

# Progesterone release and incidence of bacteria following disinfection and reuse of controlled internal drug release devices (CIDRs)

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## Abstract

Controlled internal drug release (CIDR) devices have been reused in cattle estrous synchronization protocols. However, the effectiveness of the CIDR and incidence of vaginal bacteria secondary to reuse remains unclear. Therefore, 2 experiments were conducted to evaluate plasma progesterone and vaginal bacteria utilizing used CIDRs disinfected prior to reuse. In Exp. 1, cows received a new CIDR (CONT) or a used CIDR disinfected by 1 of 3 methods: autoclaved (AUTO), dishwasher (DISH), or chlorhexidine (CHLO). No difference was detected in plasma progesterone ( $P = 0.24$ ) concentration between treatment groups. Daily progesterone concentrations differed during CIDR treatment ( $P < 0.001$ ) on d 0, 3, and 7. In Exp. 2, vaginal mucous samples were collected pre- and post-synchronization. Cows received CONT, AUTO, or CHLO treated CIDRs. Bacterial loads in vaginal mucus cultured on TSA blood agar did not differ by treatment ( $P = 0.29$ ) nor by day ( $P = 0.69$ ). CHLO-treated CIDRs had higher bacterial gram-negative colony counts than those disinfected with AUTO or CONT ( $P = 0.0035$ ), and bacterial load was higher on d 7 than d 0 ( $P = 0.0025$ ) for vaginal mucous samples cultured on EMB agar. Based on the results of these studies, twice-used treated CIDRs do not reduce progesterone plasma concentrations, however, risk of bacterial contamination of the vagina must be considered.

**Key words:** progesterone, bacteria, reuse, CIDR

## Introduction

The use of artificial insemination in cattle has been one of the most influential tools to increase genetic improvement in the cattle industry. By utilizing a synchronization protocol for artificial insemination, the breeding season and

calving season have potential to be shortened.<sup>13</sup> Development of the controlled internal drug release (CIDR) device has revolutionized cattle estrous synchronization and has become a crucial part of many synchronization protocols available to producers.<sup>5</sup> At the same time, CIDRs account for the greatest percent of the overall cost associated with synchronization protocols;<sup>17</sup> therefore, the ability to reuse CIDRs could dramatically reduce producer costs. Rathbone et al<sup>15</sup> reported that following a 7-d period of use, a CIDR still retained sufficient progesterone for another 7-d period. Each insert initially contains 1.38 grams of progesterone, and 0.72 g of residual progesterone remained after 7 d of use.<sup>15</sup> This provides approximately half the initial progesterone, allowing potential for reuse and decreasing cost of synchronization.<sup>1</sup>

Caution must be used to prevent disease transmission when reusing CIDRs. In a 2006 report, microbial swabs of CIDR inserts after removal yielded intense growth of bacteria, with the predominant species being *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Trueperella pyogenes* (*T. pyogenes*), formerly known as *Arcanobacterium pyogenes*.<sup>14</sup> These bacterial species may explain increases in vaginal discharge observed in cows following insertion of new CIDRs.<sup>4,7,19</sup> LeBlanc and colleagues,<sup>10</sup> in combination with Dubuc and others,<sup>6</sup> reported that mucopurulent or purulent vaginal discharge is indicative of uterine disease and is associated with decreased fertility.

The potential to reuse CIDRs could provide economic cost savings to the producer, but only if an appropriate sanitation technique can be identified that reduces risk of bacterial infections, while not decreasing progesterone in circulation. Thus, the following experiments were conducted to 1) identify potential sanitation techniques that could be implemented in a production setting and 2) determine the efficacy of sanitation techniques at preventing the growth of bacteria following the reuse of a CIDR.

## Material and Methods

Experiments were conducted under the approval of the Tarleton State University Institutional Animal Care and Use Committee (IACUC) protocol number 10-007-2016. All cattle were housed in Stephenville, TX, as part of the Tarleton State University cow herd.

### Exp. 1

Twenty-nine Angus-cross (*Bos taurus*) females were available for Rep. 1, with an average age of 3.9 years. Rep. 1 was conducted from 28 November through 07 December 2016. Forty-six Angus-cross females with an average age of 3.9 years were available for Rep. 2. Rep. 2 was conducted from 20 March through 29 March 2017. Cows were housed in pastures with *ad libitum* access to Coastal bermudagrass (*Cynodon dactylon* [L.] Pers.) hay and water in Rep. 1. Cows in Rep. 2 were housed in pastures with *ad libitum* access to native rangeland forage and water.

Animals were blocked by age and randomly assigned to 1 of 4 treatment groups: 1) new CIDR<sup>a</sup> (CONT), 2) reused CIDR sterilized in an autoclave (AUTO), 3) reused CIDR processed in a dishwasher (DISH), and 4) reused CIDR sanitized with chlorhexidine<sup>b</sup> (CHLO). The sample sizes for Rep. 1 and Rep. 2 was 7 and 12 in the CONT group, 7 and 11 in the CHLO group, 7 and 12 in the DISH group, and 8 and 11 in the AUTO group, respectively. The reused CIDRs were previously used once in beef cows for 7 d. After removal, all CIDRs were washed with water at room temperature (73.4°F; 23°C) and scrubbed with a plastic bristled brush to remove foreign matter. In the CHLO treatment, inserts were soaked in 1.3% chlorhexidine/water solution at 122°F (50°C) for 2 h, removed, rinsed with water at room temperature, and allowed to air dry. The CIDRs in the DISH group were placed in a dishwasher and processed through one hot 158°F (70°C) cycle (on high) using approximately 3.38 oz (100 ml) of chlorhexidine solution as the detergent (placed in the detergent dispenser in the dishwasher). Once the dishwasher cycle was complete, CIDRs were removed and allowed to air dry. Vaginal inserts in the AUTO group were individually packaged in self-sealing sterilization pouches and autoclaved<sup>c</sup> at 249.8°F (121°C) and 724 mmHg of pressure for 20 min.<sup>22</sup> CIDRs in CHLO and DISH groups were allowed to air dry and were placed in individual zipper-sealed bags for storage before use. CIDRs in the AUTO group were stored in individual sterilization pouches. All CIDRs were stored at room temperature for approximately 6 mo for Rep. 1 and approximately 11 mo for Rep. 2.

Blood samples were collected via coccygeal venipuncture using 0.3 oz (10 mL) heparinized vacutainer tubes and 20-gauge needles at 0, 72, and 168 ± 2 h after CIDR insertion. Blood was placed on ice in a cooler and transported immediately to the lab after collection, and centrifuged<sup>d</sup> at 39.2°F (4°C) at 2000 RPM for 20 min. Plasma was separated using disposable transfer pipettes into 0.03 oz (1 mL) microcentrifuge tubes and stored at 68°F (20°C) until analysis. Samples

were analyzed for concentrations of progesterone using a chemiluminescence assay automated system.<sup>e,2</sup>

Cattle were synchronized using a modified version of the 7-d Co-Synch + CIDR<sup>9</sup> protocol. Cattle received CIDR treatment for 7 d, but unlike the traditional protocol they received a single intramuscular injection of prostaglandin F<sub>2α</sub><sup>f</sup> on d 6 prior to CIDR removal. During CIDR insert, non-sterile (no antimicrobial treatment) lubricant was applied to each CIDR. Fixed-timed artificial insemination (FTAI) occurred 52 ± 3-h post-CIDR removal in conjunction with a single intramuscular injection of gonadotropin-releasing hormone (GnRH).<sup>g</sup> Pregnancy diagnosis was performed via transrectal ultrasonography<sup>h</sup> 35 d following FTAI.

Data were analyzed using SAS Studio version 3.4<sup>i</sup> and were compared using analysis of variance for repeated measures. Between-subject effects were group, treatment, and progesterone, with time being the repeated measures effect. Alpha was set at  $P \leq 0.05$ ; differences were further analyzed using Fisher's least squares means test. The data are presented as mean ± standard error.

### Exp. 2

Thirty-six Angus-cross cows and 7 heifers were utilized, with an average age of 5.1 years. Cattle were maintained on 45 acres with *ad libitum* access to native rangeland forage and water. Animals were supplemented with 30% protein tubs with minerals, a salt block as a limiter, and provided 3 round bales of Coastal bermudagrass hay every other day. Cattle were synchronized utilizing the same protocol as in Exp. 1.

Animals were blocked by age and previous calving dates, and randomly allotted to 1 of 3 treatment groups: 1) CONT (new CIDRs which served as a control group similar to Exp. 1), 2) AUTO, and 3) CHLO. Sample size for the study consisted of 17 CONT, 12 AUTO, and 14 CHLO. All reused CIDRs were previously used once in beef cows for 7 d in May, June, or August 2017 and cleaned prior to individual disinfection treatments. Disinfection treatment procedures were applied as previously described in Exp. 1. All CIDRs were stored at room temperature for 7 mo before use.

During CIDR insert, each CIDR was lubricated with non-sterile lubrication (no antimicrobial treatment). Cattle were evaluated for standing estrus and AI performed 12 h following onset of estrus ( $n = 12$ ). Cattle that did not express estrus by 72 h after CIDR removal received FTAI ( $n = 31$ ) in conjunction with a single intramuscular injection of GnRH.<sup>f</sup> Pregnancy diagnosis was performed via transrectal ultrasonography<sup>g</sup> 35 d after FTAI.

Vaginal mucous samples were collected prior to CIDR insertion (0 h) and post-CIDR removal (168 ± 2 h) using the Metricheck<sup>®</sup> device.<sup>j</sup> Prior to insertion, the vulva exterior was cleaned with dry paper towels, and the rubber hemisphere was inserted and advanced to the anterior vagina, then raked caudally<sup>12</sup> and removed from the vagina at an approximate 30° angle to ensure fluid remained in the hemisphere. Vaginal mucus was transferred from the hemisphere to a 0.5 oz

(15 mL) conical tube via disposable transferrable pipettes and placed on ice in a cooler. Samples were immediately transported to the laboratory after collection and stored at 39.74°F (4.3°C) until culture. The stainless steel rod was disinfected in a diluted chlorhexidine/water solution between each cow and dried with a paper towel. Rubber hemispheres were removed after each individual cow was sampled, and a new hemisphere was attached to the rod prior to use in the following cow. Between collection days, rubber hemispheres were disinfected by soaking in approximately 3.9 gal (15 L) of hot water (122°F; 50°C) with roughly 6.7 oz (200 mL) of chlorhexidine solution for 2 h, removed, scrubbed with a plastic bristle brush, and rinsed with water at room temperature, and allowed to air dry before being stored in a zippered bag. Between collection days, the stainless steel rod was autoclaved in a self-sealing sterilization bag at 249.8°F (121°C) and 724mmHg of pressure for 30 min, and then stored.

Bacteria were cultured under aerobic conditions on eosin methylene blue (EMB) agar plates for gram-negative bacterial counts, and on trypticase soy agar plates with 5% sheep blood (SBA) for total bacterial counts. Prior to plating vaginal mucous samples, EMB and SBA plates, pipettes, sterile deionized water, sterile disposable plastic inoculating loop, and beakers were exposed to ultraviolet light (UV) within a biosafety hood for 10 min as an additional sterilization measure. Thereafter, the UV light was turned off and vaginal mucus samples were placed in the safety hood. Fluid samples were first vortexed for approximately 10 sec, then 100 µL of vaginal mucus was pipetted onto EMB and SBA plates and then spread with a disposable plastic inoculating loop. All plates were placed in an incubator in an aerobic environment at 98.6°F (37°C) for 18 to 24 h. Plates were removed from incubation and evaluated at room temperature. Bacteria were identified based on colony color on EMB plates to discern coliforms vs non-coliform bacteria. The presence of beta-hemolytic clearings (halos) on SBA plates was used to determine toxin-producing species. Bacterial load was determined by colony and halo numbers. Further dilution was needed for plates where colony counts were too dense and could not be identified. Dilutions were performed at 1:10, 1:100, 1:1,000, 1:10,000, and 1:100,000 ratios until a countable range was obtained on the agar plates.

Blood samples were collected via the coccygeal tail vein via a 10 mL heparinized vacutainer tube and 20-gauge needle at 0, 72, and 168 ± 2 h following CIDR insertion. Processing and analysis was performed as described in Exp. 1.

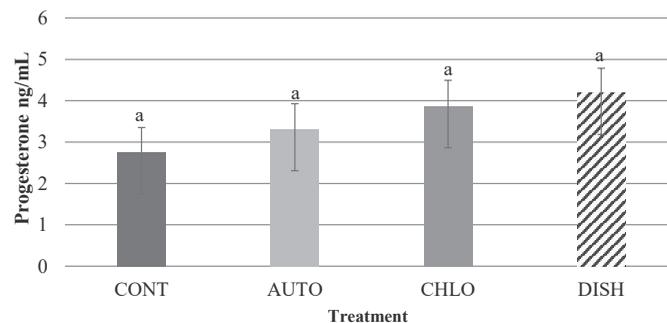
All data were analyzed using SAS version 9.4.<sup>h</sup> Multiple techniques and models were used to analyze Exp. 2 data. Regression models predicted bacterial load over progesterone concentrations and were log transformed to allow a better fit. Mixed models were used to predict mean bacterial loads based on disinfectant treatment. The dependent variable was bacterial load with treatment as the between-subject effect and time as a repeated measure. Alpha was set at  $P \leq 0.05$ ; differences were further analyzed using Fisher's least

squared means. Bacterial load data are presented as means ± standard error. Frequency of vaginal mucous scores was analyzed using the frequency procedure utilizing chi-square values to establish significant effects. Finally, mixed models were used to predict mean progesterone concentrations based on disinfectant treatment. The dependent variable was progesterone and between subject effects was treatment with time as a repeated measure. Alpha was set at  $P \leq 0.05$  and any differences were further analyzed using Fisher's least squared means. Progesterone concentration is presented as means ± standard error.

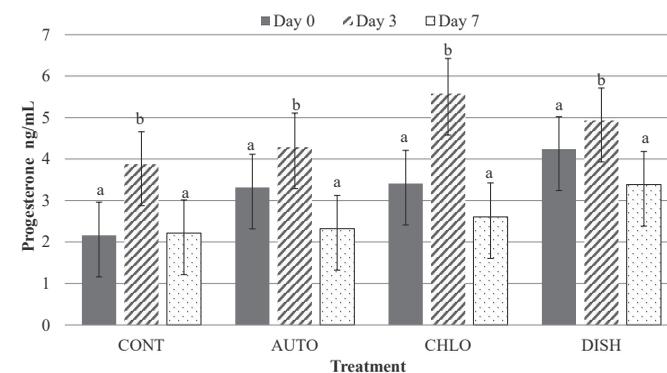
## Results

### Exp. 1

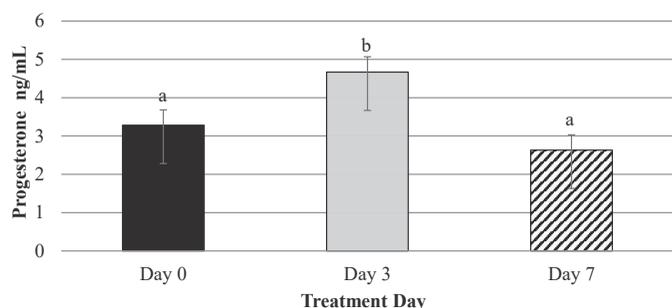
Overall plasma progesterone concentrations were not affected ( $P = 0.24$ ) by CIDR treatment (AUTO, 3.31 ± 0.62 ng/mL; CHLO, 3.86 ± 0.62 ng/mL; DISH, 4.18 ± 0.60 ng/mL) when compared to the control (CONT, 2.75 ± 0.60 ng/mL,



**Figure 1.** Overall progesterone concentrations by treatment for Control (CONT), Autoclave (AUTO), Chlorhexidine (CHLO), and Dishwasher (DISH) treated CIDR inserts. Bars with similar letters did not differ ( $P = 0.24$ ).



**Figure 2.** Plasma progesterone concentrations (ng/mL) per treatment over time. Control (CONT), Autoclave (AUTO), Chlorhexidine (CHLO), and Dishwasher (DISH) treated CIDR inserts with plasma progesterone (ng/mL) reported for d 0, 3, and 7 of synchronization protocol. Day 0 vs d 3 differed ( $P = 0.0051$ ) and d 3 vs d 7 differed ( $P < 0.0001$ ).



**Figure 3.** Overall plasma progesterone average by day. Bars with different superscripts differ ( $P < 0.001$ ).

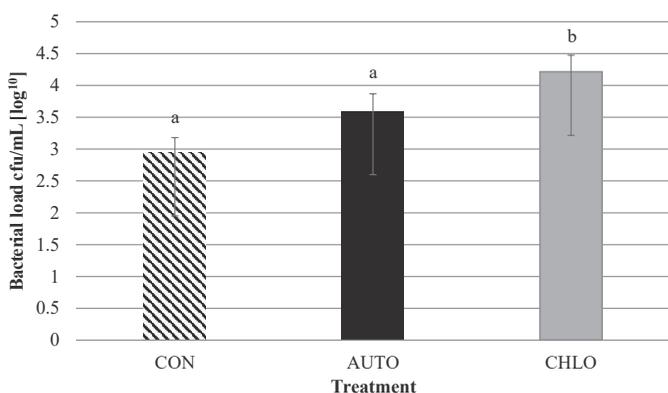
Figure 1). Differences in progesterone concentrations were reported between d 0 and 3 ( $P = 0.0051$ ), and d 3 and d 7 ( $P < 0.0001$ , Figure 2). Overall, progesterone concentrations differed across days of treatment ( $P < 0.001$ , Figure 3).

#### Exp. 2

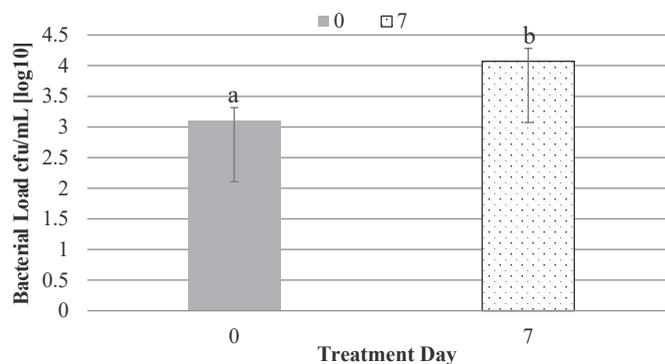
There were no differences in total bacterial load between treatments when comparing bacteria cultured on SBA to individual sanitizing treatments ( $P = 0.29$ ), total bacterial load by day ( $P = 0.69$ ), and total bacterial load to treatment by day ( $P = 0.25$ ). In addition, treatments did not impact vaginal mucous scores ( $P = 0.28$ ).

There were higher gram-negative colony counts on EMB agar plates from vaginal mucosal samples from cows that received CHLO treated CIDRs compared to samples from cows receiving inserts in the CONT or AUTO groups ( $P = 0.0035$ ; Figure 4). In addition, across all treatments, there was a higher gram-negative bacterial load on d 7 than d 0 ( $P = 0.0025$ ; Figure 5). No differences in bacterial load were found between treatment groups by day of treatment for vaginal mucosal samples cultured on EMB ( $P = 0.33$ ).

Similar to Exp. 1, no difference between treatments (data not shown) was found in overall plasma progesterone



**Figure 4.** Overall bacterial load (cfu/mL) for EMB cultures by treatment for Control (CONT), Autoclave (AUTO), and Chlorhexidine (CHLO) treated CIDRs. Bars with different superscripts differ ( $P = 0.0035$ ).



**Figure 5.** Overall bacterial load (cfu/mL) for EMB cultures by day. Bars with different superscripts differ ( $P = 0.0025$ ).

concentrations between cows receiving CIDRs from the CONT, AUTO, or CHLO group ( $P = 0.23$ ), but overall d 3 progesterone concentrations were higher compared to d 0 and d 7 ( $P < 0.0001$ ).

## Discussion

#### Exp. 1

There were no differences in plasma progesterone between CIDR sanitation treatments, therefore reuse of CIDRs may reduce costs associated with synchronization protocols. However, others have reported differences in serum progesterone concentrations in reused CIDRs.<sup>5,13,22</sup> Previous studies have shown that autoclaved devices have higher initial progesterone release compared to new CIDR inserts.<sup>13,22</sup> Zuluaga and Williams speculated this was due to formation of crystalline progesterone on the surface of the insert after autoclaving. In the current study a numerical, but not significant, increase of serum progesterone in the DISH-treated CIDRs was found compared to other treatments, which may be associated with the heat effect as described by others.<sup>22</sup> It is possible that heat during the sterilization/disinfection process may alter the structural integrity of the insert, thereby increasing progesterone release in reused devices.<sup>22</sup> However, it does not explain why the AUTO-treated group had numerically lower progesterone levels than the CHLO group.

There was a time effect, with increased plasma progesterone concentration on d 3 in all treatment groups, and overall regardless of treatment (Figures 2 and 3, respectively). However, this hormonal pattern follows expectations in a synchronization program. Others have demonstrated a similar pattern in multiple animal models with new and reused devices.<sup>8,11,18</sup> These researchers reported increased concentrations of progesterone on d 3 compared to d 0 and d 7. Even though the current study did not track ovarian activity, it can be speculated that similar follicular dynamics played a role in the current study. Long et al<sup>11</sup> reported a similar decline in progesterone concentration on d 7,

but overall lower concentrations than found in the current study. However, Long and coworkers utilized ovariectomized animals, which may explain the lower plasma progesterone concentrations. The current protocol utilized prostaglandin injections on d 6 with CIDR removal occurring on d 7, therefore it is likely that decreased progesterone on d 7 resulted from corpus luteum luteolysis and decreasing progesterone release from the CIDR.

#### Exp. 2

Few reports exist regarding disease transmission associated with the reuse of CIDR inserts. Furthermore, the efficacy of methods to disinfect reused CIDRs for preventing disease transmission has not been proven.<sup>22</sup> Padula and Macmillan demonstrated that changes in both the uterine and vaginal microflora occurred after CIDR insertion of 14 d in early postpartum beef cows.<sup>14</sup> Microbial swabs of the inserts after removal yielded intense growth of bacteria, where isolation of *P. aeruginosa* and *T. pyogenes* was most common.<sup>14</sup> *Trueperella pyogenes*, *Streptococcus* species, and coliforms were found in another study after a 7-d treatment with a vaginal insert in heifers.<sup>7</sup> In the current study, there was an increase in overall bacterial load for bacteria cultured on EMB agar on d 7 ( $P = 0.0025$ ), which would include *Pseudomonas* and coliforms, but not for bacteria cultured on SBA ( $P = 0.69$ ). This includes all species of bacteria listed and agrees with that of Padula and Macmillan,<sup>14</sup> Fischer-Tenhagen,<sup>7</sup> and others that reported increased bacterial presence after CIDR insertion. Bacteria were cultured on SBA and MacConkey agar (similar to EMB) plates both aerobically and anaerobically in the study by Padula and Macmillan.<sup>14</sup> Others have speculated that the presence of bacteria after treatment with CIDR inserts may be due to the protruding tail of the device<sup>20</sup> where bacteria could ascend into the genital tract.<sup>7</sup> This hypothesis presents an additional question for future efforts utilizing reused CIDRs and current disinfection methods.

In the present study, there was a difference between EMB-cultured bacterial load and disinfection methods, where CHLO-treated inserts had the highest bacterial load. There was no difference between bacterial load and disinfection method for samples cultured on SBA ( $P = 0.29$ ). Zuluaga and Williams reported that autoclaving used CIDRs may be the best option as it maximally reduces the risk of disease transmission.<sup>22</sup> In contrast, Muth-Spurlock<sup>13</sup> and others reported no signs of infection after the use of once- and twice-used CIDR inserts that were disinfected with chlorhexidine solution. The present study agrees with that of Zuluaga and Williams,<sup>22</sup> where AUTO treated CIDRs had lower bacterial loads than those in the CHLO group.

A correlation between vaginal mucous characteristics and the prevalent bacterial species present in the vaginal fluid has been documented in post-partum cows with clinical disease (metritis/endometritis)<sup>3,16,21</sup> and in cattle exposed to a vaginal insert.<sup>7</sup> Despite differences in clinical status, vaginal mucous discharge can be informative of this bacterial

species present in both healthy and clinically infected cows. Although bacterial species were not specifically analyzed in the current study, vaginal mucous characteristics can provide insight into potential species that may have colonized in the vaginal mucus. Uterine samples of purulent vaginal discharge from cows predominantly contained phylotypes affiliated with Bacteroides, Proteobacteria, Firmicutes, Actinobacteria, Fusobacteria, and Spirochaetes.<sup>3</sup> Vaginal mucous character scores of 2 (muco-purulent discharge) were associated with the presence of *Fusobacterium necrophorum*, and scores of 3 (purulent) were associated with recognized uterine pathogens *T. pyogenes* and *Proteus* species.<sup>21</sup> In the present study, presence of *E. coli* and other coliforms was documented on EMB agars, as indicated by colony color change as well as halo formation on SBA agars, indicating toxin-producing bacteria. Species identification was not done in this study, but is warranted for future studies. Bichahlo and colleagues reported that total bacterial load was not associated with the presence of pus in the vagina.<sup>3</sup> Furthermore, bacterial species specificity and species intrinsic factors may be more relevant to the development and appearance of purulent material in the cow postpartum than bacterial load.<sup>3</sup> However, Sheldon and colleagues advocate examination of vaginal contents for the presence of pus to aid in the diagnosis of endometritis.<sup>16</sup>

#### Conclusions

Results of the present studies suggest that the concentrations of plasma progesterone in reused CIDRs confirms previous findings for reutilization. In both experiments, progesterone concentration in CIDRs disinfected with chlorhexidine did not differ from concentrations in new CIDRs, suggesting that this method of disinfecting reused CIDRs may be a practical and effective sanitization procedure for producers. While the dishwasher method of disinfecting CIDRs may hold promise, few producers have access to an autoclave. Bacteria were cultured from vaginal mucus in both control and treatment groups, therefore producers should be aware of the risk of infection, whether using new or reused CIDRs. This study indicates proper disinfection (autoclaved or soaked in chlorhexidine) of reused CIDRs may have similar bacterial growth rates compared to new, unused CIDRs. Future research should further investigate methods to reduce risk of bacterial infection when using CIDRs. In addition, more work should focus on ovulation and pregnancy rates following the reuse of CIDRs.

#### Endnotes

<sup>a</sup> Eazi-Breed CIDR, Zoetis, Inc., Kalamazoo, MI

<sup>b</sup> Nolvasan, Zoetis Inc., Kalamazoo, MI

<sup>c</sup> Tuttnauer 2540e, Heidolph North America, Wood Dale, IL

<sup>d</sup> Eppendorf 5810r, Hamburg, Germany

<sup>e</sup> Immulite 2000 XPi Immunoassay System, Siemens Healthcare, Malvern, PA

<sup>f</sup> Lutalyse, Zoetis, Inc., Kalamazoo, MI  
<sup>g</sup> Cystorelin, Boehringer Ingelheim Animal Health, Duluth, GA  
<sup>h</sup> Aloka Prosound 2 with a linear Aloka probe 5 MHz, Aloka Co., Ltd., Wallingford, CT  
<sup>i</sup> SAS Institute, Inc., Cary, NC  
<sup>j</sup> Datamars, Auckland, New Zealand

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