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Abstract

The objective of this study was to determine whether a second injection of gonadotropin-releasing hormone (GnRH) at 54-h timed AI (TAI) of a CO-Synch/CIDR (controlled internal device release) protocol improves fertility in replacement beef heifers. Heifers (n = 375) at three locations (Colorado, Wyoming, and South Dakota) were stratified by BW within body condition score (BCS) and randomly allotted to one of two treatments. All heifers received 100 µg of GnRH and a CIDR insert (d −7), followed by CIDR removal and 25 mg of prostaglandin F₂α (PG; d 0). On d 2, control and treatment heifers underwent TAI at 54 h post-PG, and treatment heifers received a second 100-µg injection of GnRH. Blood samples were collected from all heifers at Colorado and Wyoming (d −17 and −7) to determine pubertal status. Ultrasonography was used to determine ovulation rate after TAI from a subsample of heifers (Colorado, n = 19; Wyoming, n = 49). No treatment × location interaction (P > 0.10) occurred and pooled TAI pregnancy rates were similar (P > 0.01) for control (46%) vs treatment (55%) heifers. Pubertal rates were greater (P < 0.01) for heifers at Colorado (97.4%) than for heifers at Wyoming (46.4%); however, TAI pregnancy rates were similar (P > 0.10) for pubertal and prepubertal heifers. Ovulation rates tended to be different (P = 0.10) for treatment (81.3%) than for control (62.5%) heifers. We conclude that the second injection of GnRH at TAI in the CO-Synch/CIDR protocol does not increase pregnancy rates to TAI in beef heifers, but that it may be economically viable and may guard against reduced fertility.

(Key Words: Beef Heifers, Ovulation Synchronization, Fixed-Time Artificial Insemination, Controlled Internal Device Release.)

Introduction

Approaches to estrous synchronization in beef heifers have included the use of melengestrol acetate, gonadotropin-releasing hormone (GnRH), prostaglandin F₂α, (PG), and combinations of these. Pregnancy rates from fixed-time AI (TAI) have varied widely because of day of cycle when synchronization begins, number of heifers ovulating (Ovsynch; Pursley et al., 1994; Moreira et al., 2000), and
time of ovulation (GnRH/PG/GnRH; Pursley et al., 1995). Estrous synchronization protocols that involve GnRH/PG and administration of a second injection of GnRH at 48-h TAI (CO-Synch; Geary and Whittner, 1998) improved ovulation synchronization and fertility in beef cows (Thompson et al., 1999) but resulted in somewhat lesser fertility in beef heifers undergoing TAI at 48 h when compared with insemination following a standing estrus (Schmitt et al., 1994). Pregnancy rates from a GnRH/PG protocol increased when heifers received GnRH at 54-h TAI compared with no GnRH administered at TAI (Twagiramungu et al., 1995b). Twagiramungu et al. (1995a) reported that emergence of a new ovarian follicular wave occurs when either a large follicle continues its regression by atresia, GnRH administration causes ovulation, or a new wave emerges spontaneously before PG-induced luteolysis. A dominant follicle from the new ovarian follicular wave then ovulates following PG-induced luteolysis 24 to 32 h after the second injection of GnRH (Pursley et al., 1995). Addition of a controlled internal devise release (CIDR) insert to the GnRH/PG protocol improved fertility in beef heifers (Mapletoft et al., 2003); a majority of heifers expressed estrus between 48 and 72 h after PG (Schmitt et al., 1996; Martinez et al., 2002; Richardson et al., 2002). Also, increased progesterone concentrations decrease luteinizing hormone (LH) pulse frequency, allowing ovulation of a dominant follicle following its decline (Anderson et al., 1996). By administering a CIDR insert with a CO-Synch protocol, the value of a second GnRH injection at TAI for improving fertility in beef heifers is unknown. The objectives of this study were to determine whether the second injection of GnRH at 54-h TAI of the CO-Synch/CIDR protocol improves fertility in replacement beef heifers.

Materials and Methods

Experimental Design. Nulliparous crossbred beef heifers from a cooper-
assay CV for serum samples were 12.9 and 9.6%, respectively, across two assays.

**Statistical Analysis.** Preliminary analysis of treatment, location, technician, and all possible interactions on TAI pregnancy rates revealed no significant differences in pregnancy rates to TAI for technician or technician × treatment interactions; therefore, AI technician was not included in the final analysis. Response variables included estrous cycling status, final pregnancy rate (Colorado and Wyoming), and TAI pregnancy rate. Fixed effects included in all models were treatment and location. Covariates included BW and BCS. For TAI pregnancy data, estrous cycling status was included as an additional main effect for Colorado and Wyoming data only. All possible two-way (estrous cycling status) and three-way (TAI pregnancy rate) interactions were modeled for each outcome. Statistical procedures were appropriate to binary observations using the Proc GENMOD procedure in SAS® (SAS Inst., Inc., Cary, NC). A sire effect was also included in all models as a random effect. Significance of main effects was determined using Chi-square at P<0.05.

Data collected on incidence of ovulation for heifers at the Colorado and Wyoming locations were analyzed using Proc GLM in SAS. Fixed effects included treatment, location, and treatment × location interaction on incidence of ovulation; significance was determined at P<0.05.

**Results and Discussion**

Timed AI pregnancy rates for the two treatment groups for all three locations combined are summarized in Figure 1. Final breeding season pregnancy rates for heifers at Colorado (97.4%; 38 of 39) and Wyoming (89.1%; 106 of 119) were not different (P>0.10; data not shown), and final breeding season pregnancy rates were not determined at South Dakota because of management restrictions. The CIDR retention rate for all heifers at all locations was 100%. No treatment × location interaction occurred (P>0.10) and overall TAI pregnancy rates did not differ (P>0.10) among heifers at Colorado (53.9%), South Dakota (47.4%), and Wyoming (55.2%).

Pregnancy rates to TAI were not different (P>0.10) for heifers in the control (46.2%) vs treatment (55.0%) group, respectively. Twagiramungu et al. (1995b) reported greater pregnancy rates when GnRH was administered at 54 h TAI compared with no GnRH given at TAI in beef heifers synchronized with a GnRH/PMSG protocol. However, a CIDR insert was not incorporated into the GnRH/PMSG protocol. With application of a CIDR insert in a GnRH/PMSG protocol, a majority of beef heifers expressed estrus between 48 and 60 h after PG and CIDR removal (Schmitt et al., 1996; Martinez et al., 2002), thus improving fertility of those heifers synchronized. If the majority of heifers in the present study would have expressed estrus 48 to 60 h after PG, an endogenous LH surge would have occurred 3 to 7 h later, followed by ovulation occurring 25 to 27 h later (Hendricks et al., 1970; Grier et al., 1991). If such were the case, administering a second injection of GnRH at 54-h TAI would have occurred during the natural endogenous LH surge and may not have improved fertility. Perhaps induced ovulation at 54 h among treatment heifers that had not yet exhibited estrus resulted in a premature ovulation of smaller follicles with lesser pregnancy rates (Perry et al., 2005) than delayed ovulation and fertilization by aged spermatozoa, as may occur among some control heifers.

Overall, 97.4 and 46.4% of Colorado and Wyoming heifers, respectively, were pubertal (P<0.01; Table 1). No differences were observed (P>0.10) in the proportion of heifers cycling between control (54.9%) and treatment (57.3%) groups. The proportion of pubertal and prepubertal heifers pregnant to TAI did not differ (P>0.10) between the control (53.1 and 54.5%) and treatment (57.5 and 54.3%) groups, respectively (Table 1). These results coincide with reported advantages of progestogens administered to cattle not cycling at the beginning of the breeding season (Odde, 1990). Increased progesterone...
TABLE 1. Estrous cycling and timed AI pregnancy rates (PR) of heifers bred at the Colorado and Wyoming locations for each treatment group and treatment groups combineda.

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>Treatment</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage estrous cyclingb</td>
<td>19/19 (100)</td>
<td>19/20 (95)</td>
<td>38/39 (97.4)c</td>
</tr>
<tr>
<td>CO</td>
<td>30/63 (47.6)</td>
<td>28/62 (45.2)</td>
<td>58/125 (46.4)c</td>
</tr>
<tr>
<td>WY</td>
<td>45/82 (59.8)</td>
<td>47/82 (57.3)</td>
<td>96/164 (58.5)</td>
</tr>
<tr>
<td>Overall</td>
<td>65/111 (58.3)</td>
<td>66/112 (57.8)</td>
<td>131/223 (58.5)</td>
</tr>
<tr>
<td>PR, estrous cyclingc</td>
<td>10/19 (52.6)</td>
<td>11/19 (57.9)</td>
<td>21/38 (55.3)</td>
</tr>
<tr>
<td>CO</td>
<td>16/30 (53.3)</td>
<td>16/28 (57.1)</td>
<td>32/58 (55.2)</td>
</tr>
<tr>
<td>WY</td>
<td>26/49 (53.1)</td>
<td>27/47 (57.5)</td>
<td>53/96 (55.2)</td>
</tr>
<tr>
<td>Overall</td>
<td>42/79 (53.1)</td>
<td>43/75 (56.8)</td>
<td>85/154 (55.2)</td>
</tr>
<tr>
<td>PR, prepubertal</td>
<td>0/0 (0.0)</td>
<td>0/1 (0.0)</td>
<td>0/1 (0.0)</td>
</tr>
<tr>
<td>CO</td>
<td>18/33 (54.5)</td>
<td>19/34 (55.9)</td>
<td>37/67 (55.2)</td>
</tr>
<tr>
<td>WY</td>
<td>18/33 (54.5)</td>
<td>19/35 (53.4)</td>
<td>37/68 (54.4)</td>
</tr>
</tbody>
</table>

aControl heifers were synchronized with 100 µg of gonadotropin-releasing hormone (GnRH) on d −7 plus a controlled internal device release (CIDR) insert for 7 d and 25 mg of prostaglandin F2α (PG) on d 0 with CIDR removal, followed by timed AI (TAI) 54 h after PG. Treatment heifers were synchronized with same protocol as control heifers, plus incorporation of a second injection of GnRH (100 µg) at 54 h TAI.
bPercentage of heifers cycling at both Colorado (CO) and Wyoming (WY) locations based on progesterone concentrations of blood serum ≥1 ng/mL for either d −17 or −7. Estrous cycling data were not available for heifers at the South Dakota location.
cPregnancy rates for heifers determined to be pubertal and prepubertal.

* RAW = random effects analysis.

Implications

Administration of a second injection of GnRH at 54-h TAI in a Co-
Synch/CIDR protocol did not statistically improve pregnancy rates in replacement beef heifers, and heifer puberty status does not appear to affect pregnancy rates to this protocol. The economic value of incorporating a second injection of GnRH at 54-h TAI remains questionable. Addition of the second GnRH injection at TAI may be an option to decrease the risk of low fertility. Depending on the cost of inputs, fertility of semen, and ultimately the cost of a low pregnancy rate outcome, it may be considered as a means of reducing risk. These results warrant further investigation to determine factors leading to the cause of pregnancy rate variations observed in response to GnRH administration at TAI.

Acknowledgments

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Figure 2. Effects of treatment (shaded bars) and control (open bars) protocols on ovulation rates in heifers scanned by transrectal ultrasonography at controlled internal device release (CIDR) removal (d 0) and at 48 h (d 2) and 88 h after CIDR removal (d 4) from Colorado and Wyoming locations only. The CIDR/CO-Synch protocol consisted of 100 µg of gonadotropin-releasing hormone (GnRH) on d −7 given concurrently with a CIDR insert, CIDR removal, and 25 mg of prostaglandin F2α on d 0. On d 2, control and treatment heifers are TAI at 54 h, and treatment heifers receive a second injection (100 µg) of GnRH at TAI. Raw mean percentages for ovulation rates between overall control and treatment heifers without a common letter (a) differ (P<0.05).

Literature Cited


