# A two-injection prostaglandin $F_{2\alpha}$ presynchronization treatment decreases pregnancy rates of cycling replacement beef heifers

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**ABSTRACT:** Improving artificial insemination (AI) pregnancy rates in replacement heifers improves the genetic advancement within a herd. Heifers that have completed at least three estrous cycles prior to breeding have greater pregnancy rates compared to acyclic females. Therefore, it was hypothesized that a presynchronization treatment program consisting of two injections of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) prior to the start of the CO-Synch + 5 d CIDR protocol would initiate earlier attainment of puberty and more estrous cycles prior to AI, thus increasing AI pregnancy rates. All heifers were managed the same at two locations over the course of 2 yr. Heifers were randomly assigned to receive either the two-injection $PGF_{2\alpha}$ presynchronization treatment (PreSynch; $n = 105$) or no presynchronization (Control; $n = 106$) prior to the start of estrous synchronization. On the first day of the trial, reproductive tract scores (RTSs), pelvic areas, body condition scores, and weights were collected on all heifers. All heifers were synchronized with the CO-Synch + 5 d CIDR protocol and fixed-time artificially inseminated with semen from a bull of known fertility. Blood samples were collected three consecutive times at 7 d intervals starting 45 d prior to estrous synchronization to determine the onset of puberty via analyzing progesterone concentrations. Pregnancy status to AI was assessed using ultrasonography diagnosis at approximately 30 and 60 d post insemination. Data were analyzed using PROC MIXED of SAS and reported as least square mean. The PreSynch treatment decreased AI pregnancy rates (52.2% vs. 38.1 ± 6.3% for Control vs. PreSynch, respectively; $P = 0.06$) and did not result in earlier attainment of puberty in beef heifers ($P > 0.05$). The PreSynch treatment did not impact pregnancy rates in heifers with an RTS of 3 or 4 ($P > 0.05$). However, PreSynch heifers with an RTS of 5 had decreased pregnancy rates (68.3% vs. 46.9 ± 10.1% for Control vs. PreSynch, respectively; $P < 0.05$). Finally, PreSynch heifers with increased body condition of 6 had decreased pregnancy rates when compared to Control heifers (37.5% vs. 62.5 ± 11.6%, respectively; $P < 0.05$). On the basis of these data, implementation of heifer breeding soundness examination at least 3 wk prior to the start of the breeding season may be beneficial for selecting replacement females; however, presynchronization with prostaglandins immediately prior to estrous synchronization will negatively affect AI pregnancy rates in cycling pubertal heifers.

**Key words:** cattle, estrous synchronization, heifer growth, pregnancy



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## INTRODUCTION

The continued success of a cow-calf operation is dependent on establishment of pregnant replacement heifers. For these heifers to calve at 24 mo of age, an early onset of puberty is of critical importance to maximize lifetime productivity (Núñez-Dominguez et al., 1991). This requires heifers to be bred by 15 mo of age; however, it has been estimated that less than 65% of beef heifers achieve puberty by this age (reviewed by Lamb, 2013).

Puberty is defined as the first ovulatory estrus (heat) and subsequent luteal function (reviewed by Moran et al., 1989). Few methods exist to determine pubertal status and reproductive capability of heifers including body weight (BW), pelvic area, and reproductive tract scores (RTSs). Typically, it is recommended that a target weight for heifers to attain is 60% to 65% of their mature BW by the start of the breeding season to avoid impairment of reproductive performance (reviewed by Patterson et al., 1992). Pelvic area is a measurement used when selecting heifers before the start of the breeding season. Pelvic measurements can be used to successfully identify abnormally small or large pelvises; however, it is not necessarily used to determine pubertal status. Rather, this measurement is best used to identify heifers with increased risk for dystocia (reviewed by Dziuk and Bellows, 1983). Finally, an RTS is a subjective measurement that involves palpation of the reproductive tract and ovarian structures per rectum and assigns a score from 1 to 5 (1 being a prepubertal heifer with an infantile tract and 5 being a pubertal heifer with a palpable corpus luteum (CL) present; Anderson et al., 1991). This measurement is repeatable, accurate at determining pubertal status, and a good predictor of a heifer's response to synchronization (Anderson et al., 1991; Rosenkrans and Hardin, 2003).

Although pubertal estrus is the first opportunity for a heifer to conceive, fertility is not optimal. In fact, it has been shown that pregnancy rates are significantly improved when heifers are inseminated on their third estrus compared to artificial insemination (AI) at pubertal estrus (Byerley et al., 1987). Furthermore, it has been suggested that the use of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) may induce estrus before insemination and increase pregnancy rates (Young et al., 1984). A presynchronization (PreSynch) protocol typically involves two injections of $PGF_{2\alpha}$ 14 d apart to synchronize animal's estrous cycle prior to breeding. Previous studies have shown that a presynch protocol has the potential to increase pregnancy rates following timed artificial insemination (TAI) in cycling heifers (Colazo et al., 2004; Small et al., 2010). Therefore, the objective of this study was to compare pregnancy rates of beef heifers subjected to a presynch protocol (treatment) or not (control) prior to the CO-Synch + 5 d CIDR protocol followed by TAI. It was hypothesized that the presynch treatment will increase pregnancy rates in heifers.

## MATERIALS AND METHODS

### *Animals and Treatments*

The study was conducted over a 2-yr period (2015 to 2017) at the North Carolina State University Butner Beef Cattle Field Laboratory (BBCFL) in Butner, NC, and at the Upper Piedmont Research Station (UPRS) in Reidsville, NC. Nulliparous Angus and SimAngus heifers 10 to 14 mo of age ($n$ = 211) were used in this experiment. All animals were handled in accordance with procedures approved by the Animal Care and Use Committee from North Carolina State University. The *Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching* (FASS, 1999) was used for animal care during these studies (IACUC protocol No. 14-079-A).

Heifers were blocked by age and weight then randomly allotted into either the presynchronization treatment group or the control group. The heifers assigned to the presynchronization treatment group (PreSynch; $n$ = 105) received two injections of $PGF_{2\alpha}$, 21 and 7 d prior to the start of the estrous synchronization. The heifers assigned to the control group (Control; $n$ = 107) were treated the same but did not receive the additional two injections of $PGF_{2\alpha}$ (see experimental timeline in Figure 1). All animals were submitted to the CO-Synch + 5 d + controlled internal drug release protocol (CIDR; Zoetis, Florham Park, NJ) and TAI 60 ± 4 h post CIDR removal was performed with semen from bulls of known fertility by an experienced (university staff) inseminator. Cattle were exposed to natural service sires 14 d following AI to evaluate overall pregnancy rates for the breeding season. All animals described earlier were maintained on a roughage diet consisting of ad libitum access to either pasture or hay and water.

As attainment of critical BW is essential for heifer development, weekly BW and body condition scores (BCSs) (scale of 1 to 9; Eversole et al., 2005) were collected from the start of the presynchronization period through estrous synchronization. Hip height was measured at the start of the trial and frame scores (FS) were calculated using the

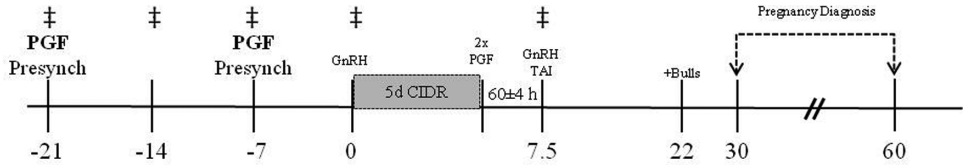


**Figure 1.** Experimental timeline: weekly animal measurements (‡) on all heifers included blood sampling, body weights, body condition scores started 21 d prior to estrous synchronization. PreSynch heifers ($n$ = 105) received two injections of prostaglandin $F_{2\alpha}$ 21 and 7 d prior to the start of the estrous synchronization, whereas Control heifers ($n$ = 107) were treated the same but did not receive the additional two injections of $PGF_{2\alpha}$. All heifers were submitted to the 5-d CO-Synch + controlled internal drug release protocol (CIDR; Zoetis) and timed AI (TAI) 60 ± 4 h post CIDR removal. Heifers were exposed to natural service sires (+Bulls) 14 d after TAI. Pregnancy diagnosis was by ultrasonography on d30 and 60 after TAI.

following equation: FS = 0.4723 (ht) − 0.0239 (days of age) + 0.0000146 (days of age)$^2$ + 0.0000759 (ht) (days of age) − 11.7086 (adapted from Hammack and Gill, 2001).

### *RTSs and Pelvic Area Measurements*

RTSs were determined via rectal palpation, and the pelvic area was measured by a single trained professional at the start of the presynchronization period. Scores were between 1 and 5, with 1 and 2 being reproductively immature (prepubertal), 3 having some reproductive capability and relatively close to reaching puberty (peripubertal), and 4 and 5 are reproductively mature (pubertal; Anderson et al., 1991). Pelvic area (cm$^2$) was determined for all heifers using a Rice pelvimeter (Lane Manufacturing, Denver, CO; Bennett et al., 2008).

### *Ultrasonography, Blood Collection, and Radioimmunoassay*

Transrectal ultrasonography using a 7.5 MHz linear array transducer (SonoSite M-Turbo; SonoSite Inc., Bothell, WA) was performed to determine pregnancy status at 30 and 60 d post-insemination. Blood samples were taken, via jugular venipuncture with 18-gauge needles and sterile vacutainer serum tubes without additive (Becton Dickinson, Franklin Lakes, NJ) every 7 d starting the day of the first injection of $PGF_{2\alpha}$ and continued through the start of estrous synchronization to determine the onset of puberty. During collection, blood was placed on ice for no longer than 3 h before being centrifuged for 20 min (1,580 × $g$; Clay Adams DYNAC Centrifuge) for separation. Serum was extracted and stored at −20 °C until analysis. Blood samples were analyzed for concentration of progesterone (P4) to identify the presence of a CL and cyclicity. A P4 concentration > 1.0 ng/mL for 2 consecutive weeks was used as an indicator of puberty attainment and cyclicity. In yr 2, additional blood samples were collected for analysis of P4 at TAI to estimate the proportion of animals that failed to undergo complete luteolysis. Animals with circulating concentrations of P4 > 0.5 ng/mL at insemination were considered to have failed to undergo luteolysis as reported by Cruppe et al. (2014). Concentrations of P4 were determined using a commercially available RIA kit (Coat-a-Count Progesterone; Siemens, Los Angeles, CA) as previously described by Burke et al. (2003). The intra- and interassay coefficient of variation was 2.9% and 12.3%, respectively.

### *Statistical Analysis*

Pregnancy rate to AI was defined as the number of animals diagnosed pregnant at the first pregnancy diagnosis following AI divided by the total number of animals submitted to AI. The MIXED Model procedure (SAS Institute Inc., Cary, NC) was used to analyze all binominal data (AI pregnancy rate and onset of puberty [$n$ = 211], luteolysis by the time of insemination [$n$ = 117]). The model included year, location, animal BW, BCS, AI technician, and treatment, with treatment as the fixed effects. Year, location, animal BW, BCS, RTS, and AI technician were considered random for all data analyses. Effects of BW, BCS, AI technician, treatment, and the appropriate interactions on AI pregnancy rate were initially evaluated within location and by the respective interactions with treatment. Terms 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. A statistical significance was reported at a $P < 0.05$. A tendency was reported at a $0.05 < P < 0.1$.

## RESULTS

### *Pubertal Status and Cyclicity*

Although PreSynch heifers were significantly younger compared to Control heifers (12.9 vs.

13.2 ± 0.08 mo, respectively; $P < 0.05$), this did not appear to alter other characteristics associated with pubertal status including percent mature BW, RTS, and pelvic area (Table 1). Moreover, blood samples for P4 collected at d −21, −14, −7 and 0 from heifers at each location indicated that attainment of puberty did not differ ($P > 0.05$; Figure 2), and the percent of heifers cycling at the start of estrous synchronization did not differ (65.3% vs. 72.8 ± 4.2% for Control vs. PreSynch, respectively; $P = 0.21$; Table 1). Concentrations of P4 indicated that 146 heifers were cycling and 66 heifers were acyclic by the start of synchronization (d 0).

**Table 1.** Characteristics of heifers enrolled in either the control or presynchronization program prior to CO-Synch + 5 d CIDR and TAI protocol

| | Control[1] | PreSynch[2] | SEM[3] | *P* value[4] |
|---|---|---|---|---|
| Growth parameter | | | | |
| Age, mo | 13.2[a] | 12.9[b] | 0.08 | 0.0422 |
| Weight, kg | 331.3 | 330.5 | 4.9 | 0.9044 |
| BCS[5] | 5.46* | 5.33* | 0.048 | 0.0644 |
| Frame score[6] | 4.46 | 4.57 | 0.086 | 0.3669 |
| MBW, %[7] | 63.5 | 63.3 | 0.93 | 0.8986 |
| Reproductive parameter | | | | |
| RTS[8] | 4.2 | 4.2 | 0.08 | 0.8384 |
| Pelvic area, cm$^2$ | 183.5 | 185.6 | 2.0 | 0.4588 |
| Cycling, %[9] | 65.3 | 72.8 | 4.2 | 0.2091 |

[a,b]Indicate significant differences ($P < 0.05$).

*Denotes a statistical tendency ($0.05 < P < 0.1$).

[1]Control heifers received 100 µg injection of GnRH (Factrel; gonadorelin hydrochloride; Zoetis Animal Health, Parssipany, NJ) at CIDR (EAZI-BREED CIDR; 1.38 g P4; Zoetis Animal Health) insertion [d 0], a 25 mg injection of $PGF_{2\alpha}$ (Lutalyse, dinoprost tromethamine; Zoetis Animal Health) administered at CIDR removal [d 5] and a second injection 8 h later, and an injection of GnRH and fixed-time AI (TAI) 60 ± 4 h later.

[2]PreSynch heifers were treated the same as Controls but received two additional 25-mg injection of $PGF_{2\alpha}$ 14 d apart starting 21 d prior to the first GnRH injection [d −21].

[3]SEM: Standard error of the mean between Control and PreSynch treatments within row.

[4]*P* value represents difference between Control and PreSynch treatments within row.

[5]BCS = body condition score; 1–9 scale according to Eversole et al. (2005).

[6]Frame score = calculated using the following equation [FS = 0.4723 (ht) − 0.0239 (days of age) + 0.0000146 (days of age)$^2$ + 0.0000759 (ht) (days of age) − 11.7086] according to Hammack and Gill (2001).

[7]MBW = mature body weight represented as the percentage of dam body weight.

[8]RTS = reproductive tract score; 1–5 scale according to Anderson et al. (1991).

[9]Cycling = the percentage of heifers that had serum progesterone concentrations greater than 1.0 ng/mL for 2 consecutive weeks prior to the start of estrous synchronization..

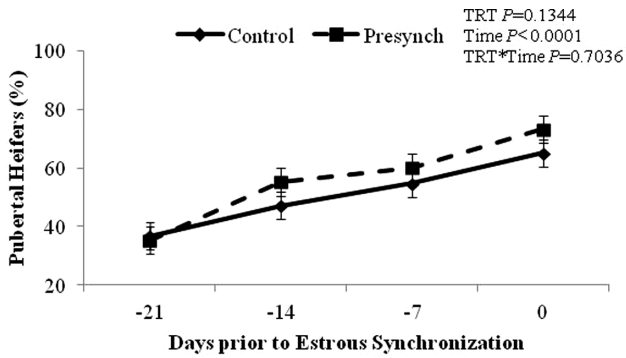


**Figure 2.** Puberty attainment (±SEM) of PreSynch heifers receiving two injections of prostaglandin $F_{2\alpha}$ 21 and 7 d prior to the start of the estrous synchronization whereas Control heifers did not receive prostaglandin $F_{2\alpha}$. Heifers were considered pubertal once serum progesterone concentrations were greater than 1.0 ng/mL for 2 consecutive weeks, and puberty attainment was declared at the second week of increased progesterone (Perry et al., 1991). Different letters indicate significant differences ($P < 0.05$), whereas * denotes a statistical tendency ($0.05 < P < 0.1$).

### *Pregnancy Outcomes*

The PreSynch treatment tended to have a negative effect on pregnancy rates from TAI (52.2% vs. 38.1 ± 6.3% for Control vs. PreSynch, respectively; $P = 0.06$; Table 2). However, no differences were observed for overall pregnancy rates ($P > 0.05$). Of the cyclic animals, heifers in the PreSynch treatment had significantly lower pregnancy rates when compared to Control heifers (39.0% vs. 56.5 ± 8.1%, respectively; $P < 0.05$; Table 2). The PreSynch treatment had no effect on pregnancy rates in acyclic animals ($P > 0.05$).

Treatment did not affect pregnancy rates in heifers with RTS of 3 and 4 ($P > 0.05$). However, the PreSynch treatment reduced pregnancy rates in heifers with an RTS of 5 (46.9% vs. 68.3 ± 10.1% for PreSynch vs. Control, respectively; $P < 0.05$; Table 2). Although there was a tendency in heifers that were 12 mo of age in the PreSynch treatment having lower pregnancy rates when compared to Control (16.3% vs. 56.7 ± 16.3%, respectively; $P = 0.06$), this was not observed in the other age groups (Table 2). Interestingly, there was a location × treatment × RTS interaction with the PreSynch treatment improving pregnancy rates in heifers with an RTS of 5 at BBCFL, however not at UPRS ($P < 0.05$; Figure 3).

There was a year effect with PreSynch heifers having lower pregnancy rates in 2016 compared to Control heifers (35.7% vs. 61.6 ± 10.7%, respectively; $P < 0.05$; Table 2); however, no differences were observed in 2017 ($P > 0.05$). Finally, both BCS and pelvic area had an impact on pregnancy rates. In heifers with a BCS of 6, the PreSynch

**Table 2.** Effect of presynchronization program, prior to CO-Synch + 5 d CIDR and TAI, on AI pregnancy rates in beef heifers

| Reproductive parameters | *n* | Control[1] (%) | PreSynch[2] (%) | SEM[3] | *P* value[4] |
|---|---|---|---|---|---|
| Pregnancy rates | | | | | |
| TAI | 211 | 52.2* | 38.1* | 6.3 | 0.0571 |
| Overall | 211 | 96.3 | 93.0 | 2.3 | 0.3100 |
| Expression of estrus | | | | | |
| Acyclic | 66 | 32.4 | 28.6 | 12.2 | 0.7519 |
| Cyclic | 146 | 56.5[a] | 39.0[b] | 8.1 | 0.0307 |
| Reproductive tract score[5] | | | | | |
| RTS 3 | 49 | 44.8 | 35.3 | 14.1 | 0.4968 |
| RTS 4 | 60 | 42.3 | 30.9 | 13.4 | 0.3959 |
| RTS 5 | 99 | 68.3[a] | 46.9[b] | 10.1 | 0.0361 |
| Year | | | | | |
| 2016 | 95 | 61.6[a] | 35.7[b] | 10.7 | 0.0165 |
| 2017 | 116 | 41.9 | 39.6 | 10.2 | 0.8171 |
| Location | | | | | |
| BBCFL | 92 | 52.3 | 35.7 | 10.6 | 0.1314 |
| UPRS | 119 | 51.3 | 39.7 | 9.7 | 0.2322 |
| Age (mo) | | | | | |
| 11 | 14 | 59.7 | 67.8 | 27.6 | 0.7690 |
| 12 | 48 | 56.7* | 16.3* | 16.3 | 0.0557 |
| 13 | 85 | 51.1 | 50.0 | 12.9 | 0.9333 |
| 14 | 64 | 40.7 | 25.8 | 15.8 | 0.3231 |
| Breed | | | | | |
| Angus | 181 | 50.6 | 40.2 | 9.6 | 0.2850 |
| Sim Angus | 30 | 53.6 | 44.3 | 18.3 | 0.6137 |
| Body condition score[6] | | | | | |
| BCS 5 | 121 | 42.9 | 33.3 | 9.0 | 0.2932 |
| BCS 6 | 87 | 62.5[a] | 37.5[b] | 11.6 | 0.0320 |
| Pelvic area | | | | | |
| 140–159.9 $cm^2$ | 22 | 41.7 | 33.3 | 21.7 | 0.7019 |
| 160–179.9 $cm^2$ | 50 | 41.7 | 40.0 | 14.9 | 0.9112 |
| ≥180 $cm^2$ | 137 | 56.7[a] | 33.3[b] | 8.8 | 0.0087 |

BBCFL = Butner Beef Cattle Field Laboratory; UPRS = Upper Piedmont Research Station.

[a,b]Indicate significant differences ($P < 0.05$).

*Denotes a statistical tendency ($0.05 < P < 0.1$).

[1]Control heifers received 100 µg injection of GnRH (Factrel; gonadorelin hydrochloride; Zoetis Animal Health) at CIDR (EAZI-BREED CIDR; 1.38 g P4; Zoetis Animal Health) insertion [d 0], a 25 mg injection of $PGF_{2\alpha}$ (Lutalyse, dinoprost tromethamine; Zoetis Animal Health) administered at CIDR removal [d 5] and a second injection 8 h later, and an injection of GnRH and fixed-time AI (TAI) 60 ± 4 h later.

[2]PreSynch heifers were treated the same as Controls but received two additional 25-mg injection of $PGF_{2\alpha}$ 14 d apart starting 21 d prior to the first GnRH injection [d −21].

[3]SEM = standard error of the mean between Control and PreSynch treatments within row.

[4]*P* value represents difference between Control and PreSynch treatments within row.

[5]RTS = reproductive tract score; 1–5 scale according to Anderson et al. (1991).

[6]BCS = body condition score; 1–9 scale according to Eversole et al. (2005).

treatment reduced pregnancy rates when compared to the Controls (37.5% vs. 62.5 ± 11.6%, respectively; $P < 0.05$; Table 2); however, no differences were observed in heifers with a BCS of 5 ($P > 0.05$). In heifers with a pelvic area of 180 $cm^2$ or greater, the PreSynch treatment reduced pregnancy rates when compared to the Controls (33.3% vs. 56.7 ± 8.8%, respectively; $P < 0.05$; Table 2).

### *Response to Synchronization*

To determine if heifers were responding to the CO-Synch + 5 d CIDR estrous synchronization protocol, blood samples were collected at TAI in yr 2 of the study. It was identified that a proportion (9.5%) of heifers failed to undergo complete luteolysis following the second $PGF_{2\alpha}$ injection of in the 5-d protocol as indicated by P4 concentrations >1 ng/mL at TAI (d 7.5). There was no difference

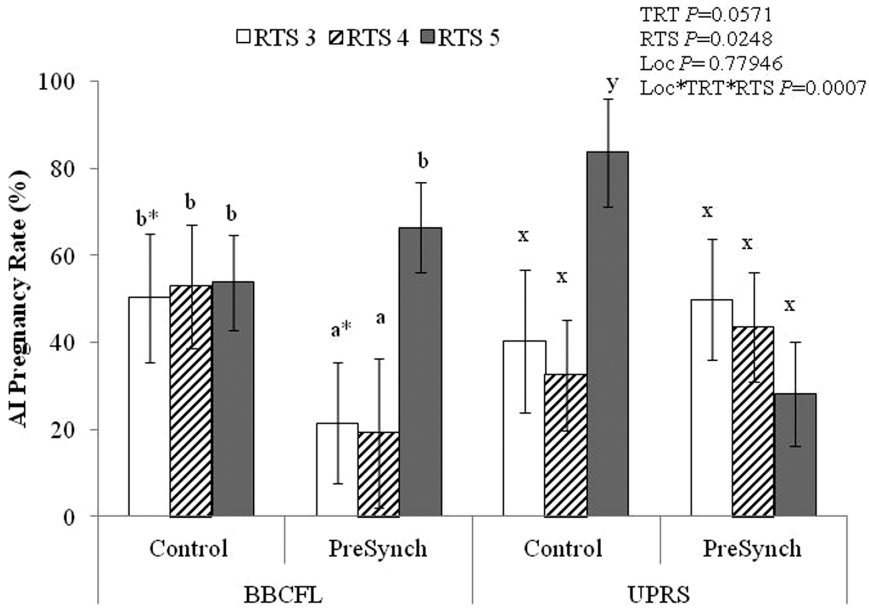


**Figure 3.** Effect of treatment (TRT, Control vs. PreSynch), reproductive tract score (RTS 3, white bars; RTS 4, hashed bars; RTS 5, gray bars), and location (BBCFL vs. UPRS) on TAI pregnancy rates. Different letters indicate significant differences ($P < 0.05$), whereas * denotes a statistical tendency ($0.05 < P < 0.1$).

($P > 0.05$) in the proportion of heifers that failed to undergo complete luteolysis when animals received the presynchronization protocol (PreSynch; 12%) compared to those that did not receive the presynchronization program (Control; 6%). Although the incidence of luteolysis failure in this study is relatively low, it was greater than the number of beef heifers that failed to undergo complete luteolysis as reported by Cruppe et al. 2014.

## DISCUSSION

The overall goal of a presynchronization program is to ensure that animals undergo a few estrous cycles before the first insemination. A standard two-injection $PGF_{2\alpha}$ presynchronization protocol is extensively used in postpartum dairy cows (Ferguson and Galligan, 1993). It has been demonstrated that cows that ovulate earlier in postpartum have an increase number of estrous cycles before the first insemination, and thus have increased fertility (Thatcher and Wilcox, 1973; Darwash et al., 1997). However, there are two limitations to this presynchronization protocol, first is $PGF_{2\alpha}$ alone does not benefit acyclic cows (Moreira et al., 2000) and second is that follicular growth is not precisely synchronized (Souza et al., 2008). Geary and Whittier (1998) were the first to report that the Ovsynch protocol, which uses both $PGF_{2\alpha}$ and gonadotropin-releasing hormone (GnRH), can induce a fertile ovulation in anestrous cattle. In addition, the inclusion of progestin and GnRH in conjunction with $PGF_{2\alpha}$ can either induce or synchronize ovulation and increase pregnancy rates in postpartum beef cows and heifers, regardless of their cyclicity (reviewed by Lamb et al., 2010).

It has been shown that pregnancy rates are significantly improved when heifers are inseminated on their third estrus compared to those at pubertal or first estrus (Byerley et al., 1987). Therefore, we hypothesized that beef heifers subjected to a presynchronization program before a TAI protocol would increase the number of estrous cycles before the first insemination and enhance pregnancy rates. It appears, however, contrary to our hypothesis that a presynchronization protocol reduces pregnancy rates in beef heifers. Specifically, heifers deemed cyclic prior to TAI and having an RTS of 5 had reduced pregnancy rates when subjected to a presynchronization protocol. A plausible explanation would be that these heifers had a functional CL on treatment days (d −21 and −7), thus by initiating the CO-Synch + 5 d CIDR protocol 7 d following the second $PGF_{2\alpha}$ PreSynch injection, only subordinate follicles would be present. The ability for GnRH to induce ovulation and initiate a new follicular wave is dependent on follicle size (Geary et al., 2000; Atkins et al., 2005), and it has been shown that subordinate follicles (<10 mm) are unresponsive to GnRH (Ryan et al., 1998; Sartori et al., 2001). Therefore, a presynchronization protocol has the potential to be effective in cycling heifers if the first injection of GnRH is initiated 2 to 3 d following the second $PGF_{2\alpha}$ PreSynch injection.

This would ensure that a dominant follicle is present on d 0, and following GnRH administration a new follicular wave will emerge as indicated by Roche et al. (1999). In this study, response to the CO-Synch + 5 d CIDR protocol in yr 2 was 94% of the Control heifers and 88% of the PreSynch heifers as indicated by ≤ 0.5 ng/mL of P4 at insemination. This signifies complete luteolysis in heifers following the second $PGF_{2\alpha}$ injection of the CO-Synch + 5 d CIDR protocol. Therefore, it is not surprising that there were no differences in AI pregnancy rates in yr 2 between treatments (41.9% vs. 39.6% for Control and PreSynch, respectively). Typically, an estrous synchronization program can result in pregnancy rates of approximately 50% to 60% if the majority of females respond to treatment and display estrus (Sprott and Carpenter, 2007). However, in yr 2 with the majority of heifers responding to synchronization (91%) this resulted in only 41% pregnancy rates. The poorer AI pregnancy rate observed was most likely attributed to the inability to synchronize follicular waves. This is not surprising because it has been shown that when given GnRH at random stages of the estrous cycle, 75% of postpartum beef cows will respond; however, only 48% of beef heifers respond (reviewed by Lamb et al., 2010).

It has been previously described that there is a relationship between RTS and estrous response; specifically that as RTS increases (4 and 5) in heifers, response to estrous synchronization improves. Moreover, heifers with a greater RTS were more developed by being heavier and having larger pelvic areas compared to heifers with lower RTS (Patterson and Bullock, 1995). In addition to RTS, it is well known that body condition plays a role in fertility, specifically it is important for initiating cyclicity and pregnancy rates are improved with increasing BCS (Rae et al., 1993; Gutierrez et al., 2014). In this study, there were no significant differences in average BW, BCS, RTS, and pelvic area in heifers subjected to either treatment (Control vs. PreSynch; Table 1); therefore, these factors that play a role in fertility do not confound results observed by treatment. However, the more developed heifers (RTS 5, BCS 6, and pelvic area ≥ 180 $cm^2$) subjected to the PreSynch treatment in this study had lower pregnancy rates compared to developed Control heifers (Table 2). These results are similar to those of Kasimanickam et al. (2016) where beef heifers with RTS 5 subjected to a double $PGF_{2\alpha}$ protocol had reduced reproductive performance compared to heifers subjected to either a CIDR-$PGF_{2\alpha}$ or Select Synch protocol.

In conclusion, it appears that the two-injection $PGF_{2\alpha}$ presynchronization program negatively affects cycling beef heifers by lowering pregnancy rates. Ultimately, this study identified the importance of reproductive tract scoring in heifers and understanding the period of the estrous cycle an animal is in when trying to synchronize. Future research is necessary to elucidate if the utilization of a presynchronization protocol can improve pregnancy rates in heifers with RTS 5 if an estrous synchronization protocol using GnRH is initiated approximately 3 d post-second injection of $PGF_{2\alpha}$.

*Conflict of interest statement*. None declared.

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