrations of progesterone ranging from .3 to 1 ng/mL, suggesting the possible presence of luteinized follicles; and 3) no detectable concentrations of progesterone (< .2 ng/mL; below the assay sensitivity). Sixty-eight percent of cows in the 7-11 Synch group had concentrations of progesterone > 1 ng/mL at the time of the last PG injection (30/44: anestrous 10/15 and cyclic 20/29), and 32% had concentrations of progesterone between .3 and 1 ng/mL (14/44: anestrous 5/15 and cyclic 9/29). For cows in the Select Synch group, 58% had concentrations of progesterone > 1 ng/mL (26/45: anestrous 9/17 and cyclic 17/28); 22% had concentrations of progesterone between .3 and 1 ng/mL (10/45: anestrous 6/17 and cyclic 4/28); and 20% had no detectable concentrations of progesterone (9/45: anestrous 2/15 and cyclic 7/28). All cows in the 7-11 Synch group had detectable progesterone concentrations (> .3 ng/mL) compared with 80% (36/45) in the Select Synch group. These data suggest that the 7-11 Synch protocol successfully induced CL or resulted...
in the formation of lutenized follicles capable of responding to PG.

**Discussion**

Precise control of estrous cycles in cattle requires the synchronization of follicular growth and synchronized luteal regression. The new treatment tested in these experiments was designed to 1) shorten the treatment period compared to a 14/17-d MGA/PG program without reducing fertility and 2) improve synchrony of estrus by synchronizing development and ovulation of follicles from the first wave of development compared to currently available estrus synchronization protocols.

A high percentage of cattle can be expected to exhibit estrus 3 to 5 d after short-term feeding of MGA (Moody et al., 1978; Patterson et al., 1986; Beal et al., 1988; Chenault et al., 1990). These cattle would be on d 6 to 8 of the estrous cycle at the time of the proposed second injection of PG 11 d later (Exp. 1). Consequently, CL should respond to the injection of PG and the existing dominant follicles should ovulate. Cattle that failed to respond to the second PG injection in Exp. 1 had either a cystic follicle (n = 1) or early developing CL (n = 8).

Early developing CL were caused by delayed estrus and ovulation after the last feeding of MGA. Although a decline in concentrations of progesterone was observed after the last day of MGA feeding and the first PG injection, timing of estrus and ovulation was delayed in cows that did not respond to this treatment. Variation in expression of estrus following MGA feeding may be related to differences in clearance of MGA among individual cattle (Kojima et al., 1995). In fact, MGA can be stored in adipose tissue and released at different rates for individual cattle after withdrawal of MGA from the feed (Neff, 1983); therefore, body condition and/or amount of MGA consumed would affect clearance of MGA and consequently the timing of estrus and ovulation after the last day of MGA feeding. Another possibility for delayed estrus is that some animals may have been in the latter portion of the follicular wave at the end of MGA feeding at the time PG was administered. Hence, initiation of the new follicular wave occurred after the first injection of PG, which resulted in delayed expression of estrus.

An injection of GnRH was added to this estrus synchronization protocol 4 d after the last day of MGA feeding to ensure ovulation or luteinization of dominant follicles and synchronization of first-wave follicular development, and to determine whether synchrony of estrus could be improved (Exp. 2). Timing of the GnRH injection was determined by the expected day of MGA clearance based on results from Exp. 1 and previous literature (Moody et al., 1978; Patterson et al., 1986; Beal et al., 1988; Chenault et al., 1990).

The relationship between follicular development and timing of GnRH injection in estrus synchronization protocols may differ between anestrous and cyclic cows; however, both cyclic and anestrous cows responded equally well to the 7-11 Synch protocol. Although the number of anestrous cows in the present study was small, timing of estrus, estrus response, and pregnancy rates of anestrous cows were similar to those of cyclic cows.

The 7-11 Synch protocol resulted in a higher degree of estrus synchrony and greater AI pregnancy rates during a 24-h peak response period (42 to 66 h) compared to the Select Synch protocol. The interval to peak estrus response (42 to 66 h) in 7-11 Synch cows was similar to that observed in Exp. 1. These results agree with previous studies in which cyclic cattle were injected with PG during the early (d 5 to 7) portion of the estrous cycle (Tanabe and Hann, 1984; Watts and Fuquay, 1985). These data indicate that the 7-11 Synch protocol effectively synchronized the first wave of follicular development, resulting in a fertile estrus in both anestrous and cyclic cows.

Concentrations of progesterone at the time of the last injection of PG demonstrated that the injection of GnRH in the 7-11 Synch protocol successfully resulted in ovulation or luteinization of dominant follicles followed by initiation of new follicular waves. Either CL or luteinized follicles induced by GnRH would be capable of responding to PG; therefore, a relationship may exist between concentrations of progesterone before PG and improved synchrony of estrus for cows treated with the 7-11 Synch protocol. Consequently, follicles from the first wave ovulated after the second injection of PG, resulting in an earlier estrus response compared to the interval typically observed in cows injected with PG during the mid- to late estrous cycle (approximately 70 to 75 h: Tanabe and Hann, 1984; Watts and Fuquay, 1985). Although a decreased estrus response was reported in earlier studies in which cattle were injected with PG during d 5 to 7 of the estrous cycle (Tanabe and Hann, 1984; Watts and Fuquay, 1985), cows assigned to the 7-11 Synch protocol demonstrated excellent synchrony of estrus when PG was administered on approximately d 7 of the estrous cycle. Further study is necessary to confirm effectiveness of the 7-11 Synch protocol in anestrous cows and peripubertal heifers. It would seem that 7-11 Synch may offer the potential for fixed-time AI programs because of the high degree of estrus synchrony exhibited by cows on this treatment.

In general, GnRH-PG-based protocols are more economical and less labor-intensive than other protocols currently available (Twagiramungu et al., 1992; Pursley et al., 1995). The drawback of these protocols is that approximately 5 to 15% of the cyclic cows exhibit estrus prior to and immediately after the time PG is administered, resulting in the need for increased length of time to detect estrus or decreased response during the synchronized period after PG injection (Pursley et al., 1995; Twagiramungu et al., 1995). In Exp. 2, 9% (4/45) of the cows in the Select Synch group exhibited estrus after the GnRH injection and before PG (~30 to 0 h), and another 9% (4/45) exhibited estrus immediately after the injection of PG (0 to 18 h), necessitating a prolonged period of estrus detection and AI. Additionally, these
cows did not have detectable concentrations of progesterone at the time of PG; 4 of these cows (one anestrous and three cyclic) exhibited estrus after GnRH and before PG (~30 to 0 h). Another four cows (four cyclic) exhibited estrus immediately after injection with PG (0 to 18 h), and one anestrous cow did not respond to the treatment. These observations indicate that those cows did not respond to the injection of GnRH and exhibited estrus regardless of the treatment. This is in agreement with the previous report that Select Synch-treated cows that exhibit estrus early are in the late portion of the estrous cycle (d15 to 17) at the time GnRH is administered (Downing et al., 1998).

The advantages of MGA for synchronization of estrus are ease of administration and low cost. Furthermore, MGA recently received clearance from the FDA for use in reproductive classes of beef cattle and dairy heifers (Federal Register, 1997); therefore, research of estrus synchronization methods involving MGA bears increased significance and marked relevance to current industry needs. However, other progestin treatments (i.e., controlled intravaginal drug release [CIDR], progesterone releasing intravaginal device [PRID], or Norgestomet implants [as in Syncro-Mate-B treatment]) could be used in place of MGA feeding in the 7-11 Synch system, offering a variety of alternatives to fit individual needs. In summary, the 7-11 Synch protocol improved synchrony of estrus in both cyclic and anestrous cows without reducing fertility. This protocol provides potential future application in estrus synchronization and fixed-time AI programs for use in beef cattle production systems.

Implications

The advantages of the 7-11 Synch protocol compared to a 14/17-d melengestrol acetate/prostaglandin $F_2\alpha$ program include 1) shorter treatment period and 2) improved synchrony of estrus. Improved synchrony of estrus should reduce labor costs associated with estrus detection and offset the increased treatment cost of this protocol compared to other estrus synchronization protocols currently available. The drawback associated with 7-11 Synch is that cattle need to be worked four times to successfully administer the treatment and artificially inseminate the cows. The 7-11 Synch protocol, however, provides potential future application in estrus synchronization and fixed-time artificial insemination programs for use in beef cattle production systems.

Literature Cited


