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Effects of short-term feeding of MGA combined with GnRH and $PGF_{2\alpha}$ on reproduction of beef cows

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Abstract

Short-term feeding of melengesterol acetate (MGA) was used in two trials to improve synchrony of ovulation, increase pregnancy rates to first service TAI (experiment 1), and shorten the interval from $PGF_{2\alpha}$ injection to estrus in beef cows (experiment 2). In experiment 1 a total of 192 beef cows were assigned to either the Co-Synch (n = 95) or the MGA: 7-11Synch + TAI (n = 97) protocols. MGA: 7-11Synch + TAI cows were fed MGA (0.5 mg/h/day) for 7 days and administered $PGF_{2\alpha}$ on the last day of MGA feeding. On day -7, four days later, cows in both groups (Co-Synch and MGA: 7-11Synch + TAI) were administered GnRH followed with $PGF_{2\alpha}$ on day 0, then GnRH was administered 48 h later and concurrently inseminated. Overall pregnancy rate was 97/192 (51%). Number of cows with functional CL at day 0 was greater ($P > 0.001$) in the MGA: 7-11Synch + TAI group (94/97) than the Co-Synch group (76/95) suggesting that the MGA: 7-11Synch + TAI protocol successfully induced ovulations and formation of functional CL or luteinized follicles capable of responding to $PGF_{2\alpha}$. In experiment 2 purebred Angus cows (n = 109) and purebred Simmental cows (n = 63) were included in Select Synch (n = 84) or MGA: 7-11Synch (n = 88) protocols. Cows in the MGA: 7-11Synch group were fed MGA (0.5 mg/h/day) for 7 days and $PGF_{2\alpha}$ was administered on the last day of MGA feeding (day -11). On day -7, four days later, all cows (Select Synch and MGA: 7-11Synch) were administered GnRH followed with $PGF_{2\alpha}$ on day 0. Within -24 to 96 h of $PGF_{2\alpha}$ administration, 128/171 (75%) cows exhibited estrus and were inseminated. First service conception and pregnancy rates during the first synchronization period (day -1 to day 4) were not different ($P > 0.10$) between the two groups. Overall conception and pregnancy rates were 99/128 (77%) and 99/171 (58%), respectively. Cows in the Select Synch group had a shorter ($P < 0.05$) interval to estrus (54.5 ± 22.2 h) than cows in the MGA: 7-11Synch protocol (61.2 ± 14.9 h). The MGA: 7-11Synch protocol resulted in a higher degree of estrus synchrony (49/56, 88%) in a 24 h peak response period (48 to 72 h) after $PGF_{2\alpha}$ injection compared to Select Synch cows (46/67, 69%). Tighter synchrony of estrus suggests that MGA: 7-11Synch offers the potential for fixed-time AI programs.

Keywords: beef cows, Co-Synch, MGA: 7-11Synch, Select Synch.

Introduction

Methods of estrous synchronization are being developed continuously to manipulate estrus and estrous cycle in beef cows. Precise control of estrus and estrous cycles requires effective control of both luteal and follicular functions (Kojima *et al*., 2000). Efforts to develop a more effective estrus synchronization protocol have focused on synchronizing follicular waves by injecting GnRH, followed 7 days later by an injection of $PGF_{2\alpha}$ (Ovsynch; Pursley *et al*., 1995; Co-Synch; Geary *et al*., 1998; and Select Synch; Downing *et al*., 1998). Melengesterol acetate (MGA) has also been included in the diets of beef cows to control the estrous cycle. The original MGA-based protocol included feeding MGA for 14 days with natural services 10 days later (Patterson *et al*., 2000). Pregnancy rates were improved when a single injection of $PGF_{2\alpha}$ was administered 17 days after cessation of MGA feeding. Further studies (Brown *et al*., 1988; Lamb *et al*., 2000) found that extending the interval from 17 days to 19 days from MGA withdrawal to $PGF_{2\alpha}$ injection would increase pregnancy rates to first services. An injection of GnRH given 10 to 12 days after MGA withdrawal and 7 days before $PGF_{2\alpha}$ injection was introduced (MGA Select; Wood *et al*., 2001). The MGA Select® was then modified to include a second injection of GnRH 72 h before timed AI (Patterson *et al*., 2001, 2006). Improved rates of synchrony of heifers synchronized with MGA-PGF protocols and comparable pregnancy rates (52%) to those bred upon detection of estrus (50%) created an opportunity for fixed-time AI (Kesler, 2007). Kojima *et al*. (2000) fed MGA for 7 days with PGF2α administered on the last day of MGA feeding and GnRH was administered 4 days later followed by a second injection of $PGF_{2\alpha}$ 11 days after MGA withdrawal. The GnRH was administered to ensure ovulation or luteinization of dominant follicles and synchronization of the first follicular wave development. Synchrony of estrus within a 24 h period (42 to 66 h) was greater in 7-11Synch (91%) than in Select Synch (69%) cows. AI-pregnancy rates were greater for 7-11Synch (66%) than Select Synch (40%) as well (Kojima *et al*., 2000).

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Our objectives were to determine if short-term MGA feeding in the 7-11 Synch protocol would improve synchrony of estrus and pregnancy rates in beef cows after fixed time insemination (experiment 1) and shorten the interval from $PGF_{2\alpha}$ injection to estrus (experiment 2).

Materials and Methods

Experiment 1

Animals and management

Cross-bred beef cows from the Dixon Springs Agricultural Center (Simpson, Illinois; n = 192) were included in this study. Cows were 2-11 years old and 12-102 days postpartum with calves. At the start of the experiment, cows had body condition scores of 4 to 8 (BCS; 1 to 9 scale, 1 = emaciated and 9 = obese). All cows were randomly assigned to either the Co-Synch (n = 95) or MGA: 7-11Synch (n = 97) estrus synchronization protocols with fixed time AI (MGA: 7-11Synch + TAI). The experimental design is presented in Fig. 1. Cows in the MGA: 7-11Synch + TAI treatment group were fed MGA (0.5 mg/h/day; Pharmacia Animal Health; Kalamazoo, MI) beginning on day -17 and concluding 7 days later on day -11. On the last day of MGA feeding (day -11), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$; 25 mg i.m.; 5 ml Lutalyse®; Pharmacia Animal Health; Kalamazoo, MI) was administered. Treatment schedules after day -11 are identical in both Co-Synch and MGA: 7-11Synch + TAI protocols. On day -7 all cows (Co-Synch and MGA: 7-11Synch + TAI) received a GnRH (100 µg i.m; 2 ml Cystorelin®; Merial; Iselin; NJ) injection followed with an injection of $PGF_{2\alpha}$ (25 mg) on day 0. All cows were administered a second injection of GnRH on day 2, 48 h after $PGF_{2\alpha}$ injection, and then artificially inseminated with frozen thawed semen.

Blood samples were collected via jugular venipuncture on day 0, prior to the injection of $PGF_{2\alpha,}$ to determine the presence of a functional corpus luteum (CL). Blood samples were again collected on day 2 to determine the luteolytic response to injection of $PGF_{2\alpha}$. Blood samples were stored on ice water at -4°C until centrifugation at 2000 x g within 6 h after collection (Wiseman *et al.*, 1983). Sera were harvested after centrifugation and stored at -20°C until assayed for progesterone (P4) concentrations using a validated ELISA protocol with intra- and inter assay CV of 2.7 and 13.8% and sensitivity of 0.1 ng/ml (Kesler *et al*., 1990). Cows with P4 concentrations of >1.0 ng/ml (day 0) were considered to have had a functional CL. Incidence of luteolysis was determined to have occurred if P4 concentrations were≥ 1ng/ml (day 0) and fell to <1.0 ng/ml (day 2). Pregnancy was determined via transrectal ultrasonography 47-56 days after fixed-time insemination (day 2).

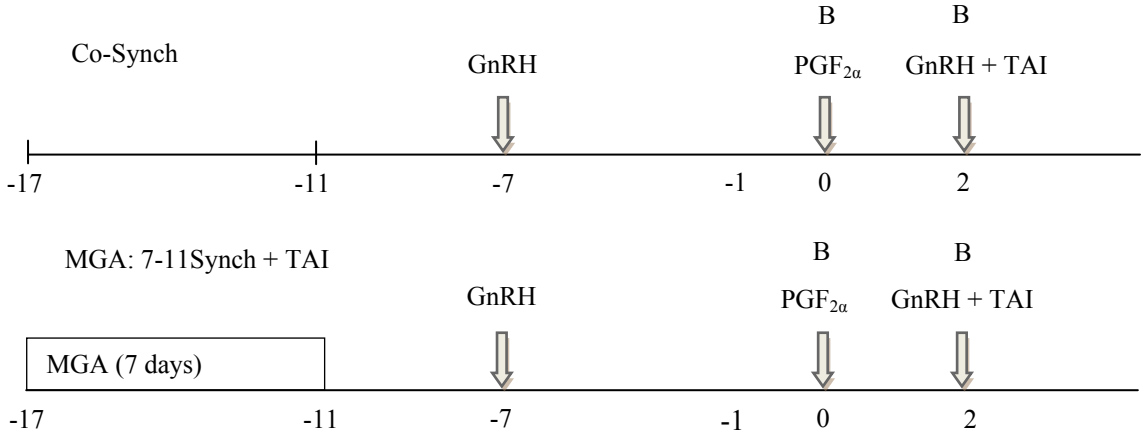

Figure 1. Treatment protocols applied to beef cows before first service in Experiment 1. Blood (B) samples were collected from all cows on days 0 and 2. The MGA: 7-11Synch + TAI cows were fed MGA (0.5 mg/h/day) for 7 days. GnRH (100 µg) and $PGF_{2\alpha}$ (25 mg) were administered as illustrated. All cows were fixed-time inseminated on day 2 after GnRH administration.

Statistical analysis

Data were analyzed using the General Linear Models (GLM) procedure of SAS (1999) to determine effect of treatment within the experiment on the dependent variables in the analyses including number of animals with functional CL, incidence of luteolysis (number of animals with CL that regressed), and number of animals pregnant to fixed-time insemination. One cow was not present for the pregnancy examination and was eliminated from these analyses.

Experiment 2

Animals and management

Purebred Angus cows from the University of Illinois Beef Unit (Urbana, Illinois; n = 109) and purebred Simmental cows from the Orr Beef Research Center (Baylis, Illinois; n = 63) were included in this

study. Cows were 2-10 years old and 20-100 days postpartum with calves. All animals were randomly assigned to either the Select Synch (n = 84) or MGA: 7-11Synch (n = 88) estrus synchronization protocols without fixed time AI. The experimental design is illustrated in Fig. 2. Cows in the MGA: 7-11Synch treatment group were fed MGA (0.5 mg/h/day; Pharmacia Animal Health; Kalamazoo, MI) beginning on day -17 and concluding 7 days later on day -11. On the last day of MGA feeding (day -11), $PGF_{2\alpha}$ (25 mg i.m; 5 ml Lutalyse®; Pharmacia Animal Health; Kalamazoo, MI) was administered. Treatment schedules for injections of GnRH and $PGF_{2\alpha}$ after day -11 are identical in both Select Synch and MGA: 7-11Synch protocol. On day -7 all cows were administered GnRH (100 μg i.m; 2 ml Cystorelin®; Merial; Iselin; NJ), and then an injection of $PGF_{2\alpha}$ (25 mg) was given on day 0.

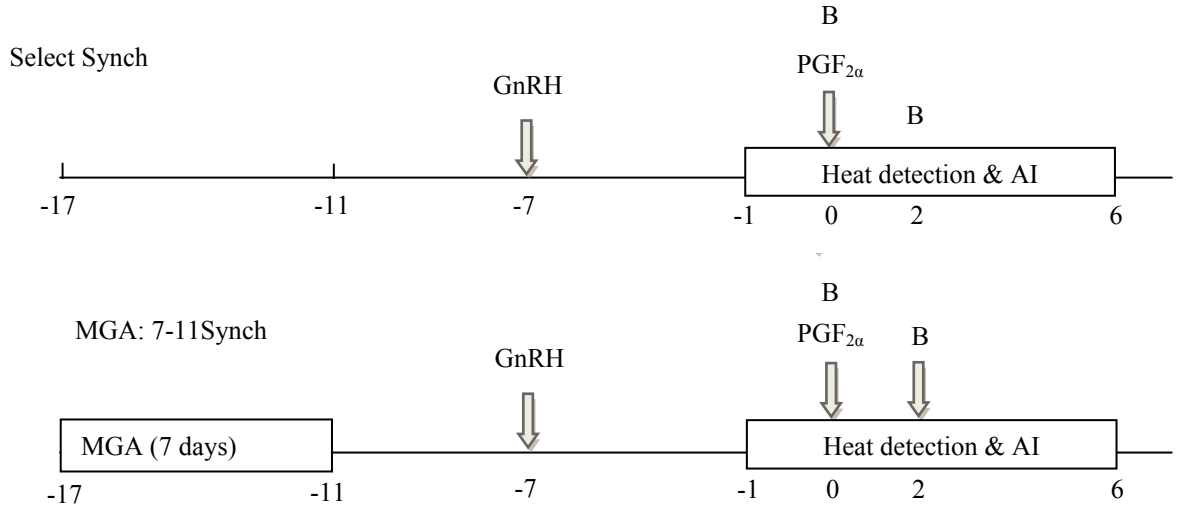

Figure 2. Treatment protocols applied to beef cows before first service in Experiment 2. Blood (B) samples were collected from all cows on days 0 and 2. The MGA: 7-11Synch cows were fed MGA (0.5 mg/h/day) for 7 days. GnRH (100 μg) and $PGF_{2\alpha}$ (25 mg) were administered as illustrated. All cows were inseminated after heat detection.

Estrus detection was performed on all animals beginning one day before the second injection of $PGF_{2\alpha}$, and continuing for 7 days (day -1 to day 6), for both treatment groups. At the University of Illinois Beef Unit, animals were observed for signs of behavioral estrus thrice daily (0600, 1200, and 1900) for a minimum of 30 min. Heat Watch® (HeatWatch, DDx, Inc., Denver, CO) was used to aid in detection of estrus at the Orr Center. Heat Watch® patches were applied at the time of GnRH injection (day -7). At both locations, cows that stood to be mounted were determined to be in estrus and were artificially inseminated 8-12 h later according to the AM/PM rule.

Blood sampling and progesterone assay were carried out as in experiment 1. Cows that did not exhibit estrus by day 6 were not artificially inseminated, and were re-synchronized using modified Co-Synch protocol with heat detection prior to insemination. GnRH (100 μg) was administered on day 6 and an injection of $PGF_{2\alpha}$ (25 mg) was given seven days later (day 13). Animals were observed for estrus and bred according to the AM/PM rule. On day 15, all cows not inseminated were administered a second injection of GnRH (100 μg) and concurrently inseminated.

Pregnancy was determined via transrectal ultrasonography 43-50 days after the first synchronization period (day -1 to day 6). Pregnant animals that were in estrus during a 5 day interval (day -1 to day 4) were considered pregnant to the first synchronization period. Animals that were either in estrus day -1 to day 14 or were fixed-time inseminated on day 15, after $PGF_{2\alpha}$ injection and became pregnant, were considered pregnant during the first 16 days of breeding season.

Statistical analysis

The General Linear Models (GLM) procedure of SAS was used to analyze the main effect of treatment and location within the experiment. Dependent variables in the analyses included the number of animals in estrus, interval from $PGF_{2\alpha}$ to estrus, number of animals with functional CL, incidence of luteolysis (number of animals with CL that regressed), number of animals that conceived to the first synchronization period, number of animals pregnant to the first synchronization period, and number of animals pregnant to the first AI in the first 16 days of breeding. Interval from $PGF_{2\alpha}$ to estrus was measured in hours.

Results

Experiment 1

Pregnancy rates, percentages of cows with

induced ovulation in response to the GnRH (day -7) and a functional CL (day 0) and incidence of luteolysis data for cows in the Co-Synch and MGA: 7-11Synch + TAI treatment groups are summarized in Table 1. First service pregnancy rates were not different (P > 0.35) between treatment groups. Overall pregnancy rate was 97/192 (51%). The number of cows with functional CL at $PGF_{2\alpha}$ (day 0) was greater (P > 0.001) in the MGA: 7-11Synch + TAI treatment group (94/97; 97%) than the Co-Synch group (76/95; 81%). These data suggest that the MGA: 7-11Synch + TAI protocol successfully induced ovulations and consequent formation of functional CL or resulted in the formation of luteinized follicles capable of responding to $PGF_{2\alpha}$. Incidence of luteolysis was not different (P > 0.60) between treatment groups and averaged 85%.

Table 1. Percentages of pregnant cows, cows in which ovulation was induced in response to GnRH (day -7) and formed functional CL (day 0), and incidence of luteolysis after $PGF_{2\alpha}$ on day 0 for cows synchronized with Co-Synch and MGA: 7-11Synch + TAI protocols. Experiment 1.

Treatment	First service AI pregnancy rate (%)	CL at $PGF_{2\alpha}$ (%)	Luteolysis (%)
Co-Synch	45/95 (48)	76/95 (81)[a]	66/76 (87)
MGA: 7-11Synch + TAI	52/97 (54)	94/97 (97)[b]	79/94 (84)
Combined	97/192 (51)		145/170 (85)

[a, b] Column values with different superscripts differ P < 0.01.

Experiment 2

Estrus, conception and pregnancy rates for cows in the Select Synch and MGA: 7-11Synch treatment groups are summarized in Table 2. The number of cows exhibiting estrus did not differ (P > 0.35) between the two synchronization protocols. Within -24 to 96 hours of $PGF_{2\alpha}$ administration (day -1 to day 4), a total of 128/171 (75%) cows exhibited estrus. First service conception and pregnancy rates during the first synchronization period (day -1 to day 4) were not different (P > 0.10) between the two treatment groups. Overall conception and pregnancy rates between days -1 and 4 were 99/128 (77%) and 99/171 (58%), respectively. There was, however, a location effect for estrus (P < 0.02) and pregnancy rate (P < 0.01) and a tendency towards a location effect for conception rate (P = 0.06). The total pregnancy rate (cows serviced between day -1 and day 15) also did not differ (P > 0.25) between treatment groups. In the first 16 days of breeding, a total of 127/171 (74%) cows were pregnant to the first AI. Again, there was a difference (P < 0.01) between locations. The intervals from the last $PGF_{2\alpha}$ injection (day 0) to estrus for Select Synch and MGA: 7-11 Synch were 54.5 ± 22.2 h and 61.2 ± 14.9 h, respectively. Cows in the Select Synch treatment group had a shorter interval to estrus (P < 0.05) than cows treated according to the MGA: 7-11Synch protocol. Again, there was a difference (P < 0.001) between locations.

Table 2. Fertility parameters of cows synchronized with Select Synch and MGA: 7-11Synch protocols. Experiment 2.

Treatment	Estrus response within -24 to 96 h of $PGF_{2\alpha}$ (%)	First service AI conception rate (%)	First service AI pregnancy rate (%)	First service AI pregnancy rate in first 16 day of breeding (%)
Select Synch	66/83 (80)	54/66 (82)	54/83 (65)	65/83 (78)
MGA: 7-11Synch	62/88 (70)	45/62 (73)	45/88 (51)	62/88 (70)
Combined	128/171 (75)	99/128 (77)	99/171 (58)	127/171 (74)

Discussion

The objectives of the two experiments were to determine if short-term MGA feeding in the 7-11Synch protocol would improve synchrony and pregnancy rates in beef cows after fixed time insemination (experiment 1) and shorten the interval from $PGF_{2\alpha}$ injection to estrus (experiment 2). It is well-established that estrous synchronization is an effective way to shorten the calving season and thus produce a more uniform calf crop (Dziuk and Bellows, 1983). Furthermore, females that conceive to a synchronized estrus calve earlier in the calving season and wean older and heavier calves compared to calves from non-synchronized females (Shafer *et al*., 1990). Although these benefits to estrous synchronization are well known, few beef cattle producers utilize estrous synchronization in their herds primarily because of the labor required for estrous detection (Britt, 1987). Recent surveys indicate that less than 5% 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 (Patterson *et al*., 2004). Development of estrus synchronization protocols that leads to more

adoption of the TAI in the beef industry is of crucial importance for the attempts to improving the genetic make up of beef cows.

Greater percentage of heifers were pregnant after the MGA:7-11 Co-Synch treatment (67%) than after the Co-Synch treatment (31%) demonstrating the potential of achieving acceptable pregnancy rates using timed artificial insemination in beef heifers (Thompson *et al*., 2003). The inclusion of a progestin in the Co-Synch protocol (Co-Synch + CIDR) yielded similar pregnancy rates to estrous detection protocols and is considered a reliable TAI protocol that eliminates detection of estrus when inseminating beef cows (Larson *et al*., 2006). Others showed improved pregnancy rates resulting from fixed-time AI after treatment with the Co-Synch + CIDR when insemination occurred 66 h as opposed to 48 and 54 h after CIDR removal and $PGF_{2\alpha}$ (Bremer *et al*., 2004). Acceptable pregnancy rates in the current study would enable more beef producers to include TAI in their synchronization programs and hence benefit from superior genetics at costs comparable to purchasing bulls

The use of GnRH 4 days after first $PGF_{2\alpha}$ (end of MGA feeding) successfully ovulated the first wave or turned it over resulting in more cows with functional CL on day 0 (second $PGF_{2\alpha}$ injection). The data obtained are in concert with those reported earlier by Kojima *et al*. (2000). Incidence of luteolysis was not different between the two groups, 87% (Co-Synch) vs. 84% (MGA: 7-11Synch + TAI), in the first study. This could be explained in part due to the fact that the MGA: 7-11Synch + TAI protocol produced more ($P < 0.01$) cows with CL on day 0 but those cows were not responsive to $PGF_{2\alpha}$ injection because these cows were in estrus later than 6 days after MGA withdrawal and their newly-formed CLs were not in a mature or responsive stage at the time of $PGF_{2\alpha}$ injection. Further, Fralix *et al*. (1996) reported that up to 20% of anestrous cows experienced short-term estrous cycles prior to $PGF_{2\alpha}$ administration on day 17 after MGA withdrawal. The number of cows with CL before $PGF_{2\alpha}$ administration was not affected ($P > 0.24$) by treatment and averaged 80%. Incidence of luteolysis was similar ($P > 0.70$) between treatment groups and averaged 90%.

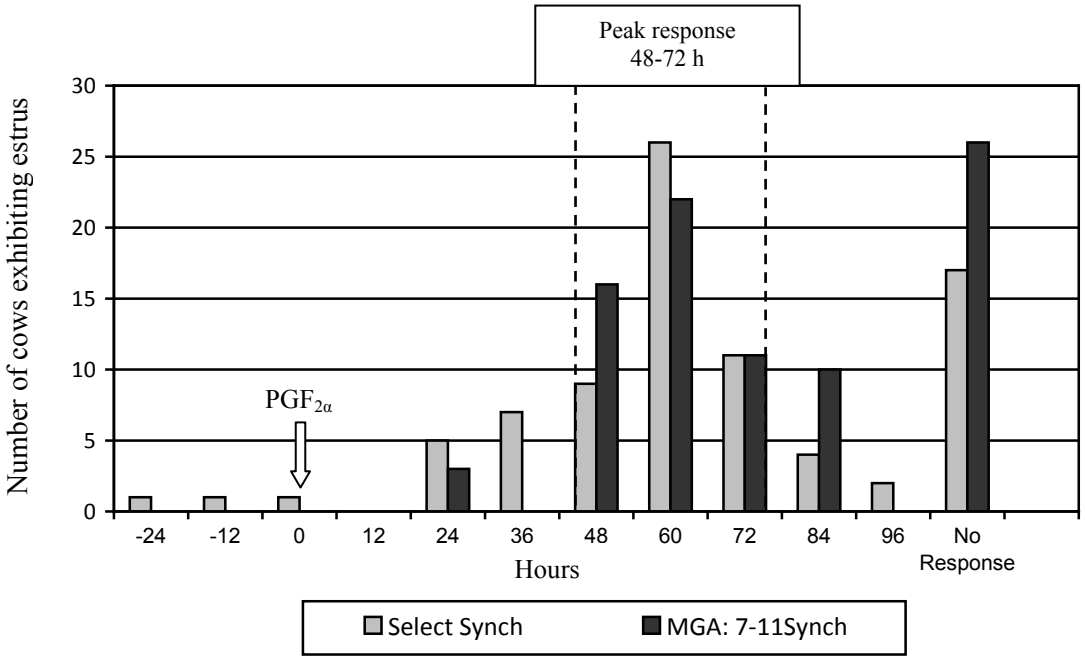

Figure 3. Distribution of estrus response in cows treated with either the Select Synch or MGA: 7-11Synch protocols. $PGF_{2\alpha}$ was administered on hour 0. Cows were observed for behavioral estrus 3 times a day (0600, 1200, and 1900). Dashed-line box represents the 24-h peak response period (48-72 h)

In experiment 2, short-term feeding of MGA combined with injections of $PGF_{2\alpha}$ and GnRH, as specified in the MGA: 7-11 Synch protocol, improved synchrony of estrus without reducing fertility when compared to Select Synch. During the 5-day synchronization period (-24 to 96 h), 3 cows (5%) in the Select Synch group exhibited estrus prior to and immediately after the time of $PGF_{2\alpha}$ (-24 to 0 h), and 2 cows (3%) exhibited estrus at 96 h. Cows that displayed estrus prior to or immediately at the time of $PGF_{2\alpha}$ injection in the Select Synch group (5%) must have been in a later stage of their estrous cycle (days 15 to 17) at the time of GnRH injection (day -7) and did not ovulate to GnRH and displayed estrus prematurely prior to $PGF_{2\alpha}$ injection (Downing *et al*., 1998). All 62 cows in the MGA: 7-11Synch group exhibited estrus in a 60 h time frame (24 to 84 h; Fig. 3) and none displayed estrus prior to or immediately at the time of $PGF_{2\alpha}$

injection. In a similar data set by Kojima *et al*. (2000), 9% of cows in the Select Synch treatment exhibited estrus after GnRH and before $PGF_{2\alpha}$ (-30 to 0 h), and another 9% of the cows exhibited estrus immediately after $PGF_{2\alpha}$ injection (0 to 18 h). Such a sporadic and premature display of estrus necessitates prolonged period of estrus detection and AI. The fact that none of the MGA: 7-11Synch cows in the second experiment displayed estrus before $PGF_{2\alpha}$ injection is considered an improvement of synchrony and grouping of estrus in a narrower peak response period providing the possibility for fixed time AI with comparable pregnancy rates. The intervals from the last $PGF_{2\alpha}$ injection (0 h) to estrus for Select Synch and MGA: 7-11Synch protocols were 54.5 ± 22.2 h (mean ± s.d.) and 61.2 ± 14.9 h, respectively. There was no significant difference in estrus response, conception rate, or pregnancy rate between the two groups. Based on the results from experiment 2 and similar estrus data reported in earlier studies (Kojima *et al*., 2000), estrus detection in the MGA: 7-11Synch protocol would only be necessary from 24 to 84 h vs 24 to 96 h for Select Synch.

In conclusion, the cost of feeding MGA is negligible, and only one additional injection of $PGF_{2\alpha}$ is required, making MGA: 7-11Synch less labor-intensive and more economical than GnRH-$PGF_{2\alpha}$-based protocols such as Select Synch. The tighter synchrony of estrus suggests that MGA: 7-11Synch offers the potential for fixed-time AI programs. Based on the results from experiments 1 and 2, there are several ways to use MGA: 7-11Synch to maximize pregnancy rates while utilizing a fixed-time insemination program.

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