Elsevier Editorial System(tm) for Theriogenology
Manuscript Draft

Manuscript Number: THERIO-D-09-00682R2

Title: Effect of timing of second prostaglandin F2� administration in a 5-day, progesterone-based CO-Synch protocol on AI pregnancy rates in beef cows

Article Type: Original Research Article

Keywords: Beef cattle; Estrus synchronization; CIDR; Prostaglandin; Pregnancy rate

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Abstract: The objective was to compare the timed AI pregnancy rate of Angus-cross beef cows synchronized with a 5-d CO-Synch + CIDR (a progesterone-releasing intravaginal insert) protocol and given two doses of PGF2� (PGF), with the first dose in conjunction with CIDR withdrawal on Day 5, and the second dose given either early or late relative to the first dose. All cows (N=1782) at 16 locations received 100 �g of GnRH + CIDR on Day 0. Cows received 25 mg of PGF concurrent with removal of the CIDR on Day 5, and were randomly allocated within locations to receive a second PGF either early (N=881; from 0:30 to 3.54 h) or late (N=901; 4:30 to 8:09 h) relative to the first PGF treatment. On Day 8 (72 h after CIDR removal), all cows were inseminated and concurrently given 100 �g of GnRH. Cows were fitted with a pressure-sensitive mount detection device (Kamar) at CIDR removal. Cows were observed twice daily through Day 7 and at the time of AI on Day 8 for estrus and Kamar status (estrus - red, partial and lost Kamar versus no estrus - white Kamar) was recorded. Accounting for location, season, AI sire, cow observed in estrus or not at or before timed AI, and treatment by cows observed in estrus interaction, timed AI pregnancy rates were greater for the late (6:27 ± 00:2 h) than the early (2:15 ± 00:3 h) interval, 57.2 vs 52.7% respectively (P<0.05). In conclusion, cows that received the second PGF late after the first PGF on the day of CIDR removal in a 5-d CO-Synch + CIDR synchronization protocol had significantly higher timed AI pregnancy rates than those receiving the second PGF early after the first PGF.

Manuscript

Effect of timing of second prostaglandin $F_{2\alpha}$ administration in a 5-day, progesterone-based CO-Synch protocol on AI pregnancy rates in beef cows

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Acknowledgements

The authors extend their gratitude to the staff of Virginia Department of Corrections for their support with data collection. The authors also thank Select Sires Inc, Plain City, OH, USA for providing partial financial support and Dale Moore, Washington State University, for review of this manuscript.

Abstract

The objective was to compare the timed AI pregnancy rate of Angus-cross beef cows synchronized with a 5-d CO-Synch + CIDR (a progesterone-releasing intravaginal insert) protocol and given two doses of $PGF_{2\alpha}$ (PGF), with the first dose in conjunction with CIDR withdrawal on Day 5, and the second dose given either early or late relative to the first dose. All cows (N=1782) at 16 locations received 100 μg of GnRH + CIDR on Day 0. Cows received 25 mg of PGF concurrent with removal of the CIDR on Day 5, and were randomly allocated within locations to receive a second PGF either early (N=881; from 0:30 to 3.54 h) or late (N=901; 4:30 to 8:09 h) relative to the first PGF treatment. On Day 8 (72 h after CIDR removal), all cows were inseminated and concurrently given 100 μg of GnRH. Cows were fitted with a pressure-sensitive mount detection device (Kamar) at CIDR removal. Cows were observed twice daily through Day 7 and at the time of AI on Day 8 for estrus and Kamar status (estrus – red, partial and lost Kamar versus no estrus – white Kamar) was recorded. Accounting for location, season, AI sire, cow observed in estrus or not at or before timed AI, and treatment by cows observed in estrus interaction, timed AI pregnancy rates were greater for the late (6:27 ± 00:2 h) than the early (2:15 ± 00:3 h) interval, 57.2 vs 52.7% respectively (P<0.05). In conclusion, cows that received the second PGF late after the first PGF on the day of CIDR removal in a 5-d CO-Synch + CIDR synchronization protocol had significantly higher timed AI pregnancy rates than those receiving the second PGF early after the first PGF.

Key words: Beef cattle; Estrus synchronization; CIDR; Prostaglandin; Pregnancy rate

1. Introduction

Synchronization of estrus, ovulation, or both in beef cows shortens the calving season and facilitates the use of AI and thereby the opportunity to introduce desired genetics into a herd. Numerous estrus synchronization protocols using $PGF_{2\alpha}$ (PGF), GnRH, and/or a progestin have been developed to induce cyclicity and successfully synchronize estrus in beef cows [1-4].

The CO-Synch protocol, in which PGF is given 7 d after GnRH, followed by a second GnRH injection and timed AI (TAI) 60 h after PGF, yielded pregnancy rates >50% [5]. Often, an exogenous progestin, e.g. a progesterone-releasing intravaginal insert (CIDR), was used during the 7 d interval from the initial GnRH injection to PGF [6]. When the interval was shortened from 7 to 5 d in a CO-Synch + CIDR program, the timed AI pregnancy rate increased to 70.4%, which was 10.5% greater than that achieved with a 7-d program [7]. Due to the shortened interval from the initial GnRH to PGF in the 5 d CO-Synch + CIDR program and variability in luteolysis in some cows when a single dose of PGF was given on Day 5 of the estrous cycle, all cows received a second PGF treatment at CIDR withdrawal (Bridges et al., 2008) to increase luteolysis [7]. A recent study by Kasimanickam et al (2009) investigated whether similar pregnancy rates could be achieved for a single dose of PGF or cloprostenol (CLP) compared to two doses of PGF [8]. In that study, administration of two PGF doses resulted in timed AI pregnancy rates of 69% across six herds, whereas a single dose of PGF or CLP resulted in a 15 to 17% reduction in timed-AI pregnancy rates.

Although variable responses in luteolysis have been reported when a single dose of PGF was given on Day 5 of the estrous cycle, two doses of CLP consistently (95 to 100%) induced regression of the CL [9,10]. In non-pregnant ruminants, luteal regression is caused by PGF secreted from the uterus [11-13] in a series of five to eight discrete pulses [14-17]. There is variability among species in the duration and magnitude of pulses, but, they typically occur at 6 to 8 h intervals [18]. Studies in which a second PGF injection was administered at 7 or 12 h intervals, reported 10 and 15% increases in timed AI pregnancy rates [7,8]. Very low doses of PGF (40 to 200 ηg/h) can induce luteal regression in ewes, if given in repeated treatments [19]. Two distinct phases of uterine PGF secretion, differing in pulse magnitude, have been described in the cow, whereas in ewes, there was a gradual increase in the magnitude of pulses during luteolysis [19]. Therefore, there is rationale to investigate PGF treatments at intervals less than 8 to 12 h.

The objective of the present study was to compare the timed AI pregnancy rate of Angus-cross beef cows synchronized with a 5-d CO-Synch + CIDR protocol and given two doses of PGF, with the first dose in conjunction with CIDR withdrawal on Day 5, and the second dose given either early or late relative to the first dose.

2. Materials and methods

2.1. Cows and treatment protocol

Angus-cross beef cows (N=1782) at 16 locations belonging to the Department of Corrections, Virginia, USA, to be inseminated at a fixed time during the fall of 2008 (N= 793) and spring of 2009 (N=989) breeding seasons were included in the study. Cows were synchronized with the 5-d CO-Synch + CIDR protocol (Fig. 1). All cows received 100 μg of gonadorelin diacetate tetrahydrate (GnRH, IM; Cystorelin® sterile solution, Merial, Athens, GA, USA) and a controlled internal drug release insert (CIDR; Eazi-Breed™ CIDR® cattle insert, Pfizer Animal Health, New York, NY, USA) on Day 0, plus 25 mg of dinoprost IM (PGF, 5 mL Lutalyse® sterile solution, Pfizer Animal Health) at CIDR insert removal on Day 5 (AM). Within location, cows were randomly allocated to receive a second dose of 25 mg IM, either early (N=881) or late (N=901) relative to the first PGF treatment. Within location, cows in the early group received their first PGF dose initially and were immediately brought back to receive the second PGF treatment, whereas cows in that late group waited at least 4:30 h between the first and second PGF treatments. Kamar® Heatmount detector patches (Kamar, Inc., Steamboat Springs, CO, USA) were applied to all cows at CIDR removal. After CIDR removal, cows were observed twice daily through Day 7 for estrus and Kamar status (estrus = red Kamar, partial red Kamar and lost Kamar with mount marks vs. no estrus = white Kamar) was recorded. A cow was designated in estrus if she was observed to stand for mounting, or if she had an activated (color change from white to red), lost (with mount marks) or partially-activated Kamar. On Day 8, cows were inseminated, given 100 μg of GnRH, and Kamar status was recorded. Sires used in this study were Angus (N=2), and Simmental (N=1) breeds; within locations, sires were randomly allocated to treatments. To ensure semen quality, samples were assessed for post-thaw motility after 0 and 3 h of incubation at 37 °C (>60 and >25%, respectively) and for the percentage of intact acrosomal membranes after 3 h of incubation at 37 °C (>60%). Experienced inseminators (with prior experience of a minimum of 1000 inseminations) performed fixed time AI at all locations. Since fixed time AI occurred at the same time (within 2 d), AI technicians were different across locations. Technicians and sires were randomly assigned within locations.

Two weeks later, reproductively sound (based on a standard breeding soundness exam 1 mo prior to the breeding season) and proven (based on reproductive performance from previous breeding season) bulls were placed with the cows (approximately 1: 40 bull: cow ratio), across treatments, for the remainder of the 60 to 70 d breeding season. Cows were examined for pregnancy status 50 to 70 d after timed AI, and again at 120 d after timed AI by transrectal palpation and/or ultrasonography, to determine the number of cows pregnant to AI or to natural service.

2.2. Statistical analyses

Data were analyzed with a statistical software program (SAS Version 9.1 for Windows, SAS Institute, Cary, NC, USA). The PROC MIXED procedure of SAS was used to examine the effect of treatments (early versus late) on timed AI and breeding season pregnancy rates. Models included treatment (early versus late), AI-Sire (three), days postpartum at initiation of synchronization (30 to 60, 61 to 80 and >81 d), body condition scores (≤4 and >4), estrus status prior to or at fixed time AI (yes or no), and appropriate interactions. The locations (1 to 16) were nested within the season (fall and spring). A *P* value of 0.05 was considered significant. For model reduction, the *P* value was set at ≤0.1 for inclusion and >0.1 for exclusion until the model contained only significant main and interaction effects. The final model had treatment, AI sire, estrus status, season (location), and treatment by estrus status interaction.

A model was developed, retrospectively, to test the effect of the hourly time interval between PGF administration (PGFH; from 1 to 8 h) on timed AI pregnancy rates. Models included PGFH (0 to 1, 1 to 2, 2 to 3, 3 to 4, 4 to 5, 5 to 6, 6 to 7, and 7 to 8 h), AI-Sire (three), days postpartum at initiation of synchronization (30 to 60, 61 to 80, and >81 d), body condition scores (≤4 and >4), estrus status prior to or at fixed time AI (yes or no) and appropriate interactions. Locations (1 to 16) were nested within the season (fall and spring). A *P* value of 0.05 was considered significant. For model reduction, the *P* value was set at ≤0.1 for inclusion and >0.1 for exclusion, until the model contained only significant main and interaction effects. The final model included PGFH, AI sire, estrus status, and season (location). The AI pregnancy rates among locations were analyzed by Chi-square.

It was hypothesized *a priori* that difference between treatment groups in AI pregnancy rates would be 7%. To detect this difference, with adequate statistical power ($1-\beta = 0.8$) and statistical significance ($\alpha = 0.05$), a sample size of 796 cows per treatment group was needed.

3. Results

Age, BCS and days postpartum at initiation of synchronization for cows assigned to the two treatments were not significantly different (Table 1). The interval from CIDR withdrawal to AI and the percentage of cows in estrus at or before timed AI was not different between treatment groups (late: 53.3% (480/901); early: 52.9% (466/881); P>0.1; Table 1). However, the percentage of cows in estrus differed among locations (Fig. 2; P<0.001), but not between seasons (54.6 vs 51.9% for fall and spring respectively; P>0.1). Accounting for AI sires (P<0.04), cows' estrus status at or before timed AI (P<0.0001), treatment by cows estrus status interaction (P=0.1), and season (location; P<0.05), AI-pregnancy rates for cows that received the second PGF late (6:26:45 ± 00:1:54 h) after the first PGF had higher AI pregnancy rates than cows that received the second PGF early (2:15:19 ± 00:3:00 h) after first PGF [57.1% (514/901) vs 52.4% (461/881), respectively; P<0.05; Table 2]. The AI pregnancy rates associated with the two Angus sires [55.6% (328/590) and 57.8% (398/688)] were significantly higher than the rate associated with the Simmental sire [49.5% (249/504)]. The AI pregnancy rate for cows that exhibited estrus at or before AI was higher than for cows that failed to exhibit estrus [63.8% (604/946) vs 44.4% (371/836); $P<0.0001$].

Cows that received the second PGF at late and exhibited estrus had higher pregnancy than cows in other groups (Fig. 3; $P<0.05$). Accounting for AI sires ($P<0.05$), cows' estrus status at or before timed AI ($P<0.0001$), and season (location; $P=0.1$), the PGF hourly administration intervals differed (Table 3 and Fig. 4; $P=0.05$). Among locations, AI pregnancy rates ranged from 30.0 to 70.8% for the early group, and from 42.9 to 84.6% for the late group.

4. Discussion

Higher timed-AI pregnancy rates were achieved with a second PGF treatment 4 to 8 h (late) rather than 0 to 3.5 h (early) after the first PGF treatment at CIDR removal (in place for 5 d in a CO-Synch protocol). Cows that exhibited estrus at or prior to timed AI had higher pregnancy rates compared to cows that failed to exhibit estrus. Cows that received a second dose of PGF late and exhibited estrus had higher pregnancy rates than cows in all other groups.

Secretion of PGF by the endometrium and increased production of intraluteal PGF terminates the luteal phase in cattle [20-26]. Although exogenous PGF can induce rapid luteolysis in the mid-luteal phase, the early CL is refractory (up to d 5) to the luteolytic action of PGF. In this study, PGF administration occurred 5 d after the first GnRH injection. If the first GnRH injection induced ovulation and/or luteinization, then the CL would have been <5 d old, which may have accounted for refractoriness to a single dose of PGF.

There are several reasons given in the literature for the refractoriness of the early CL to the luteolytic dose of PGF, including lack of expression of PGF receptors and the presence of PGF catabolizing enzyme, 15-hydroxyprostaglandin dehydrogenase (PGDH), in the early CL [27-29]. In addition, changes in blood flow, alterations in gene expressions (VEGF, eNOS, angiopoietin, angiotensin, endothelin, CAMKK2, HINT1, YWHAZ, GNB1, and RGS2), and changes in NO and pO2 may also affect the luteolytic effects of PGF on a recently formed CL [30,31].

The PGF receptor mRNA is expressed in the CL at high levels throughout the estrous cycle; administration of PGF in early or mid-luteal phases inhibited PGF receptor mRNA expression [32,33]. Therefore, the refractoriness of the early CL may not be due to the lack of PGF receptors. The CL produces PGs such as cyclooxygenase (COX)-1, COX-2, PGF, and PGE synthases during the estrous cycle [29]. The administration of PGF elevated COX-2 in mid-cycle, but not in the early part of the cycle, suggesting that luteal PGF production is stimulated by exogenous PGF only in a mature CL [25,34]. The PGDH are at a higher level in the early CL compared to the midcycle CL in sheep [35]. The biological function of PGDH in the CL prevents luteal concentrations of PGF from reaching a threshold. In addition, the production of luteal PGF may relate to mechanisms involved in luteal resistance to the luteolytic effects of PGF [35].

Based on PGFM concentrations, PGF is released from the uterus in pulses occurring approximately every 12 h in association with spontaneous luteolysis in cattle [17,36-38]. Ginther et al (2009) demonstrated that at least four sequential PGF pulses at 12 h intervals are required to stimulate natural luteolysis in cattle [39]. Although variable responses in luteolysis have been noted when a single dose of PGF was given on Day 5 of the estrous cycle, based on the present and other studies, two doses of PGF might overcome the refractoriness of the early CL and induce luteolysis [7,8,10].

In the present study, cows that received a second PGF treatment from 4 to 5 h and from 6 to 7 h after the first treatment had 63.4 and 60.8% timed AI pregnancy rates, respectively. It is noteworthy that cows received the second PGF within 2 h after the first PGF yielded had >50% AI pregnancy rates. Handling cows twice within 2 h requires good handling facility, but it could be feasible in smaller (e.g. 50 head) beef herds. The herd size ranged from 23 to 237 in this study. Interestingly, cows in the late group that exhibited estrus had higher pregnancy than cows in other groups. The mean percentage of cows that exhibited estrus was significantly different among locations, but not between seasons. It was expected that cows observed in estrus were more likely to become pregnant than cows not observed in estrus. Perhaps the dynamics of cow-specific factors, e.g., age, BCS and days postpartum at initiation of synchronization within location and season, plus differences in the management and environment, may have contributed to this difference [40]. In addition, the interval from estrus to AI is critical. A recent study recommended AI 12 h after the onset of estrus, as a compromise between earlier fertilization failure and later embryo failure [40]. They concluded younger cows (≤3 y of age) had better fertility when inseminated at 56 h, whereas older cows had better fertility at any time other than 48 h, i.e. from 56 to 72 h.

In summary, cows that received a second PGF treatment late after the first PGF treatment (on the day of CIDR removal in a 5-d CO-Synch + CIDR synchronization protocol) had significantly greater timed AI pregnancy rates than those that received their second PGF early after the first PGF. Therefore, it is advisable to wait at least 4.5 h following the initial PGF treatment to administer a second PGF.

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Table 1. Descriptive statistics (mean±SEM) for beef cows that received a second PGF treatment 0 to 3.5 h (early) or 4 to 8 h (late) after the first PGF treatment (given at CIDR removal on Day 5 of a CO-Synch + CIDR protocol).

Factor	Early	Late
No. cows	881	901
Age (y)	5.30 ± 0.09	5.45 ± 0.09
Body Condition Score*	5.79 ± 0.03	5.85 ± 0.03
Postpartum interval at start of experiment (d)	70.56 ± 0.69	68.75 ± 0.68
Average interval between PGF doses (h)	2:15:19 ± 00:3:00	6:26:45 ± 00:1:54
Range interval between PGF doses (h)	0:30:00, 3.54:04	4:30:00, 8:09:31
Interval from CIDR removal to AI (h)	72:27:34 ± 5:28:40	72:25:02 ± 05:13:28

*Body Condition Score: 1 = emaciated; 9 = obese;

Table 2. Proc Mixed model on the effects of the interval between two doses of PGF (early vs late) at CIDR removal on Day 5 and co-variables on AI pregnancy rates of crossbred beef cows (N=1782) synchronized with a 5-d CO-Synch+CIDR protocol.

Effect	DF*	F Value	Pr > F
Treatment	1	4.46	0.0349
AI sire	2	3.23	0.0399
Estrus status	1	70.83	<0.0001
Treatment × estrus status	1	2.75	0.0972
Season(location)	15	1.93	0.0169

*Degrees of freedom

Table 3. Proc Mixed model effects of hourly interval between two doses of PGF and co-variables on AI pregnancy rates of crossbred beef cows (N=1782) synchronized with a 5-d CO-Synch + CIDR protocol.

Effect	DF[2]	F value	Pr > F
PGFH[1]	7	1.95	0.0504
AI sires	2	3.20	0.0412
Estrus status	1	71.46	<0.0001
Season(location)	15	1.48	0.1027

[1]Hourly PGF treatment (0 to 1, 1 to 2, 2 to 3, 3 to 4, 4 to 5, 5 to 6, 6 to 7, and 7 to 8 h)

[2]Degrees of freedom

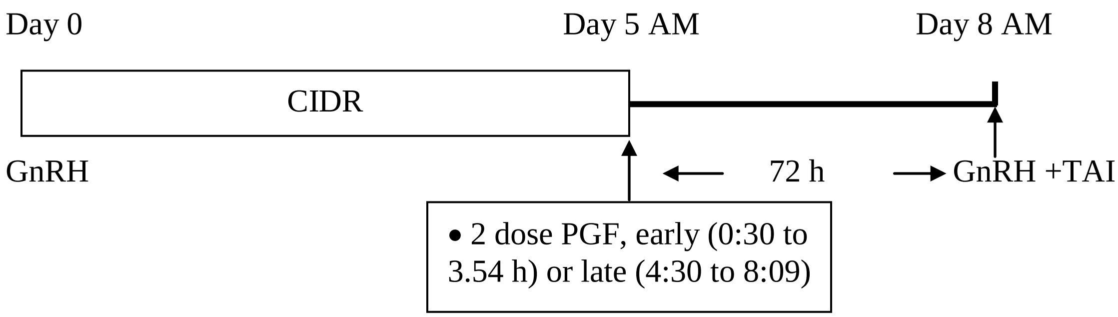

Fig. 1. Diagrammatic representation of the treatment protocol. Briefly, all cows received 100 μg of GnRH and a CIDR on Day 0, and were allocated to receive two doses of 25 mg of dinoprost, the first dose at CIDR removal (Day 5) and the second either early (0:30 to 3.54 h) or late (4:30 to 8:09) relative to the first dose. All cows were given 100 μg of GnRH and inseminated on Day 8 (72 h after CIDR removal).

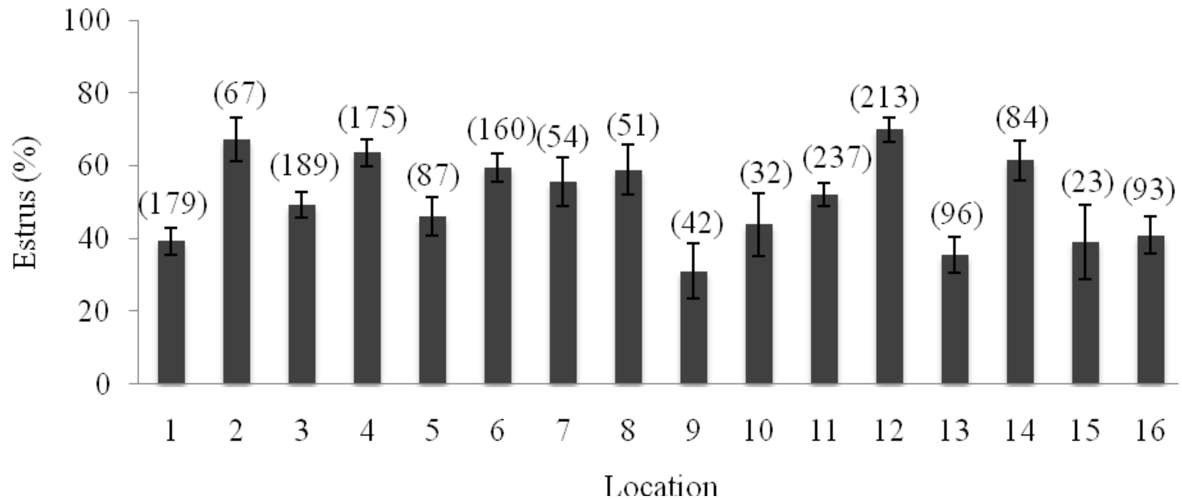

Fig. 2. Mean (±SEM) percentage of cows exhibiting estrus at or prior to timed AI in a 5-d CO-Synch protocol on AI Pregnancy in beef cows (N=1782) in 16 locations (number of cows in parenthesis). There was an effect of location ($P<0.001$).

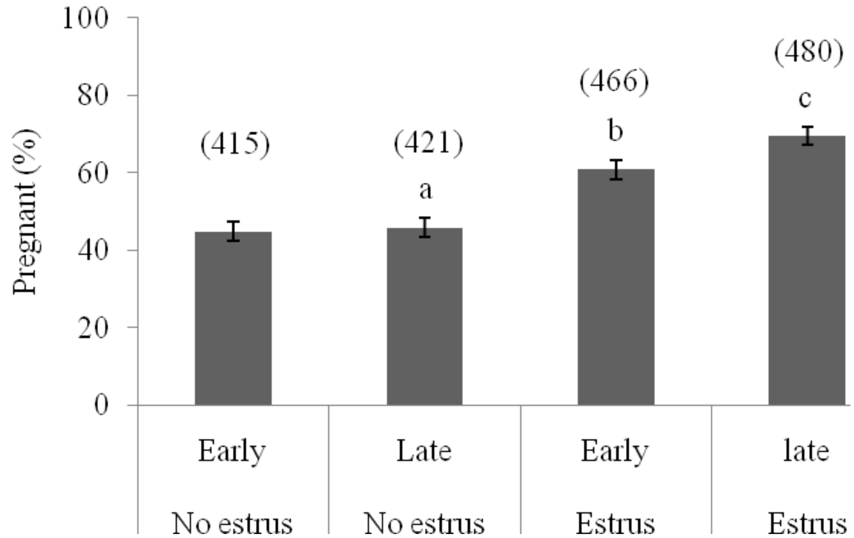

Fig. 3. Mean (+ SEM) AI pregnancy rate in beef cows that showed estrus or not and given a second PGF treatment at 0 to 3.5 h (early) or 4 to 8 h (late) after the first PGF treatment given at CIDR removal on Day 5 of a 5-d CO-Synch+CIDR protocol. The number of cows is in parentheses. There was an interaction between estrus and time of second PGF treatment ($P<0.05$)

[a-c]Columns without a common superscript differed ($P<0.05$).

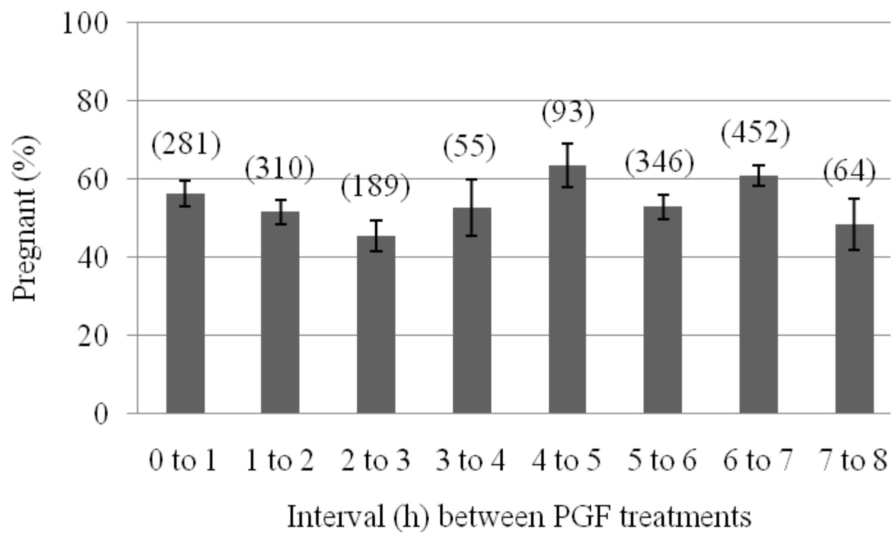

Fig. 4. Mean (± SEM) AI pregnancy rate in beef cows according to the interval between two doses of $PGF_{2\alpha}$ given at CIDR removal in a 5-d CO-Synch protocol on mean AI Pregnancy in beef cows (N=1782). The second PGF treatment was given at 0 to 3.5 h (early) or 4 to 8 h (late) after the first PGF treatment given at CIDR removal on Day 5 of a 5-d CO-Synch+CIDR protocol. The number of cows is in parentheses. There was an effect of interval between the two doses of PGF on pregnancy rates (P=0.05).

***Revision Notes**

Dated: March 24, 2010

To,

Co-Editor in Chief,

Theriogenology Journal.

Dear Dr. Kastelic,

Please find the submitted articles titled, “Effect of timing of second prostaglandin $F_{2\alpha}$ administration in a 5-day, progesterone-based CO-Synch protocol on AI pregnancy rates in beef cows” for review. The suggestions provided by the reviewers and co-editors have been incorporated in the revised version.

Thank you,

Sincerely,

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Revised

Effect of timing of second prostaglandin F2α administration in a 5-day progesterone based CO-Synch protocol on AI pregnancy rates in beef cows

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Acknowledgements

The authors extend their gratitude to the staff of Virginia Department of Corrections for their support with data collection. The authors also thank Select Sires Inc, Plain City, OH, USA for providing partial financial support and Dale Moore, Washington State University, for review of this manuscript.

Abstract

The objective of the present study was to 1. compare the timed AI pregnancy rate of Angus cross beef cows synchronized with the 5-d CO-Synch + CIDR (Controlled Internal Drug Release, a progesterone-based intravaginal insert) protocol and given two PGF2α (PGF) doses either early or late after first dose on the day of CIDR withdrawal; 2. Compare two PGF doses administered at hourly interval (up to 8 h) after first dose on the day of CIDR withdrawal. All cows (N=1782) at 16 locations received 100 μg of GnRH (Gonadotropin Releasing Hormone) + CIDR on Day 0. Cows received 25 mg of PGF at the time of CIDR insert removal on Day 5 and randomly allocated within locations to receive second PGF either early (N=881; from 0:30 to 3.54 h) or late (N=901; 4:30 to 8:09 h) from the first PGF administration. All cows were administered 100 μg of GnRH on Day 8 (72 h after CIDR removal) and were inseminated at that time. Cows were fitted with a pressure sensitive heat detection device (Kamar) at the time of CIDR withdrawal. Cows were observed twice daily through Day 7 and at the time of AI on Day 8 for estrus and Kamar status (estrus – red, partial and lost Kamar vs. no estrus – white Kamar) was recorded. Accounting for location, season, AI sire, cow observed in estrus or not at or before timed AI, and treatment by cows observed in estrus interaction, the timed AI pregnancy rates were greater for the late (6:27 ± 00:2 h) than the early (2:15 ± 00:3 h) interval, 57.2% vs. 52.7% respectively ($P<0.05$). In conclusion, cows that received the second PGF late after the first PGF on the day of CIDR removal in a 5-d CO-Synch + CIDR synchronization protocol had greater timed AI pregnancy rates than cows receiving the second PGF early after the first PGF.

Key words: Beef cows; Synchronization; CIDR; Prostaglandin; Pregnancy rate;

1. Introduction

Synchronization of estrus, ovulation or both in beef cows shortens the calving season and facilitates the use of artificial insemination in a labor-efficient way and at a fixed time. Artificial insemination provides an opportunity to introduce desired genetics into a herd. Numerous estrous synchronization protocols using PGF2α (PGF), Gonadotropin Releasing Hormone (GnRH), and/or a progestin have been developed to induce cyclicity and successfully synchronize estrus in beef cows [1-4].

The CO-Synch protocol in which PGF is administered 7 d after GnRH followed by a second GnRH injection and timed artificial insemination (TAI) 60 h after PGF yielded pregnancy rates above 50% [5]. Often, an exogenous progestin, a progesterone-based intravaginal insert (CIDR) is used during the 7 d interval from the initial GnRH injection to PGF [6]. When the interval was shortened from standard 7d to 5 d in a CO-Synch+CIDR program the timed AI pregnancy rate increased to 70.4%, which was 10.5% greater than that achieved with a 7 d program [7]. Due to the shortened interval from the initial GnRH to PGF in the 5 d CO-Synch + CIDR program and variability in luteolysis in some cows when a single dose of PGF is given on Day 5 of the estrous cycle, all cows received a second PGF treatment at CIDR withdrawal (Bridges et al., 2008) to increase luteolysis [7]. A recent study by Kasimanickam et al., (2009) investigated whether similar pregnancy rates could be achieved for a single dose of PGF or cloprostenol (CLP) compared to two doses of PGF [8]. The study concluded that administration of two PGF doses resulted in timed AI pregnancy rates of 69% across six herds whereas single dose of PGF or CLP resulted in a 15 to 17% reduction in timed AI pregnancy rates.

While variable responses in luteolysis have been noted when a single dose of PGF was given on Day 5 of the estrous cycle, two doses of CLP have consistently induced regression of the corpus luteum (95 to 100%) at 12 h interval after estrus [9,10]. In non-pregnant ruminants, luteal regression is caused by PGF secreted from the uterus [11-13]. The uterus releases PGF in a series of five to eight discrete pulses [14-17]. There is variability among species in the duration and magnitude of pulses, but, they typically occur at 6 to 8 h intervals [18]. Studies in which a second PGF injection is administered at 7 h or 12 h intervals, reported 10% and 15% increase in timed AI pregnancy rates. Very low doses of PGF (40 to 200 ηg/h) can induce luteal regression in ewes, if given in repeated treatments [19]. Two distinct phases of uterine PGF secretion, differing in pulse magnitude, have been described in the cow whereas a gradual increase in the magnitude of pulses during luteolysis has been observed in ewes [19]. It is worthy to investigate the practical application of this approach by comparing the PGF administrations at a shorter interval instead of waiting for up to 8 to 12 h.

The objective of the present study was to compare the timed AI pregnancy rate of Angus cross beef cows synchronized with the 5-d CO-Synch + CIDR protocol and given two doses of PGF either early or late in conjunction with CIDR withdrawal on Day 5.

2. Materials and methods

2.1. Animals and treatment protocol

Angus cross beef cows (N=1782) at 16 locations belonging to the Department of Corrections, Virginia, USA, to be inseminated at a fixed time during the fall of 2008, (N= 793) and spring of 2009 (N=989) breeding seasons were included in the study. Cows synchronized with the 5-d CO-Synch + CIDR protocol (Figure 1). All cows received 100 μg of gonadorelin diacetate tetrahydrate (GnRH, IM; Cystorelin® sterile solution, Merial, Athens, GA, USA) and a controlled internal drug release insert (CIDR; Eazi-Breed™ CIDR® cattle insert, Pfizer Animal Health, New York, NY, USA) on Day 0 plus 25 mg of dinoprost IM (PGF, IM, 5 mL Lutalyse® sterile solution, Pfizer Animal Health, New York, NY, USA) at the time of CIDR insert removal on Day 5, AM. Within location, cows were randomly allocated to receive a second dose of 25 mg IM, either early (N=881) or late (N=901) from the first PGF administration. Within location, cows in the early group received first PGF dose initially and immediately brought back to receive the second PGF dose and cows in late group waited at least 4:30 h between the first and second PGF dose. All cows received Kamar® Heatmount detector patches (Kamar, Inc., Steamboat Springs, CO, USA) at the time of CIDR removal. After CIDR removal, the cows were observed twice daily through Day 7 for estrus and Kamar status (estrus = red Kamar, partial red Kamar and lost Kamar with mount marks vs. no estrus = white Kamar) was recorded. A cow was determined to be in estrus if she was visually observed to stand for mounting or if she had an activated (color change from white to red), lost (with mount marks) or partially-activated Kamar. Cows were administered 100 μg of GnRH on Day 8, Kamar status was recorded and cows were inseminated at this time. Sires used in this study were Angus (N=2), and Simmental (N=1) breeds; within locations the sires were randomly allocated to treatments. As per stud center standard frozen semen samples were assessed of for post-thaw motility after 0 and 3 h of incubation at 37 °C (>60 and >25%, respectively) and for the percentage of intact acrosomal membranes after 3 h of incubation at 37 °C (>60%) to confirm maintenance of semen quality. Experienced inseminators (with prior experience of a minimum of 1000 inseminations) performed fixed time AI at all locations. Since fixed time AI occurred at the same time, within two days, AI technicians are different across locations. Technicians and sires were randomly assigned within locations.

Two weeks later, reproductively sound (based on breeding soundness exam one month prior to the breeding season) intact, and proven (based on reproductive performance from previous breeding season) bulls (approximately 1: 40 bull: cow ratio) were placed in with the cows, across treatments, for the remainder of the 60 to 70 d breeding season. Cows were examined for pregnancy status at 50 to 70 d after timed AI and again at 120 d after timed AI by rectal palpation and/or ultrasonography to determine the number of cows pregnant to AI or to rebreeding by clean-up bulls.

2.2. Statistical analyses

Data were analyzed with a statistical software program (SAS Version 9.1 for Windows, SAS Institute, Cary, NC, USA). The PROC MIXED procedure of SAS was used to examine the effect of treatments (early vs. late) on timed AI and breeding season pregnancy rates. Models included treatment (early vs. late), AI-Sire (three), days postpartum at initiation of synchronization (30 to 60, 61 to 80 and > 81 days), body condition scores (≤ 4 and > 4), estrus status prior to or at fixed time AI (yes or no) and appropriate interactions. The locations (1 to 16) were nested within the season (fall and spring). A *P* value of 0.05 was considered significant. For model reduction, the *P* value was set at ≤ 0.1 for inclusion and > 0.1 for exclusion until the model contained only significant main and interaction effects. The final model had treatment, AI sire, estrus status, season (location), and treatment by estrus status interaction.

A model was developed, retrospectively, to test the effect of hourly time interval between PGF administration (PGFH; from 1 to 8 h) on timed AI pregnancy rates. Models included PGFH (0 to 1 h; 1 to 2 h; 2 to 3 h; 3 to 4 h; 4 to 5 h; 5 to 6 h; 6 to 7 h; 7 to 8 h), AI-Sire (3), days postpartum at initiation of synchronization (30 to 60, 61 to 80 and > 81 days), body condition scores (≤ 4 and > 4), estrus status prior to or at fixed time AI (yes or no) and appropriate interactions. The locations (1 to 16) were nested within the season (fall and spring). A *P* value of 0.05 was considered significant. For model reduction, the *P* value was set at ≤ 0.1 for inclusion and > 0.1 for exclusion until the model contained only significant main and interaction effects. The final model included PGFH, AI sire, estrus status, and season (location). The AI pregnancy rates among locations were analyzed separately by Chi-square.

It was hypothesized that the AI pregnancy difference between treatment groups will be 7%. To detect the same difference in the AI pregnancy, with adequate statistical power ($1-\beta = 0.8$) and statistical significance ($\alpha = 0.05$), the study needed a sample size of 796 cows per treatment group.

3. Results

Age, BCS and days postpartum at initiation of synchronization for cows assigned to the two treatments were not significantly different (Table 1). The interval from CIDR withdrawal to AI and the percentage of cows in estrus at or before timed AI was not different between treatment groups (late: 53.3% (480/901); early: 52.9% (466/881); $P>0.1$; Table 1). However, the percentage of cows in estrus differed among the locations (Figure 2; $P<0.001$) but not between seasons (54.6% vs. 51.9% for fall and spring respectively; $P>0.1$). Accounting for AI sires ($P<0.04$), cows' estrus status at or before timed AI ($P<0.0001$), treatment by cows estrus status interaction ($P=0.1$) and season (location) ($P<0.05$), AI-pregnancy rates for cows that received the second PGF late (6:26:45 ± 00:1:54 h) after the first PGF had higher AI pregnancy rates compared to cows that received second PGF early (2:15:19 ± 00:3:00 h) after first PGF [57.1% (514/901) vs. 52.4% (461/881), respectively; $P<0.05$]. The AI pregnancy rates associated with the two Angus sires [55.6% (328/590), 57.8 (398/688)] were significantly higher than the rate associated with the Simmental sire [49.5% (249/504)]. The AI pregnancy rate for cows that exhibited estrus at or before AI was higher than cows that failed to exhibit estrus [63.8% (604/946) vs. 44.4% (371/836); $P<0.0001$].

Cows that received the second PGF at late and exhibited estrus had higher pregnancy than cows in other groups (Figure 3; $P<0.05$). Accounting for AI sires ($P<0.05$), cows' estrus status at or before timed AI ($P<0.0001$), and season (location) ($P=0.1$), the PGF hourly administration intervals were significantly different (Figure 4; $P=0.05$). Among locations, the AI pregnancy rates ranged from 30.0% to 70.8% for the early group and from 42.9% to 84.6% for the late group.

4. Discussion

Higher timed-AI pregnancy rates were achieved with a second PGF treatment 4 to 8 h (late) rather than 0 to 3.5 h (early) after the first PGF treatment at the time of removal of CIDRs in place for 5 d in a CO-Synch protocol. Cows that exhibited estrus at or prior to timed AI had higher pregnancy rates compared to cows that failed to exhibit estrus. Cows that received a second dose of PGF late and exhibited estrus had higher pregnancy rates than cows in other groups.

Secretion of PGF by the endometrium and increased production of intraluteal PGF terminates the luteal phase in cattle [20-26]. Although exogenous PGF can induce rapid luteolysis in the mid-luteal phase, there is a refractoriness of the early CL (up to d 5) to the luteolytic action of PGF. In this study PGF administration occurred 5 days following the first GnRH injection. If the first GnRH injection induced ovulation and/or luteinization then the CL would have been less than 5 days old which may be the reason for the refractoriness to a single dose of PGF.

There are several reasons given in the literature for the refractoriness of the early CL to the luteolytic dose of PGF; lack of expression of PGF receptors and the presence of PGF catabolizing enzyme, 15-hydroxyprostaglandin dehydrogenase (PGDH), in the early CL [27-29]. In addition, changes in blood flow, alterations in gene expressions (VEGF, eNOS, angiopoietin, angiotensin, endothelin, CAMKK2, HINT1, YWHAZ, GNB1 and RGS2), and the changes in NO and pO2 may also affect luteolysis of early CL after PGF [30,31].

The PGF receptor mRNA is expressed in the CL at high levels throughout the estrous cycle and administration of PGF inhibits the PGF receptor mRNA expression in both early and mid-luteal phases [32,33]. This suggests that the reason for the refractoriness of the early CL is not due to the lack of PGF receptors. The CL produces PGs such as cyclooxygenase (COX)-1, COX-2, PGF and PGE synthases during the estrous cycle [29]. The administration of PGF elevated COX-2 in mid-cycle but not in the early part of the cycle, suggesting that luteal PGF production is stimulated by exogenous PGF only in the mature CL [25,34]. The PGDH are at a higher level in the early CL compared to the mid cycle CL in sheep [35]. The biological function of PGDH in the CL prevents luteal concentrations of PGF from reaching threshold. In addition the production of luteal PGF may relate to mechanisms involved in luteal resistance to the luteolytic effects of PGF [35].

Based on PGFM concentrations, PGF is released from the uterus in pulses occurring approximately every 12 h in association with spontaneous luteolysis in cattle (17, 36-38]. Ginther et al., (2009) demonstrated that at least 4 sequential PGF pulses at 12 h are required to stimulate natural luteolysis in cattle [39]. While variable responses in luteolysis have been noted when a single dose of PGF was given on Day 5 of the estrous cycle, the results from the present study and evidence from other studies indicate that two doses of PGF might overcome the refractoriness of the early CL and induce luteolysis [7,8,10].

The cows that received a second PGF dose from 4 to 5 h and from 6 to 7 h interval after first PGF dose resulted in 63.4% and 60.8% timed AI pregnancy rates, respectively. It is noteworthy that cows received the second PGF within 2 h from first PGF yielded > 50% AI pregnancy rates. Handling cows twice within 2 h requires good handling facility and also it can be feasible in 50 head beef herds. The herd size ranged from 23to 237 in this study. Interestingly, cows in the late group that exhibited estrus had higher pregnancy than cows in other groups. The mean percentage of cows that exhibited estrus was different among locations and not different between seasons. It is expected that cows that observed in estrus having better chance of becoming pregnant compared to cows that did not observed in estrus. It is possible that the dynamics of cow level factors such as age, BCS and days postpartum at initiation of synchronization within location and season, plus differences in the management and environment may contribute to this difference [40]. In addition, time interval from estrus to AI is critical. A recent study recommended AI at 12 h after the onset of estrus was a compromise between earlier fertilization failure and later embryo failure [40]. They concluded younger cows (≤ 3 yrs of age) had better fertility when inseminated at 56 h, whereas older cows had better fertility at any time other than 48 h, from 56 to 72 h.

In summary, cows that received second PGF late after the first PGF on the day of CIDR removal in a 5-d CO-Synch + CIDR synchronization protocol had greater timed AI pregnancy rates than cows that received a second PGF early after the first PGF. In conclusion, it is advisable to wait at least 4.5 h following the initial PGF injection to administer a second PGF.

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Table 1. Descriptive statistics (Mean±SEM) for cows that received a second PGF treatment 0 to 3.5 h (early) or 4 to 8 h (late) after the first PGF treatment given at CIDR removal on Day 5 of a CO-Synch + CIDR protocol.

Factors	Early	Late
N	881	901
Age (in years)	5.30 ± 0.09	5.45 ± 0.09
Body Condition Score*	5.79 ± 0.03	5.85 ± 0.03
Days postpartum at initiation of synchronization	70.56 ± 0.69	68.75 ± 0.68
Average interval between 2 PGF doses(h)	2:15:19 ± 00:3:00	6:26:45 ± 00:1:54
Range interval between 2 PGF doses (h)	0:30:00, 3.54:04	4:30:00, 8:09:31
Interval from CIDR removal to AI (h)	72:27:34 ± 5:28:40	72:25:02 ± 05:13:28

*Body Condition Score: 1-emaciated; 9- obese;

Refer Image 1 for 5-d CO-Synch+CIDR protocol

Table 2. Proc Mixed model on effects of two doses of PGF2α treatment interval (early vs. late) at CIDR removal on Day 5 and co-variables on AI pregnancy rates of crossbred beef cows (N=1782) synchronized with a 5-d CO-Synch+CIDR protocol.

Effect	DF	F Value	Pr > F
Treatment	1	4.46	0.0349
AI Sires	2	3.23	0.0399
Estrus status	1	70.83	<0.0001
Treatment × Estrus status	1	2.75	0.0972
Season(location)	15	1.93	0.0169

DF – degrees of freedom;

Refer Image 1 for 5-d CO-Synch+CIDR protocol;

Table 5. Proc Mixed model effects of hourly interval between two doses of PGFα treatment and co-variables on AI pregnancy rates of crossbred beef cows (N=1782) synchronized with a 5-d CO-Synch+CIDR protocol.

Effect	DF	F value	Pr > F
PGFH	7	1.95	0.0504
AI Sires	2	3.20	0.0412
Estrus status	1	71.46	<0.0001
Season(location)	15	1.48	0.1027

PGFH- hourly PGF treatment as 0 to 1 h; 1 to 2 h; 2 to 3 h; 3 to 4 h; 4 to 5 h; 5 to 6 h; 6 to 7 h; 7 to 8 h;

DF - degrees of freedom;

Refer Image1 for 5-d CO-Synch+CIDR protocol;

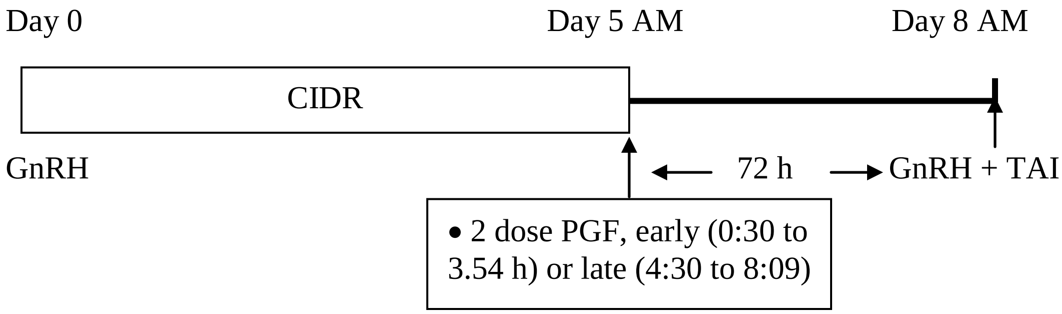

Figure 1. Diagrammatic representation of the treatment protocol

Briefly, all cows received 100 μg of gonadorelin diacetate tetrahydrate (GnRH; Cystorelin® sterile solution, Merial, Athens, GA, USA) and a controlled internal drug release insert (CIDR; Eazi-Breed™ CIDR® cattle insert, Pfizer Animal Health, New York, NY, USA) on Day 0. Within location, cows were randomly allocated to receive two doses of 25 mg of dinoprost (5 mL Lutalyse® sterile solution, Pfizer Animal Health, New York, NY, USA) either early (0:30 to 3.54 h) or late (4:30 to 8:09) interval in conjunction with CIDR insert removal on Day 5. All cows were administered 100 μg of GnRH and inseminated on Day 8, 72 h after CIDR removal.

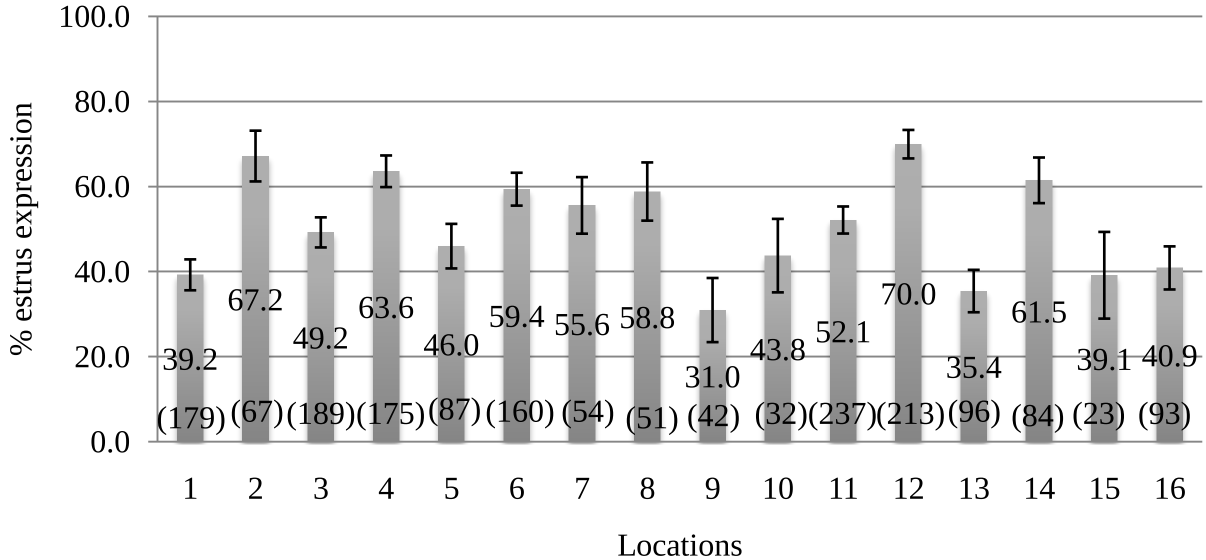

Figure 2. Mean (±SEM) percentage of cows exhibiting estrus at or prior to timed AI in a 5-d CO-Synch protocol on AI Pregnancy in beef cows (N=1782) in 16 locations.

The second PGF treatment was given at 0 to 3.5 h (early) or 4 to 8 h (late) following the first PGF treatment given at CIDR removal on Day 5 of a 5-d CO-Synch+CIDR protocol.

The mean percentage was different among the locations (P<0.001).

Refer to figure 1 for 5-d CO-Synch+CIDR protocol;

Number of cows in parenthesis;

Bar=Standard error;

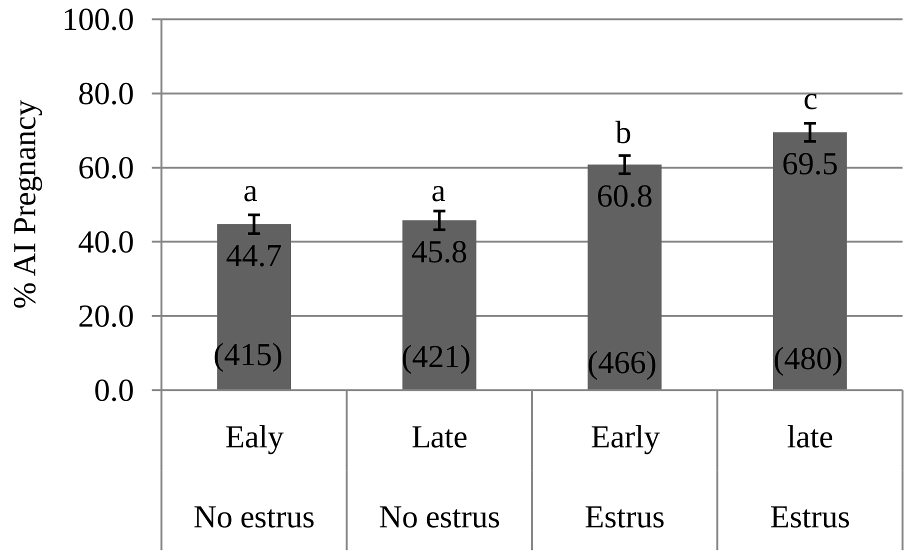

Figure 3. The interaction between estrus and a second PGF treatment at 0 to 3.5 h (early) or 4 to 8 h (late) following the first PGF treatment given at CIDR removal on Day 5 of a 5-d CO-Synch+CIDR protocol.

ab different superscripts were significant ($P<0.05$);

Refer to figure 1 for 5-d CO-Synch+CIDR protocol;

Number of cows in parenthesis;

Bar=Standard error;

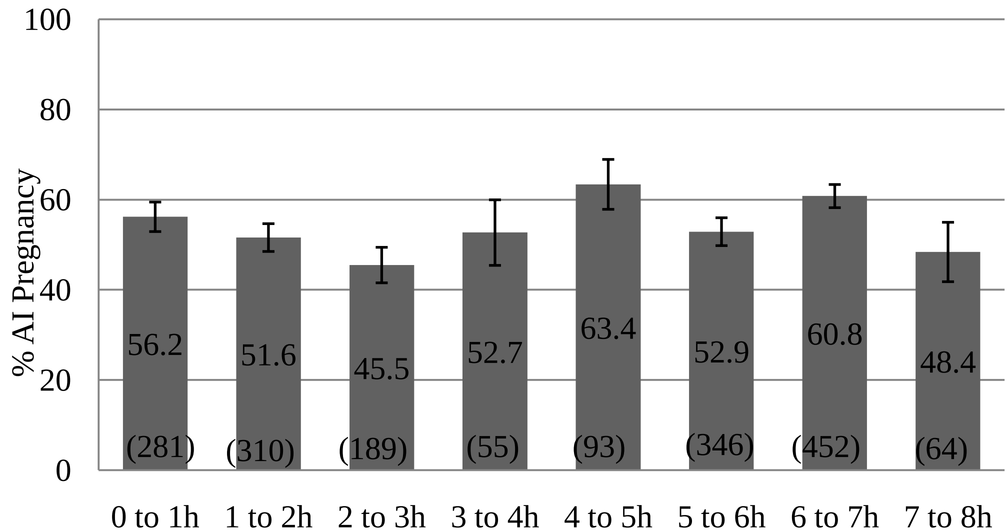

Figure 4. Effect of time interval (h) between two doses of PGF2α administered at CIDR removal in a 5-d CO-Synch protocol on mean AI Pregnancy in beef cows (N=1782).

The second PGF treatment was given at 0 to 3.5 h (early) or 4 to 8 h (late) following the first PGF treatment given at CIDR removal on Day 5 of a 5-d CO-Synch+CIDR protocol.

The timed AI pregnancy rates were different by hour (P=0.05).

Refer to figure 1 for 5-d CO-Synch+CIDR protocol;

Number of cows in parenthesis;

Bar = Standard error;

Revised highlighted

Effect of timing of second prostaglandin F2α administration in a 5-day progesterone based CO-Synch protocol on AI pregnancy rates in beef cows

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Acknowledgements

The authors extend their gratitude to the staff of Virginia Department of Corrections for their support with data collection. The authors also thank Select Sires Inc, Plain City, OH, USA for providing partial financial support and Dale Moore, Washington State University, for review of this manuscript.

Abstract

The objective of the present study was to 1. compare the timed AI pregnancy rate of Angus cross beef cows synchronized with the 5-d CO-Synch + CIDR (Controlled Internal Drug Release, a progesterone-based intravaginal insert) protocol and given two PGF2α (PGF) doses either early or late after first dose on the day of CIDR withdrawal; 2. Compare two PGF doses administered at hourly interval (up to 8 h) after first dose on the day of CIDR withdrawal. All cows (N=1782) at 16 locations received 100 μg of GnRH (Gonadotropin Releasing Hormone) + CIDR on Day 0. Cows received 25 mg of PGF at the time of CIDR insert removal on Day 5 and randomly allocated within locations to receive second PGF either early (N=881; from 0:30 to 3.54 h) or late (N=901; 4:30 to 8:09 h) from the first PGF administration. All cows were administered 100 μg of GnRH on Day 8 (72 h after CIDR removal) and were inseminated at that time. Cows were fitted with a pressure sensitive heat detection device (Kamar) at the time of CIDR withdrawal. Cows were observed twice daily through Day 7 and at the time of AI on Day 8 for estrus and Kamar status (estrus – red, partial and lost Kamar vs. no estrus – white Kamar) was recorded. Accounting for location, season, AI sire, cow observed in estrus or not at or before timed AI, and treatment by cows observed in estrus interaction, the timed AI pregnancy rates were greater for the late (6:27 ± 00:2 h) than the early (2:15 ± 00:3 h) interval, 57.2% vs. 52.7% respectively (P<0.05). In conclusion, cows that received the second PGF late after the first PGF on the day of CIDR removal in a 5-d CO-Synch + CIDR synchronization protocol had greater timed AI pregnancy rates than cows receiving the second PGF early after the first PGF.

Key words: Beef cows; Synchronization; CIDR; Prostaglandin; Pregnancy rate;

1. Introduction

Synchronization of estrus, ovulation or both in beef cows shortens the calving season and facilitates the use of artificial insemination in a labor-efficient way and at a fixed time. Artificial insemination provides an opportunity to introduce desired genetics into a herd. Numerous estrous synchronization protocols using PGF2α (PGF), Gonadotropin Releasing Hormone (GnRH), and/or a progestin have been developed to induce cyclicity and successfully synchronize estrus in beef cows [1-4].

The CO-Synch protocol in which PGF is administered 7 d after GnRH followed by a second GnRH injection and timed artificial insemination (TAI) 60 h after PGF yielded pregnancy rates above 50% [5]. Often, an exogenous progestin, a progesterone-based intravaginal insert (CIDR) is used during the 7 d interval from the initial GnRH injection to PGF [6]. When the interval was shortened from standard 7d to 5 d in a CO-Synch+CIDR program the timed AI pregnancy rate increased to 70.4%, which was 10.5% greater than that achieved with a 7 d program [7]. Due to the shortened interval from the initial GnRH to PGF in the 5 d CO-Synch + CIDR program and variability in luteolysis in some cows when a single dose of PGF is given on Day 5 of the estrous cycle, all cows received a second PGF treatment at CIDR withdrawal (Bridges et al., 2008) to increase luteolysis [7]. A recent study by Kasimanickam et al., (2009) investigated whether similar pregnancy rates could be achieved for a single dose of PGF or cloprostenol (CLP) compared to two doses of PGF [8]. The study concluded that administration of two PGF doses resulted in timed AI pregnancy rates of 69% across six herds whereas single dose of PGF or CLP resulted in a 15 to 17% reduction in timed AI pregnancy rates.

While variable responses in luteolysis have been noted when a single dose of PGF was given on Day 5 of the estrous cycle, two doses of CLP have consistently induced regression of the corpus luteum (95 to 100%) at 12 h interval after estrus [9,10]. In non-pregnant ruminants, luteal regression is caused by PGF secreted from the uterus [11-13]. The uterus releases PGF in a series of five to eight discrete pulses [14-17]. There is variability among species in the duration and magnitude of pulses, but, they typically occur at 6 to 8 h intervals [18]. Studies in which a second PGF injection is administered at 7 h or 12 h intervals, reported 10% and 15% increase in timed AI pregnancy rates. Very low doses of PGF (40 to 200 ηg/h) can induce luteal regression in ewes, if given in repeated treatments [19]. Two distinct phases of uterine PGF secretion, differing in pulse magnitude, have been described in the cow whereas a gradual increase in the magnitude of pulses during luteolysis has been observed in ewes [19]. It is worthy to investigate the practical application of this approach by comparing the PGF administrations at a shorter interval instead of waiting for up to 8 to 12 h.

The objective of the present study was to compare the timed AI pregnancy rate of Angus cross beef cows synchronized with the 5-d CO-Synch + CIDR protocol and given two doses of PGF either early or late in conjunction with CIDR withdrawal on Day 5.

2. Materials and methods

2.1. Animals and treatment protocol

Angus cross beef cows (N=1782) at 16 locations belonging to the Department of Corrections, Virginia, USA, to be inseminated at a fixed time during the fall of 2008, (N= 793) and spring of 2009 (N=989) breeding seasons were included in the study. Cows synchronized with the 5-d CO-Synch + CIDR protocol (Figure 1). All cows received 100 μg of gonadorelin diacetate tetrahydrate (GnRH, IM; Cystorelin® sterile solution, Merial, Athens, GA, USA) and a controlled internal drug release insert (CIDR; Eazi-Breed™ CIDR® cattle insert, Pfizer Animal Health, New York, NY, USA) on Day 0 plus 25 mg of dinoprost IM (PGF, IM, 5 mL Lutalyse® sterile solution, Pfizer Animal Health, New York, NY, USA) at the time of CIDR insert removal on Day 5, AM. Within location, cows were randomly allocated to receive a second dose of 25 mg IM, either early (N=881) or late (N=901) from the first PGF administration. Within location, cows in the early group received first PGF dose initially and immediately brought back to receive the second PGF dose and cows in late group waited at least 4:30 h between the first and second PGF dose. All cows received Kamar® Heatmount detector patches (Kamar, Inc., Steamboat Springs, CO, USA) at the time of CIDR removal. After CIDR removal, the cows were observed twice daily through Day 7 for estrus and Kamar status (estrus = red Kamar, partial red Kamar and lost Kamar with mount marks vs. no estrus = white Kamar) was recorded. A cow was determined to be in estrus if she was visually observed to stand for mounting or if she had an activated (color change from white to red), lost (with mount marks) or partially-activated Kamar. Cows were administered 100 μg of GnRH on Day 8, Kamar status was recorded and cows were inseminated at this time. Sires used in this study were Angus (N=2), and Simmental (N=1) breeds; within locations the sires were randomly allocated to treatments. As per stud center standard frozen semen samples were assessed of for post-thaw motility after 0 and 3 h of incubation at 37 °C (>60 and >25%, respectively) and for the percentage of intact acrosomal membranes after 3 h of incubation at 37 °C (>60%) to confirm maintenance of semen quality. Experienced inseminators (with prior experience of a minimum of 1000 inseminations) performed fixed time AI at all locations. Since fixed time AI occurred at the same time, within two days, AI technicians are different across locations. Technicians and sires were randomly assigned within locations.

Two weeks later, reproductively sound (based on breeding soundness exam one month prior to the breeding season) intact, and proven (based on reproductive performance from previous breeding season) bulls (approximately 1: 40 bull: cow ratio) were placed in with the cows, across treatments, for the remainder of the 60 to 70 d breeding season. Cows were examined for pregnancy status at 50 to 70 d after timed AI and again at 120 d after timed AI by rectal palpation and/or ultrasonography to determine the number of cows pregnant to AI or to rebreeding by clean-up bulls.

2.2. Statistical analyses

Data were analyzed with a statistical software program (SAS Version 9.1 for Windows, SAS Institute, Cary, NC, USA). The PROC MIXED procedure of SAS was used to examine the effect of treatments (early vs. late) on timed AI and breeding season pregnancy rates. Models included treatment (early vs. late), AI-Sire (three), days postpartum at initiation of synchronization (30 to 60, 61 to 80 and > 81 days), body condition scores (≤ 4 and > 4), estrus status prior to or at fixed time AI (yes or no) and appropriate interactions. The locations (1 to 16) were nested within the season (fall and spring). A *P* value of 0.05 was considered significant. For model reduction, the *P* value was set at ≤ 0.1 for inclusion and > 0.1 for exclusion until the model contained only significant main and interaction effects. The final model had treatment, AI sire, estrus status, season (location), and treatment by estrus status interaction.

A model was developed, retrospectively, to test the effect of hourly time interval between PGF administration (PGFH; from 1 to 8 h) on timed AI pregnancy rates. Models included PGFH (0 to 1 h; 1 to 2 h; 2 to 3 h; 3 to 4 h; 4 to 5 h; 5 to 6 h; 6 to 7 h; 7 to 8 h), AI-Sire (3), days postpartum at initiation of synchronization (30 to 60, 61 to 80 and > 81 days), body condition scores (≤ 4 and > 4), estrus status prior to or at fixed time AI (yes or no) and appropriate interactions. The locations (1 to 16) were nested within the season (fall and spring). A *P* value of 0.05 was considered significant. For model reduction, the *P* value was set at ≤ 0.1 for inclusion and > 0.1 for exclusion until the model contained only significant main and interaction effects. The final model included PGFH, AI sire, estrus status, and season (location). The AI pregnancy rates among locations were analyzed separately by Chi-square.

It was hypothesized that the AI pregnancy difference between treatment groups will be 7%. To detect the same difference in the AI pregnancy, with adequate statistical power ($1-\beta = 0.8$) and statistical significance ($\alpha = 0.05$), the study needed a sample size of 796 cows per treatment group.

3. Results

Age, BCS and days postpartum at initiation of synchronization for cows assigned to the two treatments were not significantly different (Table 1). The interval from CIDR withdrawal to AI and the percentage of cows in estrus at or before timed AI was not different between treatment groups (late: 53.3% (480/901); early: 52.9% (466/881); P>0.1; Table 1). However, the percentage of cows in estrus differed among the locations (Figure 2; P<0.001) but not between seasons (54.6% vs. 51.9% for fall and spring respectively; P>0.1). Accounting for AI sires (P<0.04), cows' estrus status at or before timed AI (P<0.0001), treatment by cows estrus status interaction (P=0.1) and season (location) (P<0.05), AI-pregnancy rates for cows that received the second PGF late (6:26:45 ± 00:1:54 h) after the first PGF had higher AI pregnancy rates compared to cows that received second PGF early (2:15:19 ± 00:3:00 h) after first PGF [57.1% (514/901) vs. 52.4% (461/881), respectively; P<0.05]. The AI pregnancy rates associated with the two Angus sires [55.6% (328/590), 57.8 (398/688)] were significantly higher than the rate associated with the Simmental sire [49.5% (249/504)]. The AI pregnancy rate for cows that exhibited estrus at or before AI was higher than cows that failed to exhibit estrus [63.8% (604/946) vs. 44.4% (371/836); P<0.0001].

Cows that received the second PGF at late and exhibited estrus had higher pregnancy than cows in other groups (Figure 3; P<0.05). Accounting for AI sires (P<0.05), cows' estrus status at or before timed AI (P<0.0001), and season (location) (P=0.1), the PGF hourly administration intervals were significantly different (Figure 4; P=0.05). Among locations, the AI pregnancy rates ranged from 30.0% to 70.8% for the early group and from 42.9% to 84.6% for the late group.

4. Discussion

Higher timed-AI pregnancy rates were achieved with a second PGF treatment 4 to 8 h (late) rather than 0 to 3.5 h (early) after the first PGF treatment at the time of removal of CIDRs in place for 5 d in a CO-Synch protocol. Cows that exhibited estrus at or prior to timed AI had higher pregnancy rates compared to cows that failed to exhibit estrus. Cows that received a second dose of PGF late and exhibited estrus had higher pregnancy rates than cows in other groups.

Secretion of PGF by the endometrium and increased production of intraluteal PGF terminates the luteal phase in cattle [20-26]. Although exogenous PGF can induce rapid luteolysis in the mid-luteal phase, there is a refractoriness of the early CL (up to d 5) to the luteolytic action of PGF. In this study PGF administration occurred 5 days following the first GnRH injection. If the first GnRH injection induced ovulation and/or luteinization then the CL would have been less than 5 days old which may be the reason for the refractoriness to a single dose of PGF.

There are several reasons given in the literature for the refractoriness of the early CL to the luteolytic dose of PGF; lack of expression of PGF receptors and the presence of PGF catabolizing enzyme, 15-hydroxyprostaglandin dehydrogenase (PGDH), in the early CL [27-29]. In addition, changes in blood flow, alterations in gene expressions (VEGF, eNOS, angiopoietin, angiotensin, endothelin, CAMKK2, HINT1, YWHAZ, GNB1 and RGS2), and the changes in NO and pO2 may also affect luteolysis of early CL after PGF [30,31].

The PGF receptor mRNA is expressed in the CL at high levels throughout the estrous cycle and administration of PGF inhibits the PGF receptor mRNA expression in both early and mid-luteal phases [32,33]. This suggests that the reason for the refractoriness of the early CL is not due to the lack of PGF receptors. The CL produces PGs such as cyclooxygenase (COX)-1, COX-2, PGF and PGE synthases during the estrous cycle [29]. The administration of PGF elevated COX-2 in mid-cycle but not in the early part of the cycle, suggesting that luteal PGF production is stimulated by exogenous PGF only in the mature CL [25,34]. The PGDH are at a higher level in the early CL compared to the mid cycle CL in sheep [35]. The biological function of PGDH in the CL prevents luteal concentrations of PGF from reaching threshold. In addition the production of luteal PGF may relate to mechanisms involved in luteal resistance to the luteolytic effects of PGF [35].

Based on PGFM concentrations, PGF is released from the uterus in pulses occurring approximately every 12 h in association with spontaneous luteolysis in cattle (17, 36-38]. Ginther et al., (2009) demonstrated that at least 4 sequential PGF pulses at 12 h are required to stimulate natural luteolysis in cattle [39]. While variable responses in luteolysis have been noted when a single dose of PGF was given on Day 5 of the estrous cycle, the results from the present study and evidence from other studies indicate that two doses of PGF might overcome the refractoriness of the early CL and induce luteolysis [7,8,10].

The cows that received a second PGF dose from 4 to 5 h and from 6 to 7 h interval after first PGF dose resulted in 63.4% and 60.8% timed AI pregnancy rates, respectively. It is noteworthy that cows received the second PGF within 2 h from first PGF yielded > 50% AI pregnancy rates. Handling cows twice within 2 h requires good handling facility and also it can be feasible in 50 head beef herds. The herd size ranged from 48 to 215 in this study. Interestingly, cows in the late group that exhibited estrus had higher pregnancy than cows in other groups. The mean percentage of cows that exhibited estrus was different among locations and not different between seasons. It is expected that cows that observed in estrus having better chance of becoming pregnant compared to cows that did not observed in estrus. It is possible that the dynamics of cow level factors such as age, BCS and days postpartum at initiation of synchronization within location and season, plus differences in the management and environment may contribute to this difference [40]. In addition, time interval from estrus to AI is critical. A recent study recommended AI at 12 h after the onset of estrus was a compromise between earlier fertilization failure and later embryo failure [40]. They concluded younger cows (≤ 3 yrs of age) had better fertility when inseminated at 56 h, whereas older cows had better fertility at any time other than 48 h, from 56 to 72 h.

In summary, cows that received second PGF late after the first PGF on the day of CIDR removal in a 5-d CO-Synch + CIDR synchronization protocol had greater timed AI pregnancy rates than cows that received a second PGF early after the first PGF. In conclusion, it is advisable to wait at least 4.5 h following the initial PGF injection to administer a second PGF.

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Table 1. Descriptive statistics (Mean±SEM) for cows that received a second PGF treatment 0 to 3.5 h (early) or 4 to 8 h (late) after the first PGF treatment given at CIDR removal on Day 5 of a CO-Synch + CIDR protocol.

Factors	Early	Late
N	881	901
Age (in years)	5.30 ± 0.09	5.45 ± 0.09
Body Condition Score*	5.79 ± 0.03	5.85 ± 0.03
Days postpartum at initiation of synchronization	70.56 ± 0.69	68.75 ± 0.68
Average interval between 2 PGF doses(h)	2:15:19 ± 00:3:00	6:26:45 ± 00:1:54
Range interval between 2 PGF doses (h)	0:30:00, 3.54:04	4:30:00, 8:09:31
Interval from CIDR removal to AI (h)	72:27:34 ± 5:28:40	72:25:02 ± 05:13:28

*Body Condition Score: 1-emaciated; 9- obese;

Refer Image 1 for 5-d CO-Synch+CIDR protocol

Table 2. Proc Mixed model on effects of two doses of PGF2α treatment interval (early vs. late) at CIDR removal on Day 5 and co-variables on AI pregnancy rates of crossbred beef cows (N=1782) synchronized with a 5-d CO-Synch+CIDR protocol.

Effect	DF	F Value	Pr > F
Treatment	1	4.46	0.0349
AI Sires	2	3.23	0.0399
Estrus status	1	70.83	<0.0001
Treatment × Estrus status	1	2.75	0.0972
Season(location)	15	1.93	0.0169

DF – degrees of freedom;

Refer Image 1 for 5-d CO-Synch+CIDR protocol;

Table 5. Proc Mixed model effects of hourly interval between two doses of PGFα treatment and co-variables on AI pregnancy rates of crossbred beef cows (N=1782) synchronized with a 5-d CO-Synch+CIDR protocol.

Effect	DF	F value	Pr > F
PGFH	7	1.95	0.0504
AI Sires	2	3.20	0.0412
Estrus status	1	71.46	<0.0001
Season(location)	15	1.48	0.1027

PGFH- hourly PGF treatment as 0 to 1 h; 1 to 2 h; 2 to 3 h; 3 to 4 h; 4 to 5 h; 5 to 6 h; 6 to 7 h; 7 to 8 h;

DF - degrees of freedom;

Refer Image1 for 5-d CO-Synch+CIDR protocol;

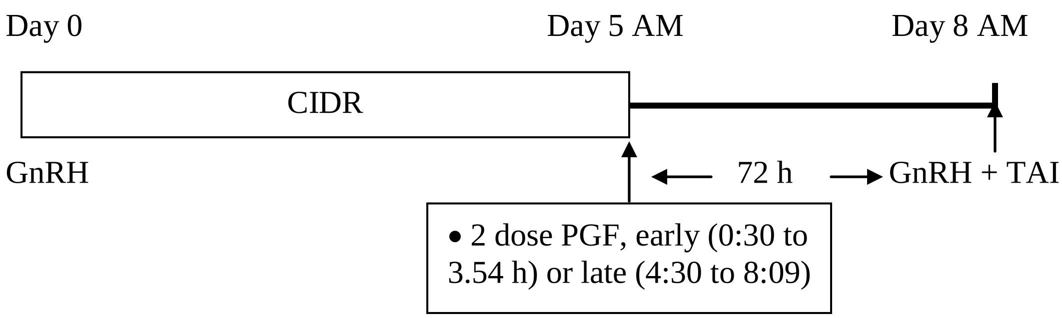

Figure 1. Diagrammatic representation of the treatment protocol

Briefly, all cows received 100 μg of gonadorelin diacetate tetrahydrate (GnRH; Cystorelin® sterile solution, Merial, Athens, GA, USA) and a controlled internal drug release insert (CIDR; Eazi-Breed™ CIDR® cattle insert, Pfizer Animal Health, New York, NY, USA) on Day 0. Within location, cows were randomly allocated to receive two doses of 25 mg of dinoprost (5 mL Lutalyse® sterile solution, Pfizer Animal Health, New York, NY, USA) either early (0:30 to 3.54 h) or late (4:30 to 8:09) interval in conjunction with CIDR insert removal on Day 5. All cows were administered 100 μg of GnRH and inseminated on Day 8, 72 h after CIDR removal.

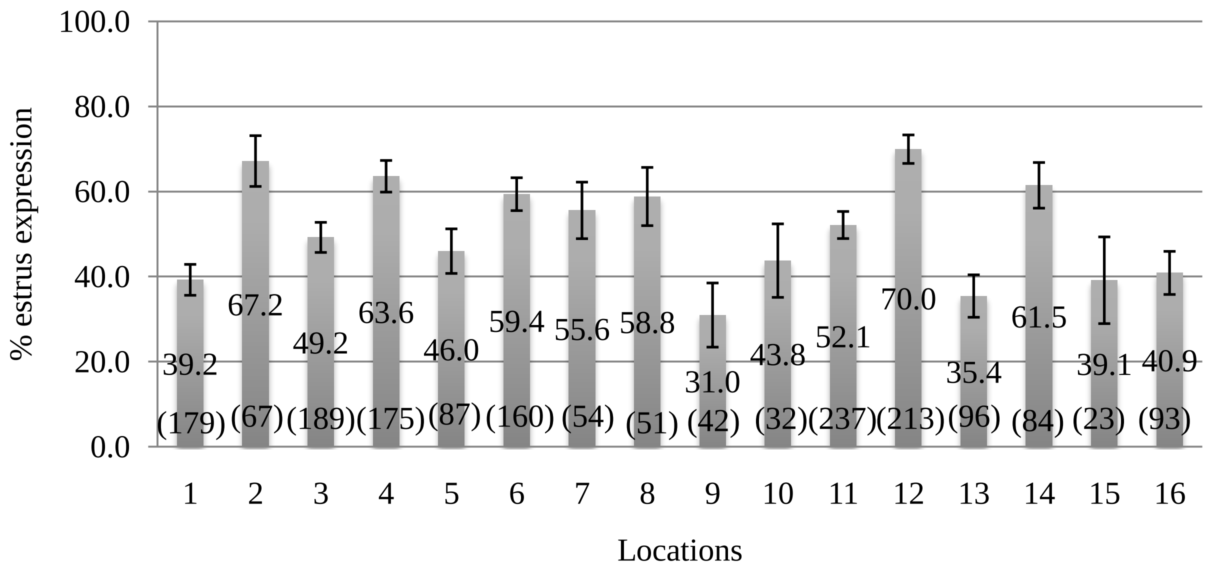

Figure 2. Mean (±SEM) percentage of cows exhibiting estrus at or prior to timed AI in a 5-d CO-Synch protocol on AI Pregnancy in beef cows (N=1782) in 16 locations.

The second PGF treatment was given at 0 to 3.5 h (early) or 4 to 8 h (late) following the first PGF treatment given at CIDR removal on Day 5 of a 5-d CO-Synch+CIDR protocol.

The mean percentage was different among the locations ($P<0.001$).

Refer to figure 1 for 5-d CO-Synch+CIDR protocol;

Number of cows in parenthesis;

Bar=Standard error;

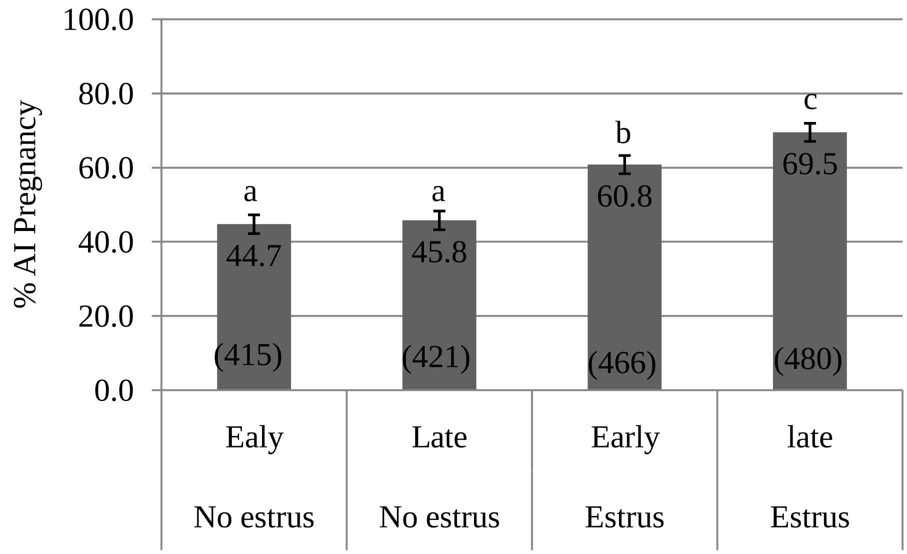

Figure 3. The interaction between estrus and a second PGF treatment at 0 to 3.5 h (early) or 4 to 8 h (late) following the first PGF treatment given at CIDR removal on Day 5 of a 5-d CO-Synch+CIDR protocol.

ab different superscripts were significant ($P<0.05$);

Refer to figure 1 for 5-d CO-Synch+CIDR protocol;

Number of cows in parenthesis;

Bar=Standard error;

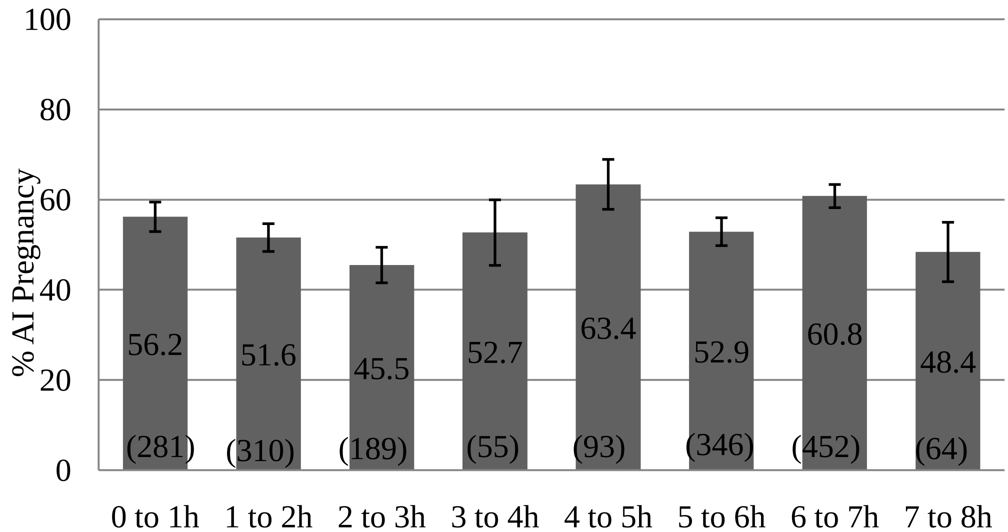

Figure 4. Effect of time interval (h) between two doses of PGF2α administered at CIDR removal in a 5-d CO-Synch protocol on mean AI Pregnancy in beef cows (N=1782).

The second PGF treatment was given at 0 to 3.5 h (early) or 4 to 8 h (late) following the first PGF treatment given at CIDR removal on Day 5 of a 5-d CO-Synch+CIDR protocol.

The timed AI pregnancy rates were different by hour (P=0.05).

Refer to figure 1 for 5-d CO-Synch+CIDR protocol;

Number of cows in parenthesis;

Bar = Standard error;

*Revision Notes

Dated: Feb 19, 2010

To

The Co-Editor,

Theriogenology Journal.

Dear Dr. Kastelic,

The authors thank the reviewers and editor for their comments. The revised manuscript addressed all the concerns raised by the reviewers.

Sincerely,

R. Kasimanickam

Reviewer #1: The manuscript contributes to the literature that already exists. The scientific content is accurate and the authors did a great work with the statistical analysis to measure the effects on the timing of the PGF.

The only comment or suggestion (Line 152) is that the authors should specify the time of day the other PGF doses were administered. The times they mention are am or pm?

Specified in Line 150 in materials and methods; first PGF shot was administered in the AM in all locations

Reviewer #2: This study shows that the administration of a second PGF treatment 0 to 3.5 h rather than 4 to 8 h after the first PGF treatment given at CIDR removal in a 5-d CO-Synch+CIDR protocol increased AI pregnancy rates and that fixed-time AI pregnancy rates were higher for cows that exhibited estrus prior to TAI. For the most part the manuscript is well written but requires some correction of English and clarification, some more detail in the Materials and Methods and some additional references and discussion before it is ready for publication.

L66 the PGF hourly administration intervals were significantly different (P=0.05).

Removed as suggested

This statement is very confusing as is L131; Both leads one to believe that a third treatment group received PGF treatments hourly. As we later find out it is a retrospective regrouping of the data from cows in the two treatment groups. I find it adds nothing to the abstract and should be removed. I am not sure it adds much to the manuscript.

L131 differences in AI pregnancy rates in cows received two doses of PGF at hourly interval from 1 to 8 h interval???????.

L131 insert the word "that" between "cows" and "received"

The sentence is removed due to this reviewer's comment…." I find it adds nothing to the abstract and should be removed. I am not sure it adds much to the manuscript". However, we retained in the analysis, results and discussion. We feel that it is important to include this aspect.

L164 rectal palpation and/or ultrasonography to identify time of conception. Revise for example:

transrectal palpation and/or ultrasonography to determine the number of cows pregnant to timed AI or rebreeding by bulls.

Clarified as suggested, line 176

L160 2 Angus sires and 1 Simmental sire were used for TAI, were all three sires used within treatments within herds and how were they balanced. What steps were taken to ensure fertility of the semen was similar among sires, treatments and location? How many technicians inseminated the cows? Were the same technicians responsible for AI at all locations?

Clarified; line 162 to 176

L161 What was the ratio of cows to bulls? Was the ratio the same among treatments? Location? What breed of bulls? Were these mature or yearling bulls? Were the bulls determined to be of breeding soundness before turnout with cows? If so, how?

Clarified; line 162 to 176

L168 Please explain how treatment effects on proportions (pregnancy rates) can be analyzed properly with the PROC MIXED procedure of SAS. The study describes the collection of data usually submitted to procedures such as PROC LOGISTIC.

The PROC MIXED was specifically designed to fit mixed effect models. It can model random and mixed effect data, repeated measures, spacial data, data with heterogeneous variances and autocorrelated observations.The MIXED procedure is more general than GLM in the sense that it gives a user more flexibility in specifying the correlation structures, particularly useful in repeated measures and random effect models. It has to be emphasized, however, that the PROC MIXED is not an extended, more general version of GLM. They are based on different statistical principles; *GLM and MIXED use different estimation methods*.

L177 This was retrospective; cows were assigned to return to the chute for the 2nd dose of PGF either immediately or 4 h later. The order of cows within these assignments was random over the time required to run the herd through the chute. Unless cows were sorted based on the time of the first PGF? It is very difficult to follow the treatment descriptions and this clarification needs to present a description that is consistent among the abstract, introduction and M & M.

Clarified in Line 190

L231 revise the first sentence PGF administration late after first dose on the day of CIDR removal in a 5-d CO-Synch+CIDR protocol resulted in higher timed AI pregnancy rate in beef cows compared to PGF administration early after first dose.

This needs revision such as:

Higher timed-AI pregnancy rates were achieved with a second PGF treatment 4 to 8 h (late) rather than 0 to 3.5 h (early) after the first PGF treatment at the time of removal of CIDRs in place for 5 d in a CO-Synch protocol.

Revised in line 208.

L240 If the first GnRH injection induced ovulation and/or luteinization then the CL would have been 5 days old …..
This statement needs to be referenced; I do not agree. Depending on the size (maturity) of the dominant follicle ovulation may follow within 1.5 d of GnRH treatment (Martinez et al.); therefore at best the CL would be aged 3.5 d at CIDR removal 5 d after GnRH.

Clarified in line 244.

L243-L248 Needs clarification and a reference:

There are several reasons given (not described) in the literature for the refractoriness of the early CL to the luteolytic dose of PGF; (delete such as) the lack of expression of PGF receptors and the presence of the PGF catabolizing enzyme 15-hydroxyprostaglandin dehydrogenase (PGDH) in the early CL (INSERT A REFERENCE).

Changed, line 246 to 248.

L263-266 While variable responses in luteolysis have been noted when a single dose of PGF was given on Day 5 of the estrous cycle (INSERT REFERENCE). Neither ovulations following the first GnRH treatment nor CL regression were determined therefore your study provided no evidence that two doses of PGF forced luteolysis of refractory CL. You must REFERENCE other work that has demonstrated this.

Referenced, line 269.

L268 delete "to mention"
deleted line 271.

L270 awkward sentence needs revision and more information:

Revised

Treating cows twice within two h required good handling facilities and may be feasible with small herds (WHAT IS SMALL? WHAT WAS THE AVERGAE SIZE OF THE HERDS USED IN YOUR STUDY?)

Given in Line 274

L269 insert the word "the" between "received" and "second PGF"

Line 272

L271 Interestingly, cows in the late group that exhibited estrus had higher pregnancy than cows in other groups. (THIS STATEMENT NEEDS FOLLOW-UP DISCUSSION ON TAI PREGNANCY RATES AND ESTRUS).

L272 The mean percentage of cows that exhibited estrus was different among locations and not different between seasons. It is possible that the dynamics of cow level factors such as age, BCS and days postpartum at initiation of synchronization within farm and season, plus differences in the management and environment may contribute to this difference (NEEDS REFERENCES; is farm the same as location?).

Revised and references in Line 278-285

L276 "In the analysis locations were nested within season to control for the variations." (THIS STATEMENT IS TROUBLESOME; what analysis? and control of what variations?. If locations (farms) were nested within season then there can be no MAIN EFFECT of season in the ANOVA (because each season did not contain all locations) and therefore L272 is not true.

Removed. Aslo clarified in line 178.

L410 Table 1. Descriptive factors for cows received second PGF early or late in conjunction with CIDR removal on Day 5 of CO-Synch + CIDR protocol. (NEEDS REVISION). Is each mean presented with its standard deviation or standard error? These are descriptive statistics for the cows in the two treatment groups; for example:

Descriptive statistics for cows that received a second PGF treatment 0 to 3.5 h (early) or 4 to 8 h (late) after the first PGF treatment given at CIDR removal on Day 5 of a CO-Synch + CIDR protocol.

Changed as suggested, line 433.

L412 the word "emaciated" is misspelled as "emaciated"

Line 436

L461 Figure 1 The x axis and y axis each need a title. What are the error bars? How many cows were at each location? Without the N the percents don't mean much. The caption needs revision; "on AI Pregnancy" does not seem to fit here.

Added

L476 Figure 2 the word "Early" is misspelled "Ealy" for the No Estrus group.

Corrected

L478 Figure 2. What are the error bars? What are the numbers within brackets?

Clarified

L478 Figure 2. The caption "Effect of interval, early (0:30 to 3.54 h) vs. late (4:30 to 8:09), between two doses PGF2<alpha> administration at CIDR removal on Day 5 for beef cows showed estrus or not on AI pregnancy during 5 d CO-Synch+CIDR protocol" Needs revision; for example:.

The interaction between estrus and a second PGF treatment at 0 to 3.5 h (Early) or 4 to 8 h (late) following the first PGF treatment given at CIDR removal on Day 5 of a 5-d CO-Synch+CIDR protocol.

Changed as suggested.

L494 Figure 3. The x axis and y axis each need a title. As with figures 2 and 3 the caption needs revision so that it does not say that the 2 doses were given in conjunction with CIDR removal. What are the error bars? What are the numbers within brackets? What hours differ?

Added and clarifications given

L514 Why is this an image and not a Figure? More specifically why would this not be Figure 1 since it is the most important description of the treatments; and subsequent results Figures 2 to 4. This is recommended.

Changed

L518 Is farm the same as location? Or were there several farms within location? For clarity refer consistently to farms or location.

Locations are farms; Changes to locations;

Co-Editor
In the abstract, you had this: 6:26:45 [this is really awkward; can you change it?]

Changed

For headings and subheadings, capitalize only first letter of first word and leave one blank line above and below.

Changed

Do not begin a sentence with a acronym (e.g. PGF)

Corrected.

In the bibliography, to indicate the range in page numbers, you have some 'regular' length hyphens, and some long dashes; please exchange the latter for the former.

Corrected.
Fig. 2 should be deleted and data reported in the text.

The authors feel that it is important to show the distribution instead of just mentioning it is different in the text, hence it is retained.

Somehow, the figure for the experimental design did not seem to be properly 'designated' (i.e. referred to; please review and revise).

Changed;

In Figs. 1 and 3, the numbers at the bottom are really difficult to read. Please delete them (if they are not essential), or if they are essential, perhaps put them above the columns?

Changed for clarity.