ABSTRACT: Eighty-one prepubertal beef heifers were used to evaluate effects of used controlled internal drug release (CIDR) insert heating methods on concentrations of progesterone after CIDR insert reinsertion. Heifers were stratified by weight and birth date and then assigned to receive a new CIDR insert (New; \( n = 10 \)) or 1 of 8 used (7 d prior use) CIDR insert treatments: 1) no processing (Used; \( n = 10 \)), 2) autoclaved (Autoclaved; \( n = 8 \)), 3) processed in dishwasher (Dishwasher; \( n = 8 \)), 4) processed in microwave for 30 s (Microwave; \( n = 10 \)), 5) processed in toaster oven (Oven; \( n = 9 \)), 6) processed in clothes dryer (Dryer; \( n = 10 \)), 7) processed in boiling water (Boiled; \( n = 8 \)), or 8) stored outdoors for 60 d (Outside; \( n = 8 \)). Used CIDR inserts were processed at 121°C for 30 min for autoclaved and oven treatments, at 121°C for boiled treatment, and for 30 min for dryer and dishwasher treatments. Blood samples were collected on d –10, immediately before CIDR insert insertion (d 0), 3 h after CIDR insert insertion (3 h), daily while CIDR insert was in place (d 1 to 11), and 24 h after CIDR insert removal (d 12) for analysis of concentrations of progesterone. Subjective color scores (1 = bright white to 5 = completely stained yellow/red) were assigned to each CIDR insert after d 11.

A treatment × time interaction (\( P < 0.0001 \)) was present for concentrations of progesterone. Concentrations of progesterone were similar (\( P > 0.10 \)) for heifers receiving a used CIDR insert compared with heifers receiving CIDR inserts processed in a dishwasher, microwave, oven, dryer, or boiling water (collectively reported as “Processed”). However, heifers receiving autoclaved CIDR inserts had greater (\( P < 0.05 \)) concentrations of progesterone from h 3 to d 3 but similar (\( P > 0.10 \)) concentrations of progesterone from d 4 to d 11 compared with heifers receiving used or processed CIDR inserts. From d 1 to 11 heifers receiving outside CIDR inserts had decreased (\( P < 0.05 \)) concentrations of progesterone compared with all other treatments. Heifers receiving autoclaved CIDR inserts had greater (\( P < 0.05 \)) concentrations of progesterone compared with all other treatments at 3 h and 1 d, whereas heifers receiving new CIDR inserts had greater (\( P < 0.05 \)) concentrations of progesterone from d 6 to 11 compared with all other treatments. Outside CIDR inserts were more discolored (\( P < 0.001 \)) compared with all other treatments. Processing used CIDR inserts with a dishwasher, microwave, oven, clothes dryer, boiling water, or full environmental exposure did not result in a pattern of concentrations of progesterone similar to that of autoclaved or new CIDR inserts.

Key words: heifers, progesterone, reused controlled internal drug release insert


INTRODUCTION

Controlled internal drug release (CIDR) inserts (EAZI-Breed CIDR insert containing 1.38 g of progesterone; Zoetis Inc., Florham Park, NJ) are an integral part of many estrus synchronization protocols. A CIDR insert is a silicone-coated nylon insert infused with progesterone that is placed in the vagina of female cattle. The progesterone diffuses through the walls of the vagina, enters circulation, and clears from the system on CIDR insert removal (Macmillan and Peterson, 1993). Residual progesterone remains in a CIDR insert after a 7-d period of use (Rathbone et al., 2002), and a portion of producers have addressed concerns over cost of synchronization protocols (NAHMS, 2009) by attempting to reuse CIDR inserts.
Autoclaves use a combination of heat, steam, and pressure to sterilize items and are common in laboratory settings. Ovariectomized beef cows receiving an autoclaved used CIDR insert had greater concentrations of progesterone at 3, 8, and 24 h after CIDR insert insertion compared with cows receiving a new or used and not autoclaved CIDR insert (Zuluaga and Williams, 2008). In addition, beef heifers receiving used CIDR inserts that were autoclaved had pregnancy rates similar to those of heifers receiving a new CIDR insert (Colazo et al., 2004).

The applicability of research evaluating autoclaving used CIDR inserts, however, is limited as autoclaves are not commonplace on commercial beef operations. In lieu of autoclave access, producers have adopted other techniques to heat and “sterilize” CIDR inserts including using a dishwasher, microwave, oven, and clothes dryer and exposing CIDR inserts to sunlight for extended periods of time. The effects of these on-farm CIDR insert heating and sterilization techniques on the physiological response of cattle subsequently receiving the CIDR inserts is unknown. Therefore, the objective of this experiment was to determine the concentrations of progesterone in prepubertal beef heifers receiving CIDR inserts processed with on-farm heating and sterilization techniques.

MATERIALS AND METHODS

Management of all cattle for the duration of the experiment was in accordance with guidelines set forth in the Guide for the Care and Use of Agricultural Animals in Agriculture Research and Teaching (FASS, 1999) and procedures were approved by the North Dakota State University’s Institutional Animal Care and Use committee.

Blood samples collected on d –10 and immediately prior to (d 0) and 1 d after treatment conclusion were used to determine whether beef heifers were cyclic before or during the time of treatment administration. When 1 or more of the 3 blood samples contained concentrations of progesterone ≥1 ng/mL, the heifer was considered to be pubertal and was not eligible to be included in the study dataset (Perry et al., 1991). The remaining prepubertal heifers (n = 81) were used to evaluate the effects of CIDR insert handling method on circulating concentrations of progesterone after insertion of a reused CIDR insert.

All used CIDR inserts had been inserted into crossbred beef cows to facilitate synchronization of estrus for a period of 7 d. After removal from the cows, all CIDR inserts were washed in warm water with a brush to remove foreign matter, rinsed in chlorhexidine solution (Nolvasan; Zoetis Inc.), and allowed to air dry. Once dry, CIDR inserts were stored in a plastic sample bag at room temperature for 6 mo before applying experimental procedures.

Heifers were stratified by weight and birth date and then assigned to receive a CIDR insert processed using 1 of 9 methods: 1) new CIDR insert with no previous use and directly from factory package (New; n = 10), 2) used CIDR insert with no heat treatment (Used; n = 10), 3) Used CIDR insert autoclaved at 121°C and 137.9 kPa for 30 min (Autoclaved; n = 8), 4) Used CIDR insert washed in a household dishwasher set on hot water setting for the duration of a normal 30 min wash cycle (Dishwasher; n = 8), 5) Used CIDR insert heated in a microwave for 30 s (Microwave; n = 10), 6) Used CIDR insert heated in a household oven set at 121°C (Oven; n = 9), 7) Used CIDR insert heated in a clothes dryer set on high for 30 min (Dryer; n = 10), 8) Used CIDR insert heated via insertion in water with a temperature of 121°C for 30 min (Boiled; n = 8), and 9) Used CIDR insert stored outdoors with direct exposure to environmental conditions (sun, wind, rain, etc.) for a period of 60 d (Outside; n = 8). Environmental conditions during the period of outdoor exposure for CIDR inserts in the Outside treatment included a mean maximum temperature of 18.0 ± 7.0°C and minimum temperature of 5.2 ± 6.0°C, and rainfall was present on 7 of 60 d during storage for a total accumulation of 24.4 mm.

Blood samples were collected into heparinized Vacutainer tubes (Becton, Dickinson and Company, Franklin Lakes, NJ) via jugular venipuncture on d –10, immediately before CIDR insert insertion (d 0), 3 h after CIDR insert insertion (d 3), daily for the duration of the time CIDR inserts were in place (d 1 to 11), and 24 h after CIDR insert removal (d 12). Blood was immediately placed on ice for 1 h, centrifuged at 1,500 × g for 15 min at 4°C, placed in sample vials, and stored at −20°C until analysis. Samples were analyzed for concentrations of progesterone via RIA (Coat-A-Count Progesterone; Siemens Healthcare Diagnostics, Los Angeles, CA). The assay kit was validated for bovine serum (Kirby et al., 1997) using an assay volume of 100 μL. A standard curve was calculated using tubes containing 0.01, 0.025, 0.05, 0.2, 0.5, 1, 2, and 4 ng/tube. Assay sensitivity for a 100-μL sample was 0.1 ng/mL. Intra- and interassay CV were 3.7 and 3.2%, respectively.

Upon removal from heifers, each CIDR insert was rinsed, placed individually into plastic samples bag, and stored at −20°C until subjective evaluation of color score. A subjective color score was developed to evaluate the degree of discoloration in each CIDR insert after being inserted for 11 d. A color score of 1 was bright white, similar to a new CIDR insert, a color score of 3 had approximately 50% of the CIDR insert body stained with a yellow/red color, and a color score of 5 was completely stained with a yellow/red color embedded through the entire CIDR insert.

Effects of treatment on concentrations of progesterone were analyzed using the mixed model procedure of
SAS (SAS Inst. Inc., Cary, NC) for repeated measures in time. Day was the repeated factor and heifer was the subject. The model included the effects of treatment, day, and their interaction. The unstructured covariance structure produced the smallest Schwarz’s Bayesian criterion was used in the analysis.

Subjective color score of each CIDR insert after d 11 of the study was evaluated using the GLM procedure of SAS. The model included the effect of treatment. Since no differences were found for either concentrations of progesterone or subjective CIDR insert color score among heifers receiving a CIDR insert processed in a dishwasher, microwave, oven, dryer, or boiling water results from these treatments were combined and collectively reported as “Processed.” Effects were determined to be significant at P < 0.05.

RESULTS

At the beginning of the study heifers had a mean weight of 276 kg (ranging from 185 to 353 kg) and a mean age of 273 d (ranging from 219 to 304 d).

Concentrations of progesterone were affected by a treatment × day interaction (P < 0.0001; Fig. 1). No differences (P > 0.10) in concentrations of progesterone were present for heifers receiving CIDR insert processed in a dishwasher, microwave, oven, dryer, or boiling water and therefore results from these respective treatments were combined and collectively reported as “Processed.” The remaining results will we discussed as comparisons among the treatments throughout the study.

Concentrations of progesterone in heifers receiving a New CIDR insert were greater (P < 0.05) on each day while CIDR inserts were in place compared with heifers receiving a Used or Processed CIDR insert or receiving a CIDR insert that remained Outside for 60 d (Fig. 1). Heifers receiving Autoclaved CIDR inserts, however, had greater (P < 0.05) concentrations of progesterone compared with all other treatments at 3 and 24 h (1 d) after CIDR insert insertion (Fig. 1). From 2 to 4 d after CIDR insert insertion, concentrations of progesterone were similar among heifers receiving an Autoclaved or New CIDR insert. On d 5 after insertion, concentrations of progesterone tended (P = 0.06) to be greater for heifers receiving a New CIDR insert compared with an Autoclaved CIDR insert, and from d 6 to CIDR insert removal (d 11) concentrations of progesterone were greater (P < 0.05) for heifers that received a New CIDR insert compared with all other treatments.

The Processed group included heifers that received a CIDR insert processed in a dishwasher, microwave, oven, dryer, or boiling water and no differences (P > 0.10) were present among any of the processing methods on any day of the study. In addition, no differences (P > 0.10) in concentrations of progesterone were present on any day of the study among heifers receiving a Used or Processed CIDR insert.

Heifers receiving Autoclaved CIDR inserts had greater (P < 0.05) concentrations of progesterone compared with heifers receiving Used and Processed CIDR inserts from 3 h to 3 d after CIDR insert insertion. Concentrations of progesterone were similar (P > 0.10) among heifers receiving Autoclaved, Used, and Processed CIDR inserts, however, from d 4 through the end of the study.

Concentrations of progesterone were greater (P < 0.05) from 3 h after CIDR insert insertion until CIDR insert removal in heifers receiving Autoclaved, New, and Processed CIDR inserts compared with heifers that received CIDR inserts that remained Outside for 60 d. Whereas concentrations of progesterone at 3 h after CIDR insert insertion only tended to be greater (P = 0.09) for heifers receiving a Used CIDR insert compared with those receiving an Outside CIDR insert, concentrations of progesterone were greater (P < 0.05) on each remaining day of CIDR insert insertion (d 1 to d 11) for heifers receiving a Used CIDR insert compared with those receiving an Outside CIDR insert.

When CIDR inserts were inserted into heifers, it was noted that the tails of CIDR inserts that remained Outside for a period of 60 d were brittle and often broke when handled. In addition, color score evaluation of CIDR inserts after being inserted for 11 d revealed that CIDR inserts that remained Outside for 60 d before insertion were more discolored (P < 0.001) compared with all other treatments (Table 1).
As we were optimizing the length of time CIDR inserts were avoided when new inserts are used. In the current study, heifers receiving a new CIDR insert compared with Used CIDR inserts, but New CIDR inserts resulted in sustained concentrations of progesterone similar to that of Autoclaved CIDR inserts. In addition, mean concentrations of progesterone on d 10 and 11 after CIDR insert insertion in heifers receiving Outside CIDR inserts were below 1.0 ng/mL, which may indicate that perhaps the ability of Outside CIDR inserts to inhibit estrus was diminished. The combination of sunlight and moisture was found to be detrimental to integrity (measured via cross-linking density) of different types of silicone sealants (Keshavaraj and Tock, 1994). In addition, silicone facial elastomers changed color after outdoor exposure (Polyzois, 1999). Although specific silicone used for sealants and facial prostheses is different than those used to coat a CIDR insert, they appear to be similarly sensitive to sunlight. In addition, exposure to sunlight may have impacted hardness of the silicone. As shore hardness of silicone increased, the rate of progesterone release decreases (Rathbone et al., 2002). Perhaps the porosity of the silicone covering on the CIDR inserts was also changed after outdoor exposure in the current study. Those CIDR inserts stored outside were more discolored compared with all other treatments, which may be a result of absorption of vaginal secretions into the silicone covering the CIDR insert.

In summary, processing used CIDR inserts with a dishwasher, microwave, oven, clothes dryer, or boiling water did not result in a pattern of concentrations of progesterone similar to that of Autoclaved CIDR inserts. Autoclaved CIDR inserts did result in greater concentrations of progesterone at 3 and 24 h after insertion compared with New CIDR inserts, but New CIDR inserts resulted in sustained concentrations of progesterone that were greater than all other treatments from Day 5 after insertion until the time of CIDR insert removal. Leaving CIDR inserts outside with full environmental exposure for 60 d resulted in discoloration and concentrations of progesterone that were lower than all other treatments and mean values that were below 1.0 ng/mL by the conclusion of the experiment.

### DISCUSSION

The “on-farm” heating and sterilization techniques (dishwasher, microwave, oven, clothes dryer, or boiling water) were not effective at increasing the progesterone release over that of a Used CIDR insert. On-farm processing beyond general cleaning and disinfecting, although at the same temperature and time, are not equivalent to processing with the combination of temperature, pressure, and steam created in an autoclave environment.

The pattern of heifers in the current study having greater concentrations of progesterone soon after insertion of an Autoclaved CIDR insert compared with a new CIDR insert has been reported previously in ovariectomized cows (Zuluaga and Williams, 2008). Similar to the current study, the main differences among ovariectomized cows receiving a new or autoclaved CIDR insert observed by Zuluaga and Williams (2008) occurred soon after CIDR insert insertion, and concentrations of progesterone in cows receiving the Autoclaved CIDR insert soon return to levels similar to those found in cows receiving a new CIDR insert. When ovariectomized beef heifers or cows received a new or used CIDR insert, concentrations of progesterone were greater over the duration of time the CIDR insert was inserted for females receiving a new CIDR insert compared with Used CIDR insert (Long et al., 2009; Gunn et al., 2011). In the current study, heifers receiving New CIDR inserts had the greatest sustained concentration of progesterone over the duration of the study. In addition, any potential issues that may arise with inserting contaminated used CIDR inserts are avoided when new inserts are used.

Although microwave ovens are widely used as means to heat and prepare food, not all materials are affected by electromagnetic radiation (Saltiel and Datta, 1999). As we were optimizing the length of time CIDR inserts needed to be processed in the Microwave treatment, several unique findings were observed. The silicone coating on the CIDR insert did not change physical states because silicone is transparent to electromagnetic radiation (Al-Hartomy et al., 2012). However, the nylon spine underneath the silicone coating changes from a solid to a semiliquid state with increased time in the microwave. Regardless of how much heat was transferred from the nylon spine to the silicone surrounding it, processing CIDR inserts in a microwave was ineffective at elevating concentrations of progesterone similar to an autoclave.

Exposing used CIDR inserts outside to full sun and other environmental elements resulted in reduced concentrations of progesterone compared with all other treatments over the course of the study. In addition, mean concentrations of progesterone on d 10 and 11 after CIDR insert insertion in heifers receiving Outside CIDR inserts were below 1.0 ng/mL, which may indicate that perhaps the ability of Outside CIDR inserts to inhibit estrus was diminished. The combination of sunlight and moisture was found to be detrimental to integrity (measured via cross-linking density) of different types of silicone sealants (Keshavaraj and Tock, 1994). In addition, silicone facial elastomers changed color after outdoor exposure (Polyzois, 1999). Although specific silicone used for sealants and facial prostheses is different than those used to coat a CIDR insert, they appear to be similarly sensitive to sunlight. In addition, exposure to sunlight may have impacted hardness of the silicone. As shore hardness of silicone increased, the rate of progesterone release decreases (Rathbone et al., 2002). Perhaps the porosity of the silicone covering on the CIDR inserts was also changed after outdoor exposure in the current study. Those CIDR inserts stored outside were more discolored compared with all other treatments, which may be a result of absorption of vaginal secretions into the silicone covering the CIDR insert.

In summary, processing used CIDR inserts with a dishwasher, microwave, oven, clothes dryer, or boiling water did not result in a pattern of concentrations of progesterone similar to that of Autoclaved CIDR inserts. Autoclaved CIDR inserts did result in greater concentrations of progesterone at 3 and 24 h after insertion compared with New CIDR inserts, but New CIDR inserts resulted in sustained concentrations of progesterone that were greater than all other treatments from Day 5 after insertion until the time of CIDR insert removal. Leaving CIDR inserts outside with full environmental exposure for 60 d resulted in discoloration and concentrations of progesterone that were lower than all other treatments and mean values that were below 1.0 ng/mL by the conclusion of the experiment.

### Table 1. Color scores of reused controlled internal drug release (CIDR) inserts processed with different heat-treating methods

<table>
<thead>
<tr>
<th>Treatment1</th>
<th>Color score2</th>
</tr>
</thead>
<tbody>
<tr>
<td>New</td>
<td>2.1 ± 0.30x</td>
</tr>
<tr>
<td>Autoclaved</td>
<td>2.7 ± 0.33x</td>
</tr>
<tr>
<td>Used</td>
<td>2.4 ± 0.33x</td>
</tr>
<tr>
<td>Processed</td>
<td>2.3 ± 0.14x</td>
</tr>
<tr>
<td>Outside</td>
<td>5.0 ± 0.34x</td>
</tr>
</tbody>
</table>

x,y Means within column differ (P < 0.05).

1 Heifers received a new CIDR (New), an unprocessed used CIDR (Used), or a used CIDR that was Autoclaved (Autoclaved), processed in a dishwasher, microwave, oven, dryer, or boiling water (Processed), or exposed to full environment for 60 d (Outside).

2 Scale of 1 to 5 (1 = entire CIDR insert white and unstained and 5 = entire CIDR stained red/yellow).
LITERATURE CITED


