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Comparison of three CIDR-based fixed-time AI protocols in beef heifers¹

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ABSTRACT: Several effective fixed-time AI (FTAI) protocols have been developed to facilitate AI in beef heifers that circumvent the need for estrus detection. Among these are the 5-d CO-Synch + controlled intravaginal progesterone insert (CIDR) protocol (5dCO), PGF_{2α} (PG) 6-d CIDR protocol (PG-6dCIDR), and 14-d CIDR-PG protocol (14dCIDR-PG). Although each of these protocols varies in duration and approach to synchronizing estrus and ovulation, each has been reported as an effective method to facilitate FTAI in beef heifers. Therefore, the objective of this study was to compare FTAI pregnancy rates in beef heifers synchronized with these 3 CIDR-based protocols. Virgin beef heifers ($n = 801$) at 4 locations were synchronized with 1 of 3 protocols: 1) 5dCO, an injection of GnRH (100 µg) and insertion of a CIDR on d -5, PG (25 mg) and CIDR removal on d 0 with a second injection of PG (>4 h after CIDR removal) on d 0 and FTAI at 72 h after CIDR removal, 2) PG-6dCIDR, PG (25 mg) on d -9, GnRH (100 µg) and insertion of a CIDR on d -6, PG and CIDR removal on d 0, and FTAI at 66 h after CIDR removal, or 3) 14dCIDR-PG, a 14-d CIDR insert from d -30 to -16, PG (25 mg) on d 0, and FTAI at

66 h after PG. All heifers received an injection of GnRH (100 µg) concurrent with FTAI. Timing of treatment initiation was offset to allow all heifers to receive FTAI concomitantly and at random. Pregnancy success was determined between 35 and 40 d after FTAI by transrectal ultrasonography. Blood samples were collected before the beginning of each protocol and at the initiation of each protocol to determine estrous cycling status (77%). Data were analyzed using the GLIMMIX procedures of SAS. As expected, because of the duration of protocols, fewer heifers in the 14dCIDR-PG treatment were pubertal at initiation of synchronization than in the 5dCO ($P < 0.05$) and PG-6dCIDR ($P = 0.10$) treatments. Fixed-time AI pregnancy success did not differ between treatments ($P = 0.14$; 62.6%, 56.9%, and 53.3% for 5dCO, PG-6dCIDR, and 14dCIDR-PG, respectively). However, heifers that had reached puberty by initiation of synchronization had greater ($P < 0.01$) pregnancy success compared to heifers that were prepubertal (60.7% and 47.3%, respectively). In summary, all 3 protocols had similar FTAI pregnancy success, and puberty status had the greatest impact on pregnancy success.

Key words: Beef heifers, estrous synchronization, CIDR, fixed-time AI

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INTRODUCTION

Several fixed-time AI (FTAI) protocols have been developed for beef heifers. These FTAI protocols forgo estrus detection by synchronizing ovulation. Ideally, these FTAI protocols will induce a compact estrous re-

sponse at a predetermined time; however, irrespective of expression of estrus, these protocols are designed to induce the ovulation of a mature dominant follicle containing a competent ovum in all treated females. For widespread adoption of these protocols, pregnancy rates following FTAI should be similar to protocols utilizing estrus detection and AI and be consistent across groups of heifers. The key targets for developing consistently effective FTAI protocols include controlling follicular dynamics, the precise onset of estrus, and maintaining normal fertility following induced ovulation and AI, regardless of endocrine status or stage of follicle wave development among females at the initiation of treatment.

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Recently, several FTAI approaches have been developed for beef heifers. These include the 5-d CO-Synch + controlled intravaginal progesterone insert (CIDR) protocol (5dCO), the PGF_{2α} (PG) 6-d CIDR protocol (PG-6dCIDR), and the 14-d CIDR-PG protocol (14dCIDR-PG). Although each of these protocols differs in their duration and approach to synchronizing estrus and ovulation, each has been proven an effective method to facilitate FTAI in beef heifers. Moreover, independent investigations with all 3 aforementioned protocols in beef heifers have demonstrated these protocols yield greater FTAI pregnancy rates than other protocols currently available (Wilson et al., 2007; Mallory et al., 2011; Perry et al., 2011). To date, however, these 3 protocols have never been directly compared to determine which method of ovulation synchronization results in the greatest FTAI pregnancy rates in beef heifers. Therefore, the objective of this experiment was to compare FTAI pregnancy rates between the 5dCO, PG-6dCIDR, and 14dCIDR-PG protocols in virgin replacement beef heifers at multiple locations.

MATERIALS AND METHODS

Locations and Heifers

Yearling virgin replacement beef heifers at 4 commercial ranches (location 1, $n = 237$; location 2, $n = 295$; location 3, $n = 156$; and location 4, $n = 116$) in 4 states (MN, SD, UT, and WY) were enrolled in this study. Within each location, heifers had been managed as a collective group since weaning or since being purchased at weaning. At locations 2, 3, and 4 heifers were developed in a dry lot, and at location 1 heifers were developed on pasture with supplemental feed. Because heifers were managed and developed by commercial operations, ages and weights of heifers at project initiation were not known. However, all heifers were spring-born virgin heifers that were between 12 and 15 mo of age at protocol initiation. Following AI, all heifers within a location were managed as a single group or managed with an equal number of heifers from each treatment in each group throughout the breeding season. All heifers were handled in accordance with procedures approved by each collaborating university's Animal Care and Use Committee.

Treatments

Within location, heifers were randomly assigned to 1 of 3 estrous synchronization treatments. Randomization to treatments occurred when the estrous synchronization of longest duration, 14dCIDR-PG, was initiated. The estrous synchronization protocols tested were 1) 5dCO, 2) PG-6dCIDR, and 3) 14dCIDR-PG. The 5dCO treatment consisted of the insertion of a CIDR (Zoetis, Florham

Park, NJ) and administration of 100 µg of GnRH (Factrel; Zoetis) on d -5 and CIDR removal and administration of 25 mg of PG (Lutalyse; dinoprost tromethamine; Zoetis) on d 0 with a second administration of PG 4 to 6 h after the initial dose. Heifers received FTAI at 72 h after CIDR insert removal concurrent with GnRH administration. Heifers enrolled in the PG-6dCIDR treatment were administered PG (25 mg) on d -9, GnRH (100 µg) and a CIDR insert on d -6, PG (25 mg) and CIDR removal on d 0, and FTAI at 66 h after CIDR removal concurrent with GnRH administration. The 14dCIDR-PG treatment consisted of the administration of a 14-d CIDR insert from d -30 to -16, PG on d 0, and FTAI at 66 h after PG concurrent with GnRH administration. At each location, treatment initiation was offset to allow all heifers to be FTAI at the same time and at random across treatments. Within each location, AI sires and AI technicians were equally used across all 3 treatments. On d 0, all heifers in locations 1 and 3 received tail paint (Tell Tail; FIL, Mount Maunganui, New Zealand). At FTAI, tail paint scores were assessed (1 = tail paint completely gone; 2 = tail paint partially gone, obvious signs of receiving some rubs from mountings; 3 = no signs of having received mounts, tail paint undisturbed; Bridges et al., 2008, 2012). At all locations, heifers were placed with fertile bulls at a minimum of 10 d following FTAI. Pregnancy was diagnosed by transrectal ultrasonography between 35 and 40 d after FTAI to determine pregnancy rates to FTAI. A second pregnancy diagnosis was conducted via transrectal ultrasonography at approximately 35 d after the conclusion of the breeding season to determine breeding season pregnancy rates.

Determination of Reproductive Status

At all locations, blood samples were collected to assess the proportion of heifers that were pubertal at initiation of their respective ovulation synchronization treatment. To determine the proportion of pubertal heifers at the initiation of the 14dCIDR-PG treatment, blood samples were taken on d -44 to -40 (depending on location) and on d -30 (CIDR insertion). In locations 1 and 3, a blood sample was also collected on d -44 to -40 and on d -30 from animals in the PG-6dCIDR and the 5dCO groups to determine pubertal status in all animals at the initiation of the 14dCIDR-PG treatment. For the PG-6dCIDR treatment, blood samples were taken on d -16, d -9 (PG administration), and d -6 (CIDR insertion) to determine pubertal status. Blood samples taken on d -16 and -5 (CIDR insertion) were used to determine pubertal status in heifers enrolled in the 5dCO treatment. Following collection, blood was centrifuged ($1,000 \times g$ for 20 min at 3°C.), and serum or plasma was recovered and stored at -20°C until RIA. Concentration of progesterone was analyzed in serum or

plasma samples in the laboratory of individual investigators according to validated RIA procedures for that laboratory (Engel et al., 2008; Horn et al., 2010). For assessment of reproductive status, heifers with at least 1 blood sample with progesterone concentrations ≥ 1.0 ng/mL were considered pubertal at treatment initiation.

Data and Statistical Analysis

Pregnancy rate to FTAI was calculated as the number of heifers diagnosed pregnant following FTAI divided by the total number of heifers receiving FTAI. Breeding season pregnancy rate was the proportion of all heifers that became pregnant after FTAI and bull exposure.

The MIXED and GLIMMIX procedure of SAS (SAS Inst. Inc., Cary, NC) was used for statistical analyses. Effects of AI sire and AI technician were confounded with location; therefore, the effects of AI sire, AI technician, treatment, and the appropriate interactions on FTAI pregnancy rate were evaluated first within location. Although the main variable of AI technician and AI sire were found to be significant at some locations, no treatment by AI sire or treatment by AI technician effect was detected at any location.

For the statistical analysis of reproductive status (GLIMMIX), the statistical model included treatment, with location included as a random variable. For statistical analysis of tail paint score (MIXED), FTAI pregnancy rates (GLIMMIX), and breeding season pregnancy rates (GLIMMIX), the model included treatment, pubertal status, and the interaction, with location included as a random variable in the statistical model. Interactions with a significance value of $P > 0.20$ were removed from the complete model in a stepwise manner to derive the final reduced model for each variable. Differences were considered to be significant when $P \leq 0.05$ and a tendency when $P > 0.05$ but $P \leq 0.10$.

RESULTS

Reproductive Status

Not surprisingly, because of the differential lengths of the protocols used and the necessity of initiating the 14dCIDR-PG treatment much earlier in the calendar year, there were differences between treatments ($P = 0.04$) in the proportion of heifers that had attained puberty by the initiation of the ovulation synchronization protocol (Fig. 1). A greater proportion ($P < 0.05$) of heifers in the 5dCO treatment were pubertal at protocol initiation than in the 14dCIDR-PG treatment, and more heifers tended ($P = 0.10$) to be pubertal at protocol initiation in PG-6dCIDR than in 14dCIDR-PG. In locations 1 and 3, where puberty attainment at initiation of 14dCIDR-

Figure 1. Proportion of heifers that had attained puberty before initiation of either the 5-d CO-Synch + CIDR (5dCO), PG 6-d CIDR (PG-6dCIDR), or 14-d CIDR PG (14dCIDR-PG) treatment. CIDR = controlled intravaginal progesterone insert; PG = PGF_{2α}. Treatment, $P = 0.04$. $a,b P < 0.05$; $a,c P = 0.10$.

PG was evaluated in all heifers, the proportion of heifers that had reached puberty did not differ between the 14dCIDR-PG (54.5%), PG-6dCIDR (46.3%), and 5dCO (57.8%) treatments.

Fixed-Time AI and Breeding Season Pregnancy Rates

Pregnancy rates to FTAI were not affected ($P = 0.14$) by treatment (Table 1). Reproductive status, irrespective of treatment, affected ($P < 0.01$) FTAI pregnancy rates (Table 1). Heifers that were pubertal at treatment initiation had greater FTAI pregnancy rates than heifers that had failed to reach puberty at treatment initiation. Breeding season pregnancy rates did not differ among the 5dCO (87%), PG-6dCIDR (87%), and 14dCIDR-PG (89%) treatments. Breeding season pregnancy rates, however, tended ($P = 0.09$) to be greater in heifers that were pubertal at the initiation of treatments (88.7%) than in heifers that were not (84.2%).

Tail Paint Scores

At locations 1 and 3, tail paint scores were recorded at FTAI. Across treatments, tail paint score was lower ($P < 0.01$) in pubertal (1.9 ± 0.05) compared to prepubertal (2.2 ± 0.08) heifers; however, there was a tendency ($P = 0.07$) for a treatment by reproductive status interaction for tail paint score (Table 2). In heifers that were prepubertal at treatment initiation, tail paint scores did not differ between treatments. In heifers that were pubertal at treatment initiation, tail paint score was decreased ($P < 0.01$) in the 5dCO compared to the 14dCIDR-PG treatment. However, pubertal status did not affect tail paint score in heifers in the 14dCIDR-PG treatment. But in both the 5dCO and PG-6dCIDR treatments, tail paint score was lower ($P < 0.05$) in pubertal heifers compared to prepubertal heifers. In an ancillary

Table 1. Fixed-time AI pregnancy rates in virgin beef heifers receiving either the 5-d CO-Synch + CIDR (5dCO), PG 6-d CIDR (PG-6dCIDR), or 14-d CIDR PG (14dCIDR-PG) ovulation synchronization protocol¹

Item	Treatment		
	5dCO ²	PG-6dCIDR ³	14dCIDR-PG ⁴
FTAI pregnancy rate, ⁵ %	62.6 (169/270)	56.9 (149/262)	53.3 (145/272)
Item	Pubertal Status		
	Pubertal	Prepubertal	
FTAI pregnancy rate, ⁵ %	60.9 ^a (366/601)	47.8 ^b (97/203)	

^{a,b}Means differ between reproductive status ($P < 0.01$).

¹CIDR = controlled intravaginal progesterone insert; PG = PGF_{2α}; FTAI = fixed-time AI.

²Heifers received 100 µg of GnRH and a CIDR insert on d -5, and on d 0 CIDR was removed and two 25-mg doses of PGF_{2α} were given >4 h apart, with the initial one given at CIDR removal. Fixed-time AI was conducted 72 h after CIDR removal concurrent with 100 µg of GnRH.

³Heifers received 25 mg of PGF_{2α} on d -9, GnRH (100 µg) and a CIDR insert on d -6, and 25 mg of PGF_{2α} and CIDR removal on d 0. Fixed-time AI was conducted 66 h after CIDR removal concurrent with 100 µg of GnRH.

⁴Heifers received a CIDR insert from d -30 to -16 and 25 mg of PGF_{2α} on d 0. Fixed-time AI was conducted 66 h after CIDR removal concurrent with 100 µg of GnRH.

⁵Fixed-time AI pregnancy rate diagnosed via ultrasonography 30 to 40 d after FTAI.

analysis, the effect of tail paint score on FTAI pregnancy rates was evaluated. Fixed-time AI pregnancy rates were influenced ($P < 0.01$) by tail paint score, with heifers with tail paint scores of 1 (62.7%) and 2 (56.1%) having greater ($P < 0.01$) FTAI pregnancy rates than heifers with a tail paint score of 3 (41.2%).

DISCUSSION

Yearling virgin heifers continue to be a difficult class of beef cattle for which to develop consistent and predictable FTAI protocols. Difficulties in controlling follicular dynamics in heifers can limit pregnancy success when implementing FTAI (Lamb et al., 2006, 2010). Independently, the 3 protocols evaluated in the present paper have been demonstrated to be effective methods to facilitate FTAI in beef heifers but had never been directly compared in a large multilocation study. Each protocol varies in its approach to control follicular dynamics and facilitate a synchronous ovulation at FTAI, yet results herein indicate that FTAI pregnancy rates in beef heifers did not differ between these protocols. Although ovulation protocol did not affect FTAI pregnancy rate, heifers failing to have reached puberty at protocol initiation had a reduction in FTAI pregnancy rates. Such results demonstrate the importance of proper heifer development programs to ensure puberty is attained before initiation of the breeding season.

Table 2. Tail paint scores in pubertal and prepubertal virgin beef heifers receiving either the 5-d CO-Synch + CIDR (5dCO), PG 6-d CIDR (PG-6dCIDR), or 14-d CIDR PG (14dCIDR-PG) ovulation synchronization protocol¹

Treatment ²	Tail Paint Score ³ (n)	
	Pubertal	Prepubertal
5dCO ⁴	1.8 ± 0.08 ^{a,x} (n = 108)	2.2 ± 0.17 ^y (n = 25)
PG-6dCIDR ⁵	1.9 ± 0.09 ^x (n = 91)	2.3 ± 0.13 ^y (n = 35)
14dCIDR-PG ⁶	2.1 ± 0.08 ^b (n = 73)	2.1 ± 0.11 (n = 60)

^{a,b}Means with a different superscript differ within a column ($P < 0.05$).

^{x,y}Means with a different superscript differ within a row ($P < 0.05$).

¹CIDR = controlled intravaginal progesterone insert; PG = PGF_{2α}.

²Treatment by reproductive status; $P = 0.07$.

³Tail paint scores: 1 = tail paint completely gone; 2 = tail paint partially gone, obvious signs of receiving some rubs from mountings; 3 = no signs of having received mounts, tail paint undisturbed.

⁴Heifers received 100 µg of GnRH and a CIDR insert on d -5, and on d 0 CIDR was removed and two 25-mg doses of PGF_{2α} were given >4 h apart, with the initial one given at CIDR removal. Fixed-time AI was conducted 72 h after CIDR removal concurrent with 100 µg of GnRH.

⁵Heifers received 25 mg of PGF_{2α} on d -9, GnRH (100 µg) and a CIDR insert on d -6, and 25 mg of PGF_{2α} and CIDR removal on d 0. Fixed-time AI was conducted 66 h after CIDR removal concurrent with 100 µg of GnRH.

⁶Heifers received a CIDR insert from d -30 to -16 and 25 mg of PGF_{2α} on d 0. Fixed-time AI was conducted 66 h after CIDR removal concurrent with 100 µg of GnRH.

The 5dCO protocol was developed on the premise that reducing the interval from CIDR insertion and GnRH to CIDR removal and PG from 7 to 5 d would allow the interval from CIDR removal to FTAI to be extended to 72 h, thus maximizing preovulatory estradiol concentrations (Bridges et al., 2008). It has been demonstrated that maximizing preovulatory estradiol concentrations during the preovulatory period increases the probability of conception in cattle (Bridges et al., 2010, 2013). Although this protocol relies on GnRH to reset follicular development at CIDR insertion, which has been demonstrated to have limited effectiveness in beef heifers (Atkins et al., 2008), the 5-d interval from GnRH to PG may limit asynchronous follicular development in females that fail to respond to the initial GnRH administration (Bridges et al., 2000). Previous studies comparing the 5-d approach to other synchronization protocols in beef heifers have demonstrated that the 5-d approach is more effective than the 7-d approach (Wilson et al., 2007; Sparks et al., 2012) and equivalent to a longer progestin approach, the 14-d CIDR Select and FTAI (Bridges and Lake, 2012). Likewise, in the current study, the 5dCO treatment did not differ from the longer 14dCIDR-PG treatment.

The PG-6dCIDR protocol was devised in an effort to improve the ability of GnRH to reset follicular waves at CIDR insertion. Administering PG 3 d before GnRH

results in low progesterone concentrations at GnRH administration in a majority of females treated. Ovulatory response is improved when progesterone concentrations are minimized at the time of GnRH administration (Perry and Perry, 2009). Studies characterizing follicular wave dynamics between the PG-6dCIDR and the CO-Synch approaches to synchronization have demonstrated that more females initiate a new follicular wave following GnRH administration in the PG-6dCIDR approach than in the CO-Synch approach to estrous synchronization (Grant et al., 2011). This improvement in controlling follicular dynamics is likely the reason for improved pregnancy success previously observed with the PG-6dCIDR protocol compared to CO-Synch protocols (Perry et al., 2012). In the current study, however, FTAI pregnancy rates in beef heifers were similar between the PG-6dCIDR and 5dCO protocols.

Unlike the 5dCO and PG-6dCIDR protocols, the 14dCIDR-PG protocol does not rely on GnRH to control follicular dynamics during synchronization. Rather, the 14dCIDR-PG approach utilizes a long-term progestin treatment to facilitate a tight synchrony of estrous expression following PG administration (Leitman et al., 2009a,b). The predecessor of the 14dCIDR-PG protocol, the CIDR Select protocol (Busch et al., 2007; Leitman et al., 2008), incorporated GnRH administration 7 d before PG and was proven to yield an improved synchrony of estrus and greater pregnancy rates than the 7-d CO-Synch + CIDR protocol in beef heifers. Subsequent experiments indicated, however, that GnRH administration was not required with the long-term CIDR approaches and removal of GnRH administration resulted in a tight synchrony of estrus (Mallory et al., 2010) and tended to improve FTAI pregnancy rates compared to when GnRH was administered (Mallory et al., 2011). In the current study, this longer approach to synchronization did not improve FTAI pregnancy rates when compared to the 5dCO and PG-6dCIDR protocols.

The 3 protocols investigated differ in their approach to ovulation synchronization; however, when implemented in numerous beef heifers across a range of locations, FTAI pregnancy rates were similar between protocols. Although FTAI pregnancy rates were reduced in prepubertal heifers, within this subset of females the protocols of shorter duration (5dCO and PG-6dCIDR) were as effective in prepubertal heifers as the longer protocol (14dCIDR-PG). This suggests an extended period of progesterone exposure is not required to effectively synchronize ovulation in prepubertal heifers.

Although FTAI pregnancy rates were not impacted by treatment, pubertal status at protocol initiation, irrespective of treatment, affected FTAI pregnancy rates. Classical studies (Byerley et al., 1987; Perry et al., 1991) have demonstrated that heifers having multiple estrous

cycles before the breeding season have an increased probability of becoming pregnant. The proportion of pubertal heifers at the start of the breeding season, however, can vary dramatically across herds (Lucy et al., 2001; Lamb et al., 2006) and is influenced by numerous factors, including age, weight, body condition, and breed (Wiltbank et al., 1966; Short and Bellows, 1971; Varner et al., 1977). Given the extended duration of the 14dCIDR-PG treatment and the necessity to initiate this protocol earlier to allow for similar day of AI among protocols, it was expected and observed that fewer heifers in this treatment would be pubertal at protocol initiation across all locations. In locations 1 and 3, where pubertal status was assessed in all heifers at initiation of the 14dCIDR-PG protocol, pubertal status at that time was similar between all treatments. Heifers that failed to initiate puberty before protocol initiation had reduced FTAI pregnancy rates, highlighting the importance of proper heifer development before the initiation of the breeding season. Although providing progesterone can stimulate pubertal attainment in previously prepubertal heifers (Gonzalez-Padilla et al., 1975; Short et al., 1976), it does not negate the importance of having a high proportion of heifers pubertal at the initiation of the breeding season to maximize pregnancy success. Similar to results in the present study, other studies in beef heifers have demonstrated improved AI pregnancy rates in pubertal compared to prepubertal heifers (Lucy et al., 2001; Wood-Follis et al., 2004; Leitman et al., 2008), yet others have observed that pubertal status at onset of the breeding season does not affect pregnancy success to AI (Lamb et al., 2006).

At locations 1 and 3, assessment of tail paint scores at FTAI was used to estimate the proportion of heifers that were in or had been in estrus before FTAI. Within pubertal heifers, tail paint score was less in the 5dCO compared to the 14dCIDR-PG treatment. In prepubertal heifers, however, tail paint scores were similar among treatments, although the number of observations in prepubertal heifers is limited. Having fewer heifers in estrus at FTAI in the 14dCIDR-PG treatment is not surprising given that by breeding at 66 h after the PG injection fewer animals had the opportunity to express estrus when compared to the longer interval from PG to FTAI in the 5dCO protocol (72 h). It is important to note that no treatment by pubertal status interaction was observed for FTAI pregnancy rates. Across treatments, tail paint score impacted FTAI pregnancy success. Not surprisingly, heifers that were in or potentially in estrus as indicated by a tail paint score of 1 or 2, respectively, had greater FTAI pregnancy rates than heifers that had no obvious signs of estrus (tail paint score of 3). Although the protocols used were designed to synchronize ovulation across all females regardless of estrus expression, even when conducting FTAI, numerous stud-

ies have demonstrated that females exhibiting estrus have greater FTAI pregnancy success than females that fail to exhibit estrus before FTAI (Perry et al., 2005, 2007). Expression of estrus indicates that adequate concentrations of preovulatory estradiol were achieved during the preovulatory period. Elevated estradiol concentrations before estrus are critical for follicular and oocyte maturation and uterine function during pregnancy (Bridges et al., 2013; Geary et al., 2013). Furthermore, concentrations of estradiol adequate to stimulate estrus can also cause alterations in uterine pH (Perry and Perry, 2008) to facilitate sperm transport (Hawk, 1983). Thus, when implementing ovulation synchronization protocols, although estrous expression is not required, maximizing the proportion of females that exhibit estrus before FTAI is critical.

In summary, FTAI pregnancy rates in virgin replacement beef heifers were similar when ovulation was synchronized with 5dCO, PG-6dCIDR, or 14dCIDR-PG. Although each protocol varies in approach and duration of synchronization, each is an effective method to facilitate FTAI. Results of the present experiment highlight the importance of proper heifer development to ensure pubertal attainment before the breeding season, as heifers that failed to reach puberty at protocol initiation had reduced FTAI pregnancy rates. Moreover, although estrus detection is not required with ovulation synchronization protocols, heifers that had signs of estrual activity had greater pregnancy success than those that did not exhibit signs of estrus.

LITERATURE CITED

- Atkins, J. A., D. C. Busch, J. F. Bader, D. H. Keisler, D. J. Patterson, M. C. Lucy, and M. F. Smith. 2008. Gonadotropin-releasing hormone-induced ovulation and luteinizing hormone release in beef heifers: Effect of day of the cycle. *J. Anim. Sci.* 86:83–93.
- Bridges, G. A., J. K. Ahola, C. Brauner, L. H. Cruppe, J. F. Currin, M. L. Day, P. J. Gunn, J. R. Jaeger, G. C. Lamb, G. H. Marquezini, R. K. Peel, A. E. Radunz, J. S. Stevenson, and W. D. Whittier. 2012. Determination of the appropriate delivery of PGF_{2α} in the 5 day CO-Synch + CIDR protocol in lactating beef cows. *J. Anim. Sci.* 90:4814–4822.
- Bridges, G. A., M. L. Day, T. W. Geary, and L. H. Cruppe. 2013. Deficiencies in the uterine environment and failure to support embryonic development. *J. Anim. Sci.* 91:3002–3013.
- Bridges, G. A., L. A. Helser, D. E. Grum, M. L. Mussard, C. L. Gasser, and M. L. Day. 2008. Decreasing the interval between GnRH and PGF_{2α} from 7 to 5 days and lengthening proestrus increases timed-AI pregnancy rates in beef cows. *Theriogenology* 69:843–851.
- Bridges, G. A., and S. L. Lake. 2012. Comparison of the CIDR Select and Five Day Select Synch + CIDR protocols that included a limited estrous detection and timed insemination for synchronizing estrus in beef heifers. *Prof. Anim. Sci.* 27:141–146.
- Bridges, G. A., M. L. Mussard, C. R. Burke, and M. L. Day. 2010. Influence of the length of proestrus on fertility and endocrine function in female cattle. *Anim. Reprod. Sci.* 117:208–215.
- Bridges, G. A., M. L. Mussard, L. A. Helser, and M. L. Day. 2000. Comparison of follicular dynamics and hormone concentrations between the 7 d and 5 d CO-Synch + CIDR program in two-year old beef cows. *J. Anim. Sci.* 87(Suppl. 1):464. (Abstr.)
- Busch, D. C., D. J. Wilson, D. J. Schafer, N. R. Leitman, J. K. Haden, M. R. Ellersieck, M. F. Smith, and D. J. Patterson. 2007. Comparison of progestin-based estrus synchronization protocols before fixed-time artificial insemination on pregnancy rate in beef heifers. *J. Anim. Sci.* 85:1933–1939.
- Byerley, D. J., R. B. Staigmiller, J. G. Berardinelli, and R. E. Short. 1987. Pregnancy rates of beef heifers bred either on puberal or third estrus. *J. Anim. Sci.* 65:645–650.
- Engel, C. E., H. H. Patterson, and G. A. Perry. 2008. Effect of dried corn distillers grains plus solubles compared to soybean hulls, in late gestation heifer diets, on animal and reproductive performance. *J. Anim. Sci.* 86:1697–1708.
- Geary, T. W., M. F. Smith, M. D. Macneil, M. L. Day, G. A. Bridges, G. A. Perry, F. M. Abreu, J. A. Atkins, K. G. Pohler, E. M. Jinks, and C. A. Madsen. 2013. Influence of follicular characteristics at ovulation on early embryonic survival. *J. Anim. Sci.* 91:3014–3021.
- Gonzalez-Padilla, E., R. Ruiz, D. LeFever, A. Denham, and J. N. Wilbank. 1975. Puberty in beef heifers. III. Induction of fertile estrus. *J. Anim. Sci.* 40:1110–1118.
- Grant, J. K., F. M. Abreu, N. L. Hojer, S. D. Fields, B. L. Perry, and G. A. Perry. 2011. Influence of inducing luteal regression before a modified controlled internal drug-releasing device treatment on control of follicular development. *J. Anim. Sci.* 89:3531–3541.
- Hawk, H. W. 1983. Sperm survival and transport in the female reproductive tract. *J. Dairy Sci.* 66:2645–2660.
- Horn, M., P. Gunn, M. Van Emon, R. Lemenager, J. Burgess, N. A. Pyatt, and S. L. Lake. 2010. Effects of natural (*RRR* α-tocopherol acetate) or synthetic (all-*rac* α-tocopherol acetate) vitamin E supplementation on reproductive efficiency in beef cows. *J. Anim. Sci.* 88:3121–3127.
- Lamb, G. C., J. E. Larson, T. W. Geary, J. S. Stevenson, S. K. Johnson, M. L. Day, R. P. Ansotegui, D. J. Kesler, J. M. DeJarnette, and D. G. Landblom. 2006. Synchronization of estrus and artificial insemination in replacement beef heifers using gonadotropin releasing hormone, prostaglandin F_{2α}, and progesterone. *J. Anim. Sci.* 84:3000–3009.
- Lamb, G. C., C. R. Dahlen, J. E. Larson, G. Marquezini, and J. S. Stevenson. 2010. Control of the estrous cycle to improve fertility for fixed-time artificial insemination in beef cattle: A review. *J. Anim. Sci.* 88(Suppl. 13):E181–E192.
- Leitman, N. R., D. C. Busch, J. F. Bader, D. A. Mallory, D. J. Wilson, M. C. Lucy, M. R. Ellersieck, M. F. Smith, and D. J. Patterson. 2008. Comparison of protocols to synchronize estrus and ovulation in estrous-cycling and prepubertal beef heifers. *J. Anim. Sci.* 86:1808–1818.
- Leitman, N. R., D. C. Busch, D. A. Mallory, D. J. Wilson, M. R. Ellersieck, M. F. Smith, and D. J. Patterson. 2009a. Comparison of long-term CIDR-based protocols to synchronize estrus in beef heifers. *Anim. Reprod. Sci.* 114:345–355.
- Leitman, N. R., D. C. Busch, D. J. Wilson, D. A. Mallory, M. R. Ellersieck, M. F. Smith, and D. J. Patterson. 2009b. Comparison of controlled internal drug release insert-based protocols to synchronize estrus in prepubertal and estrous-cycling beef heifers. *J. Anim. Sci.* 87:3976–3982.

- Lucy, M. C., H. J. Billings, W. R. Butler, L. R. Ehnis, M. J. Fields, D. J. Kesler, J. E. Kinder, R. C. Mattos, R. E. Short, W. W. Thatcher, R. P. Wettemann, J. V. Yelich, and H. D. Hafs. 2001. Efficacy of an intravaginal progesterone insert and an injection of PGF₂alpha for synchronizing estrus and shortening the interval to pregnancy in postpartum beef cows, peripubertal beef heifers, and dairy heifers. *J. Anim. Sci.* 79:982–995.
- Mallory, D. A., J. M. Nash, M. R. Ellersieck, M. F. Smith, and D. J. Patterson. 2011. Comparison of long-term progestin-based protocols to synchronize estrus prior to fixed-time artificial insemination in beef heifers. *J. Anim. Sci.* 89:1358–1365.
- Mallory, D. A., D. J. Wilson, D. C. Busch, M. R. Ellersieck, M. F. Smith, and D. J. Patterson. 2010. Comparison of long-term progestin-based estrus synchronization protocols in beef heifers. *J. Anim. Sci.* 88:3568–3578.
- Perry, G. A., and B. L. Perry. 2008. Effect of preovulatory concentrations of estradiol and initiation of standing estrus on uterine pH in beef cows. *Domest. Anim. Endocrinol.* 34:333–338.
- Perry, G. A., and B. L. Perry. 2009. Effect of the timing of controlled internal drug-releasing device insertion on the gonadotropin-releasing hormone-induced luteinizing hormone surge and ovulatory response. *J. Anim. Sci.* 87:3983–3990.
- Perry, G. A., B. L. Perry, J. H. Krantz, and J. Rodgers. 2012. Influence of inducing luteal regression before a modified fixed-time artificial insemination protocol in postpartum beef cows on pregnancy success. *J. Anim. Sci.* 90:489–494.
- Perry, G. A., B. L. Perry, and C. A. Roberts. 2011. Estrous response following the PG 6-d CIDR protocol for heifer that do and do not exhibit estrus prior to CIDR insertion and its usefulness as a fixed-time AI protocol. In: *Proc. Western Sect. Am. Soc. Anim. Sci.* Miles City, MT 56:264–267.
- Perry, G. A., M. F. Smith, M. C. Lucy, J. A. Green, T. E. Parks, M. D. MacNeil, A. J. Roberts, and T. W. Geary. 2005. Relationship between follicle size at insemination and pregnancy success. *Proc. Natl. Acad. Sci. USA* 102:5268–5273.
- Perry, G. A., M. F. Smith, A. J. Roberts, M. D. MacNeil, and T. W. Geary. 2007. Relationship between size of the ovulatory follicle and pregnancy success in beef heifers. *J. Anim. Sci.* 85:684–689.
- Perry, R. C., L. Corah, R. C. Cochran, J. Brethour, K. S. Olson, and J. J. Higgins. 1991. Effects of hay quality, breed, and ovarian development on onset of puberty and reproductive performance of beef heifers. *J. Prod. Agric.* 4:13–18.
- Short, R. E., and R. A. Bellows. 1971. Relationship among weight gains, age at puberty and reproductive performance in heifers. *J. Anim. Sci.* 32:127–131.
- Short, R. E., R. A. Bellows, J. B. Carr, R. B. Staigmiller, and R. D. Randel. 1976. Induced or synchronized puberty in heifers. *J. Anim. Sci.* 43:1254–1258.
- Sparks, B. L., S. L. Lake, P. J. Gunn, L. S. Fisher, R. P. Lemenager, J. Berry, and G. A. Bridges. 2012. Effects of PGF₂α administration at CIDR insertion on artificial insemination and pregnancy rates in beef heifers. *Prof. Anim. Sci.* 28:552–559.
- Varner, L. W., R. A. Bellows, and D. S. Christensen. 1977. A management system for wintering replacement heifers. *J. Anim. Sci.* 44:165–171.
- Wilson, K. N., M. L. Day, W. D. Whittier, R. Kasimanickam, and J. B. Hall. 2007. Comparison of 5-day or 7-day CIDR-based estrous synchronization systems for fixed-time AI in beef heifers. *J. Anim. Sci.* 85(Suppl. 2):43. (Abstr.)
- Wiltbank, J. N., K. E. Gregor, L. A. Swinger, J. E. Ingalls, J. A. Rothlisberger, and R. M. Koch. 1966. Effects of heterosis on age and weight at puberty in beef heifers. *J. Anim. Sci.* 25:744–751.
- Wood-Follis, S. L., F. N. Kojima, M. C. Lucy, M. F. Smith, and D. J. Paterson. 2004. Estrous synchronization in beef heifers with progestin-based protocols. I. Differences in response based on pubertal status at the initiation of treatment. *Theriogenology* 62:1518–1528.