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A Preliminary Study: The Effects of Vaginal Flushing in Cattle on Mitigating Vaginitis Induced by a Controlled Internal Drug Release (CIDR)

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Abstract

The use of controlled internal drug release (CIDR) devices in beef heifer timed-artificial insemination (TAI) protocols has been shown to induce various degrees of vaginitis, reducing fertility when using TAI. The objective of this experiment was to characterize CIDR-induced vaginitis in beef heifers by quantifying polymorphonuclear leukocyte (PMN) cells in the vagina before CIDR insertion, after CIDR removal, and three days post-CIDR removal. Furthermore, our objective was to evaluate the effects of flushing the vaginas of beef heifers with saline solution immediately after CIDR withdrawal, followed by immediate removal of flush content, on reducing the proportion of PMN in the vaginas by the day of TAI. Beef heifers (n = 19) from the Kansas State University Heifer Development Unit were enrolled in the study. The heifers were stratified by weight and randomly assigned to one of three groups: CIDR and Flush (n = 7), CIDR (n = 7), and negative control (n = 5). The presence of vaginitis was assessed before inserting the CIDRs using a 1-4 vaginitis scoring system that evaluated color and irritation of the vaginal mucosa. On day 0, heifers included in CIDR treatments received an intravaginal CIDR device. On day 7, CIDRs were removed and assigned a CIDR score. A sterile saline flush solution was used in the treatment group, CIDR and Flush, to lavage the vagina at CIDR removal in conjunction with using a Metricheck device to manually remove the flush from the vagina. Additionally, vaginal cytology samples were collected from all heifers on days 0, 7, and 10 using a sterile cotton swab. A difference ($P < 0.05$) in the average PMN was observed between collection days. However, no significant effects were observed for treatment ($P = 0.92$) or treatment × day interactions ($P = 0.75$).

Introduction

The vaginal microbiome of synchronized heifers is dynamic and changes in response to environmental inputs, such as the use of intravaginal controlled internal drug release (CIDR) devices (Gonzalez Moreno et al., 2016). The use of CIDRs in synchronized protocols in the beef cattle industry has been shown to induce vaginitis by recruiting an inflammatory response that increases the abundance of polymorphonuclear leukocyte (PMN) cells. The inflammatory response originates in the vagina; however, given the open state of the cervix, as is commonly observed during estrus, it is hypothesized that

PMN may migrate into the uterus and can adversely affect fertility by triggering an early inflammatory response to spermatozoa used in artificial insemination. Previous research indicated an increase in PMN at CIDR removal, characterizing a CIDR-induced vaginitis phenotype. Furthermore, lower conception rates to timed artificial insemination (TAI) were observed in beef females as the intensity of vaginitis increased (Dias et al., 2019). A greater incidence of CIDR-induced vaginitis presents management implications for fertility at TAI. The objective of this experiment was to characterize CIDR-induced vaginitis in beef heifers by quantifying inflammatory cells in the vagina on day 0, day 7, and day 10, and to evaluate if flushing the vagina with a saline solution after CIDR removal would reduce PMN cells on day 10 compared to unflushed heifers that received a CIDR. We hypothesized that the use of a CIDR would result in an increased proportion of PMN at CIDR withdrawal when compared to control heifers not receiving a CIDR. Furthermore, we hypothesized that heifers that receive a vaginal flush with sterile saline solution immediately after withdrawal would have a reduced proportion of PMN on day 10 when compared to heifers that received a CIDR but received no vaginal flush.

Experimental Procedures

Beef heifers (n = 19) from the Kansas State University Heifer Development Unit were enrolled in this study. Heifers were stratified by weight to categorize maturity and then randomly assigned to CIDR (n = 7), CIDR with Flush (n = 7), or a negative control (n = 5). This study focused on the timing of events of a 7-day CO-SYNCH+CIDR protocol, without the use of pharmacological injections. The progesterone from CIDR devices was the only exogenous form of hormone applied to treatment heifers.

All heifers had their vaginas inspected for signs of vaginitis on days 0 and 10 and were assigned a score from 1-4, where 1 = clean, pink mucosa with no mucus or secretion, 2 = clean, pink mucosa with clear mucus or secretion, 3 = reddened mucosa with small amounts of yellow mucus or purulent secretion, and 4 = reddened mucosa with large amounts of yellow mucus or purulent secretions, and blood. Control heifers also received a vaginitis score on day 7. At CIDR removal on day 7, each device was assigned a 1–4 CIDR score to assess CIDR-induced vaginitis, where 1 = clean, no mucus; 2 = clear with mucus; 3 = purulent secretion; 4 = purulent and bloody secretion combination. These scoring systems provided chute-side evaluation of vaginitis.

Vaginal swabs were collected on days 0, 7, and 10 to generate vaginal cytology slides to evaluate the abundance of PMN in the vaginal microbiome during the estrous synchronization protocol. Samples were collected by removing fecal material with a paper towel, spreading the vulvar labia apart, and inserting and rotating a sterile cotton swab against the vaginal mucosa for material collection. Then, the sample was transferred onto a microscopy slide by rotating the tip of the cotton swab onto the microscopy slide. Each slide was individually stained using modified Giemsa and then dried for analysis. Once the slides dried, vertical lines were drawn to separate the microscopy slide into three even, equal-sized chambers. Slides were analyzed for the proportion of epithelial cells and PMN by counting 100 cells per slide chamber (i.e., 37 PMN, 63 epithelial cells = 37% PMN). The final proportion of PMN per slide was calculated as the average percent PMN of the three slide chambers.

On day 7, heifers in CIDR with Flush were subjected to vaginal flush. Prior to the vaginal flush, the vulva was cleaned using paper towels, the vulvar labia were spread

apart, and a bottle containing sterile saline was inserted into the vestibule. Saline was infused by squeezing the bottle until the entire volume was infused into the vagina. Following vaginal flush, contents were manually removed using a Metricheck device.

All statistical analyses were performed in SAS version 9.4. The GLIMMIX procedure of SAS, with the heifer as the experimental unit, was used to assess vaginitis scores on day 10. The MIXED procedure was used to analyze the average PMN cells over the length of the trial. The level of significance was set at $P < 0.05$ with tendency set at $0.05 \leq P \leq 0.10$.

Results and Discussion

The average for vaginitis scores, CIDR scores, and vaginal PMN abundance per treatment are summarized in Table 1. Additionally, the average proportion of vaginal PMN by day is summarized in Figure 1. There was an effect of day ($P < 0.01$) on the proportion of vaginal PMN, with samples collected on days 7 and 10 having greater proportions of PMN when compared to samples collected on day 0. However, there were no effects of treatment ($P > 0.10$), or an interaction between treatment and day ($P > 0.10$) on the proportion of vaginal PMN. Overall, the proportion of vaginal PMN increased with the progression of the experiment, with the greatest proportion of PMN found on day 10.

Heifers that received a CIDR had purulent or bloody secretions in their CIDR (CIDR scores 3.1 and 3.3 for CIDR and Flush groups, respectively), as it is characteristic of a CIDR-induced vaginitis. Accordingly, these heifers showed an increased proportion of vaginal PMN on day 7 when compared to day 0, indicating that the use of a CIDR indeed induced the vaginitis. We hypothesized that flushing the vagina of heifers immediately after CIDR removal would result in a decreased vaginitis score on day 10 when compared to heifers that received a CIDR but received no flush. Both groups of heifers had reduced vaginitis scores on day 10 when compared to CIDR scores on day 7, indicating that flushing the vagina had no additional effect on decreasing the vaginitis score by day 10. Intriguingly, the proportion of vaginal PMN increased on day 10 when compared to day 7, which does not agree with our observations of decreased vaginitis scores on day 10.

The most confusing result from our experiment is that control heifers that received no CIDR also expressed an increased proportion of vaginal PMN as the experiment progressed. This is intriguing because these heifers did not receive the source of inflammation and had no signs of vaginitis throughout the experiment. Such discrepancies in PMN results for control heifers could partially be explained by differences in vaginal PMN according to the stage of the reproductive cycle. Furthermore, the visual identification of cells under the microscope can be a subjective measurement and could have partially influenced these results. Our research group is actively seeking more objective analyses of the abundance of vaginal PMN to better characterize this phenotype. Nevertheless, we conclude that the use of a CIDR induces vaginitis, as characterized by purulent secretions present in the CIDR at withdrawal. Additionally, we conclude that signs of vaginitis are naturally decreased following CIDR withdrawal, with no additional effects of flushing the vaginas at CIDR withdrawal on decreasing signs of vaginitis by day 10.

Implications

This study highlights opportunities for future research and practical applications in beef cattle reproductive management, such as reducing CIDR-induced vaginal inflammation and identifying when PMN levels peak can optimize the interventions and improve outcomes.

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References

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Table 1. Average vaginitis score, CIDR score, and percentage of polymorphic nuclear cells (PMN)

	Average vaginitis score[1]			Average CIDR score[2]			Average PMN cells (%)		
Group	**Control**	**CIDR**	**Flush**	**Control**	**CIDR**	**Flush**	**Control**	**CIDR**	**Flush**
Day 0	1	1.3	1.1				7.0	6.2	5.2
Day 7	1				3.1	3.3	24.2	20.4	23.6
Day 10	1	1.4	1.6				27.6	29.7	28.5
P-value							TRT[3] = 0.9183 Day <0.0001 TRT × Day = 0.7476		

[1] There were no differences between groups for vaginitis scores.
[2] There were no differences between groups for CIDR scores.
[3] TRT = Treatment.

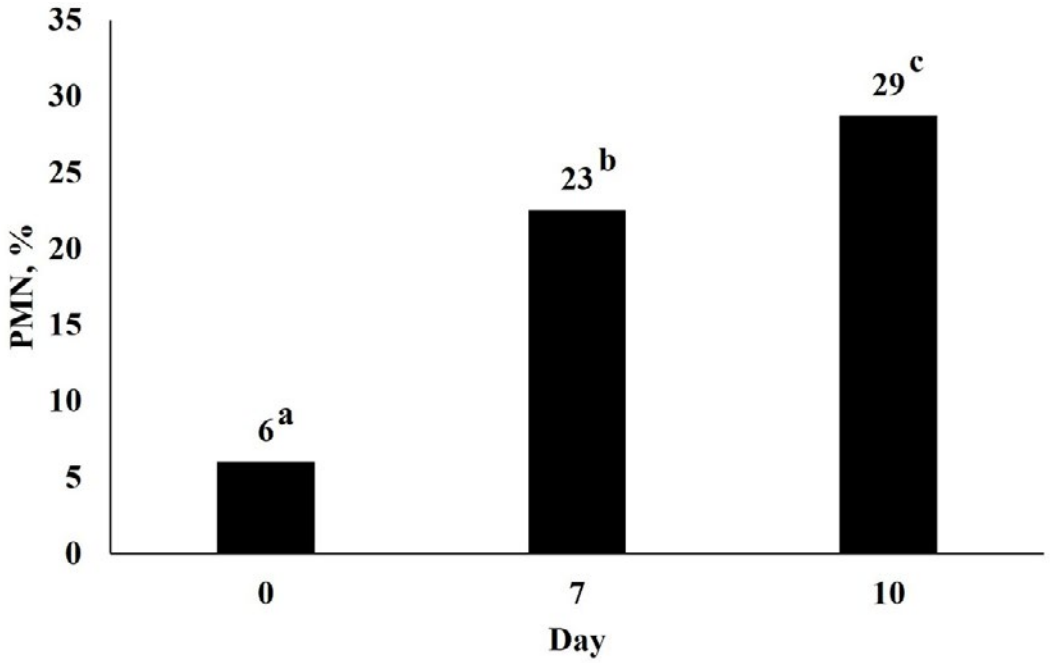

Figure 1. The overall average percentage of polymorphonuclear (PMN) leukocyte cells on each day of the trial. Means with uncommon superscripts differ ($P < 0.05$).