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FERTILITY IN BEEF HEIFERS SYNCHRONIZED USING A MODIFIED CO-SYNCH PLUS CIDR PROTOCOL WITH OR WITHOUT GNRH AT TIMED AI

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ABSTRACT: The objectives of this study were to determine if a second injection of GnRH at timed AI (TAI) increases the percentage of induced ovulations and improves pregnancy rates in beef heifers synchronized with the CO-Synch plus CIDR protocol. Nulliparous crossbred beef heifers (n = 375, BW = 362.7 kg, body condition score, BCS = 5.6) from three locations (Colorado [CO], Wyoming [WY] and South Dakota [SD]) were stratified by BW within BCS and randomly allotted to one of two treatments. All heifers received 100 µg of GnRH with a CIDR insert on day 0, followed by CIDR removal and 25 mg of $PGF_{2\alpha}$ on day 7. At 54 hours post $PGF_{2\alpha}$, heifers in the control (CON) and treatment (TRMT) groups were mass mated and heifers in the TRMT group were given a second injection of GnRH at that time. Blood samples were collected in heifers at d -10 and 0 to determine cyclicity status at CO and WY. Ultrasonography was used to determine percentage of heifers ovulating 40 h after TAI at the CO and WY locations. Cyclicity rates were higher (P < 0.01) for heifers at CO (97.4 %) vs WY (46.4 %). Pregnancy rates were similar (P > 0.10) between treatment groups and for cycling and non-cycling heifers at CO and WY; however, pregnancy rates were higher (P < 0.05) for heifers in the TRMT (54.2 %) vs CON group (40.4 %) at SD. Body weight did not affect pregnancy rates for either treatment group across all locations (P > 0.10); however, pregnancy rates tended to decrease (P = 0.08) for heifers with body weights greater than 409.1 kg (39 %) vs heifers with body weights less than 409.1 kg (53.2 %) at SD. The percentage of heifers ovulating were similar (P > 0.10) between CO and WY and ovulation rates tended to be higher (P = 0.10) for heifers in the TRMT (81.3 and 73.9 %) vs CON (62.5 and 66.7 %) groups at CO and WY. We conclude that synchronizing beef heifers with a modified CO-Synch plus CIDR protocol induces ovulation in cycling and non-cycling heifers and produces acceptable pregnancy rates at 54 h TAI. The value of incorporating a second injection of GnRH at timed AI remains questionable.

Key Words: Estrous Synchronization, GnRH, CIDR

Introduction

Past approaches to synchronization have included the use of both gonadotropins and prostaglandins; however, variations in estrous response (Moreira et al., 2000), interval to standing estrus (Geary et al., 2001) and missed standing heats (Hixon et al., 2001) influence pregnancy outcomes from fixed-time AI protocols. Some of these responses are dependent upon the stage of the estrous cycle when GnRH and/or PG are given (Geary et al., 2000). Schmitt et al. (1996) reported no differences in a 4-day estrous response period, but conception rates in beef heifers were reduced by 16 % when GnRH was removed from the beginning of a 9 d CIDR insert plus PG. Schmitt also reported that the average interval from PG to standing estrus was 47.3 h. Twagiramungu et al. (1995) reported higher pregnancy rates in beef heifers synchronized with a modified Select Synch protocol with the addition of a second injection of GnRH at 54 h timed AI vs no GnRH at TAI. With respect to GnRH, 75 % of anestrous cows formed new luteal tissue and more than 85 % ovulated in response to a second injection of GnRH 48 h after a Select Synch protocol (Thompson et al., 1999). When combining progestin with a gonadotropin and prostaglandin, estrous response was high with a reported 29 % increase in pregnancy rates for beef heifers synchronized with a CO-Synch + CIDR vs CO-Synch protocol alone (Martinez et al., 2002). The objective of this study was to determine if a second injection of GnRH increases the percentage of induced ovulations and improves fertility in beef heifers synchronized with a CO-Synch + CIDR protocol and mass mated at 54 h post PG injection.

Materials and Methods

Experimental Design. Nulliparous crossbred beef heifers from one cooperator herd in South Dakota (SD; n = 211, BW = 392.3 kg, body condition score, BCS = 5.7) and two research station herds in Colorado (CO; n = 39, BW = 324.5 kg, BCS = 5.7) and Wyoming (WY; n = 125, BW = 325 kg, BCS = 5.4) were used to determine if a second injection of GnRH 54 h following CIDR removal increases the percentage of induced ovulations and improves timed AI (TAI) pregnancy rates. Heifers were synchronized with the CO-Synch plus EAZI BREED CIDR® (CIDR; 1.38 g of progesterone) protocol and stratified by BW within BCS to be randomly allotted to one of two treatment groups. All heifers received 100 µg (i.m.) of GnRH with a CIDR insert on day 0, followed by CIDR removal and 25 mg (i.m.) of prostaglandin $F_{2\alpha}$ (PG) on day 7. At 54 hours post PG administration (d 9), heifers in the control (CON) and treatment (TRMT) groups were mass mated and heifers in the TRMT group were given a second injection of GnRH at that time. Body condition scores (1 to 9; 1 = emaciated and 9 = obese) and body weights were assessed on all heifers at time of CIDR insertion (day 0). Heifers were then assigned by weight class (90.9 kg increments) as either 1 (227.3 to 317.7 kg), 2 (318.2 to 408.6 kg) or 3 (409.1 to 499.5 kg) for each location. All heifers were diagnosed for pregnancy to

AI via transrectal ultrasonography 45 d post TAI. Cleanup bulls were turned out 8-14 d after mass mating and left in for 45 d.

Prior to synchronizing, two jugular vein blood samples were collected from all heifers at CO and WY 10 days apart (d -10 and 0) to determine cyclicity status. Heifers were assumed to be cyclical before the onset of treatments if any one of the two samples contained concentrations of serum progesterone ≥ 1 ng/mL. Serum was collected and stored at -20°C until analyzed for progesterone by solid-phase radioimmunoassay (RIA; Diagnostic Products Corp., Los Angeles, CA). Serum samples were assayed in duplicate and sensitivity of the assay was 0.08 ng/ml. Within and between assay CV for serum samples was 12.86 and 9.6 % across two assays, respectively.

Ovaries from a subset of heifers at CO (n = 19) and WY (n = 49) were examined by transrectal ultrasonography to characterize incidence of ovulation relative to treatment groups 40 h following TAI. While ultrasounding heifers at TAI, follicular cysts were detected in one heifer from CO and 3 heifers from WY. These animals were removed from the study. Follicles were classified as cystic if the diameter of the follicle was > 20 mm. Ovulation was defined as the disappearance of a large dominant follicle present on the ovary at time of insemination. Ovaries were scanned using transrectal ultrasonography (5-MHz intrarectal transducer, Aloka 500V, Corometrics, Wallingford, CT).

Heifers from CO were visually observed three times daily from day 6 (24 h prior to PG) to 10 (72 h after PG) to characterize estrous response. Estrous response was used to calculate pregnancy rates for specific times of estrus relevant to TAI.

Statistical Analysis. Preliminary analysis revealed a location effect on treatment, therefore data were not pooled and the main effects were evaluated within each location. Effects of treatment, AI technician, sire, cyclicity status and weight class on pregnancy rates were analyzed using Proc GENMOD procedure in SAS (1996). Differences in least squares means were used to compare pregnancy rates between treatments, sire and weight class. AI sire was then included in all models as a random effect with BW and BCS analyzed as covariates. Effects of BCS and BW on percent of heifers cycling were determined using Proc GENMOD. Significance was determined using Chi-square at $P < 0.05$.

All follicle data for heifers at CO and WY were analyzed using Proc GLM in SAS (1996). Effects of treatment, BCS and BW on incidence of ovulation were analyzed. Pregnancy rate differences based on treatment, incidence of ovulation, BCS and BW were analyzed. Significance was determined at $P < 0.05$.

Results and Discussion

Estrous Response. There were no heifers observed in standing estrus until 36 h after CIDR removal and average interval from PG to first observed standing estrus was 51 h (Figure 1). Estrous response was 64.1 and 71.8 % within a 24 and 36 h period beginning 36 h after CIDR removal and pregnancy rates for heifers exhibiting estrus within 36 h was 67.9 %. The percentage of heifers pregnant not exhibiting a standing estrus by 72 h after CIDR removal was 18.2 %. In heifers, estrous response rate was 65 % within a 72 h period when CIDR inserts were incorporated into a CIDR plus PG protocol (Lucy et al., 2001). Schmitt et al. (1996) and Martinez et al. (2002) reported that the average interval from PG to standing estrus in heifers was 47.3 and 47.8 h when a CIDR insert or MGA feeding was incorporated with GnRH at the time of insert/beginning of MGA; however, Richardson et al. (2002) reported average interval to estrus was 68 h in beef heifers after a CIDR+PG protocol. These responses are largely dependent upon the stage of the estrous cycle when GnRH and/or PG is given (Geary et al., 2000). However, incorporating a CIDR insert with GnRH injection at the time of insert synchronized a tight estrous response in the current study within 54 h to allow for TAI.

Cyclicity Status. The proportion of heifers cycling was lower ($P < 0.01$) for WY (46.4 %) vs CO (97.4 %) heifers (Table 1). Heifers from WY had low cyclicity rates and a 55.2 % pregnancy rate to AI which indicated the effectiveness of GnRH and a CIDR insert to induce puberty in prepubertal heifers. The progesterone insert is known to increase LH secretion which increases follicular growth and development resulting in ovulation following its removal (Anderson et al., 1996). The proportion of heifers cycling was similar ($P > 0.10$) between CON and TRMT heifers at both CO (100 and 95 %) and WY (47.6 and 45.2 %). Lucy et al. (2001) reported a 48 % synchronization rate in prepubertal beef heifers using a 7 d CIDR insert plus PG.

Fertility. Pregnancy rates to TAI were similar ($P > 0.10$) for both cycling and non-cycling heifers at CO and WY (Table 1). The proportion of cycling and non-cycling heifers pregnant to AI did not differ ($P > 0.10$) between CON vs TRMT heifers for CO and WY. Incorporating a norgestomet implant prevented short estrous cycles from naturally occurring after the first pubertal ovulation in beef heifers (Gonzalez-Padilla et al., 1975), and may be the reason differences in pregnancy rates were not observed between cycling and prepubertal heifers at both locations.

The percentage of CIDRs lost during the 7 d CIDR insert was 0 % for all locations. Final pregnancy rates for

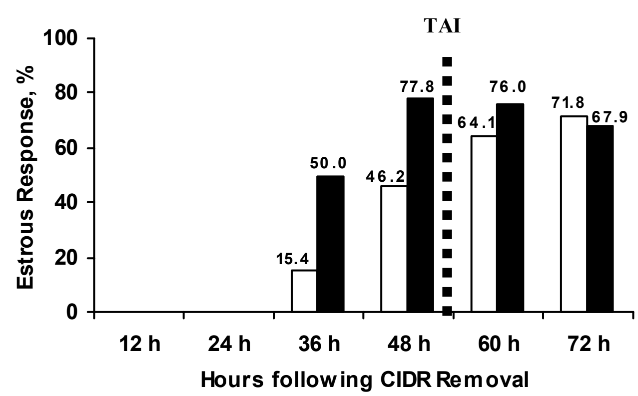

Figure 1: Cumulative estrous response (open bars) and pregnancy rates (shaded bars) for heifers detected in estrus at CO relative to TAI (54 h).

heifers at Colorado (97.4 %) and Wyoming (89.1 %) were not different ($P > 0.10$) between the two locations and final pregnancy rates were not determined at SD. Timed AI pregnancy rates for heifers from both treatments combined did not differ ($P > 0.10$) between CO (53.9 %; 21/39), SD (47.4 %; 100/211) and WY (55.2 %; 69/125). There was a treatment effect on AI pregnancy rates at the SD location, therefore data from all locations were not pooled and treatment effects were analyzed within each location. At SD, the proportion of pregnant heifers was lower ($P < 0.05$) in the CON (40.4 %) vs TRMT group (54.2 %), but differences in pregnancy rates were not observed between treatments ($P > 0.10$) for both CO and WY locations (Figure 2). The reason for decreased fertility among CON heifers at SD is unclear. While we did not determine cyclicity rate at SD, cyclicity did not affect response to treatment from heifers at CO and WY locations and thus, would not be expected to have contributed to fertility at SD.

Effects of BCS and body weight on heifer pregnancy rates were not significant ($P > 0.10$); however, average BW was heavier for SD heifers compared to CO and WY heifers. Anderson et al. (1987) reported reduced fertility in beef heifers that carried a condition score greater than 6. The subjectivity of body condition scores, within locations, may have prevented us from detecting differences in pregnancy rates. Weight differences may explain more variation in pregnancy rates, so we separated body weights for heifers at all locations into three weight classes and analyzed for differences in pregnancy rates as a fixed variable (Table 1). While 96 % of the heifers from CO and WY and 72 % of the heifers from SD weighed between 272 to 408 kg, the remaining 28 % from SD weighed between 409 to 499 kg. Weight class tended ($P = 0.08$) to affect pregnancy rates for heifers at SD, but no differences ($P > 0.10$) in pregnancy rates were observed for heifers at CO and WY locations within weight class.

The number of service sires varied within location. Pregnancy rate differences were not observed ($P > 0.10$) for CO heifers inseminated to sires A (57.9 %) and B (50 %), but 3 out of 13 AI sires used at SD and 1 out of 4 sires used at WY resulted in lower pregnancy rates within both locations ($P < 0.05$). Pregnancy rates from sires used at WY and SD ranged from 45.2 to 73 % and 31.8 to 72.2 %.

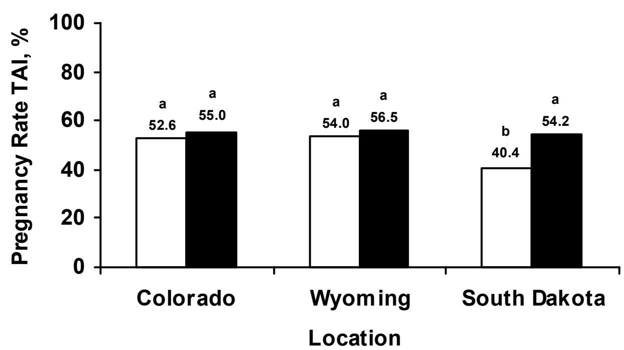

Figure 2: Effect of treatment (CON [open bars] vs TRMT[shaded bars]) on pregnancy rates to 54 h fixed time AI within location. Percentages within location without a common letter (a,b) differ ($P < 0.05$).

Dalton et al. (2001) reported significant differences between AI bulls on fertility rates when used for insemination at hours 0, 12 and 24 after first standing estrus in dairy cows.

Incidence of Ovulation after Treatments. The incidence of ovulation did not differ ($P > 0.10$) between CO and WY heifers (66.7 % and 73.9 %), however ovulation rates tended to be lower ($P = 0.10$) for heifers in the CON (62.5 and 66.7 %) vs TRMT (81.3 and 73.9 %) groups, respectively. Heifers ovulating by 40 h after TAI at CO and WY, regardless of treatment, had higher ($P < 0.01$) pregnancy rates (50 and 61.8 %) than heifers that had not ovulated (16.7 and 16.7 %). Incorporating a second injection of GnRH at timed AI in dairy cattle successfully induced ovulation 24 to 32 h after GnRH (Pursley et al., 1994), but ovulation rates in the current study from heifers receiving a CIDR, but not receiving an additional injection of GnRH (62.5 %) resulted in pregnancy rates that were not different from heifers receiving an additional GnRH at TAI. It would appear that incidence of ovulation is high without incorporating a second injection of GnRH at TAI and suggest that a tight estrous response was in close proximity to the 54 h TAI period.

Implications

Currently, there is no consistent TAI synchronization protocol that exists for controlling ovulation in beef heifers. In the current study, pregnancy rates were not improved for heifers receiving an additional GnRH injection at TAI at two locations, but were improved at the third location. Producers may be able to achieve acceptable pregnancy rates in beef heifers using a synchronization protocol that utilizes 54 h TAI, without estrous detection, with a CIDR insert plus GnRH at CIDR insertion and PG. Administering the second GnRH injection at timed AI may not improve pregnancy rates to AI, but guard against low pregnancy rates.

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Table 1: Characteristics of heifers bred TAI by location

Item	Location: Colorado (CO)		Wyoming (WY)		South Dakota (SD)		Overall	
	(no.) %							
Cyclicity[a]								
% cycling	(38/39)*	97.4	(58/125)	46.4	n/a		(96/164)	58.5
PR, cycling	(21/38)	55.3	(32/58)	55.2	n/a		(53/96)	55.2
PR, noncycling	(0/1)	0.0	(35/67)	52.2	n/a		(35/68)	51.5
Wt class, kg[b]								
1 (227.3 – 317.7)	(8/16)	50.0	(27/51)	52.9	(0/1)	0.0	(35/68)	51.5
2 (318.2 – 408.6)	(13/23)	56.5	(42/74)	56.8	(77/151)	51.0	(132/248)	53.2
3 (409.1 – 499.5)					(23/59)	39.0	(23/59)	39.0

[a]Percentage of heifers cycling based on progesterone values > 1 ng/ml taken on d -10 and 0.
Cyclicity data was not available (n/a) for heifers at the SD location because they were a cooperator herd.
Pregnancy rates (PR) for the percent of heifers cycling and non-cycling.
[b]Combined weights of heifers separated into three classes for all locations.
*Raw mean percentages within a row for % cycling lacking the common asterisks differ ($P < 0.05$).

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