Impact of aspirin feeding on ovulation, CL development and concentrations of progesterone in beef heifers

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The objective of this study was to determine the effects of feeding low doses of aspirin during estrous synchronization on ovarian response and concentrations of progesterone in beef heifers. Aspirin feeding had no impact on ovarian response during estrus synchronization. The volume of luteal tissue was decreased from day 7 to day 14 in heifers fed aspirin, compared with controls, but no impacts were observed on concentrations of progesterone during the experiment.

Summary

Twenty-eight beef heifers were used to evaluate the effects of low-dose aspirin treatment on ovarian dynamics and concentrations of progesterone. Heifers were stratified by weight, then randomly assigned to one of two treatments: 1) fed a diet to meet 100 percent of National Research Council (NRC) requirements (CON, n =14) or 2) fed a diet to meet 100 percent of NRC requirements mixed with aspirin delivered at 6.5 milligrams per kilogram (mg/Kg) of body weight (ASA, n = 14). Heifer diets were controlled using the Insentec feeding system, recording daily individual feed intake and ensuring appropriate delivery of dietary treatment to each heifer. All heifers were synchronized using the five-day CO-Synch + CIDR estrus synchronization protocol. At the time of CIDR removal (day minus 3), the diameter of the largest follicle for heifers fed aspirin (12.4 ± 0.81 millimeters [mm]) tended ($P = 0.08$) to be greater than that of control heifers (10.3 ± 0.81 mm), and no differences ($P = 0.76$) were observed between treatments in the volume of luteal tissue. At the time of GnRH administration (when heifers normally would be bred, day 0), no differences ($P = 0.59$) were observed between treatments in the diameter of the ovulatory follicle (13.7 ± 0.63 mm and 14.2 ± 0.63 mm for CON and ASA, respectively). Ultrasound evaluation seven and 14 days after final GnRH administration revealed no differences ($P \geq 0.12$) between treatments in the total volume of luteal tissue. However, the change in the total luteal tissue from day 7 to day 14 was different ($P = 0.05$) between treatments; ASA heifers (-0.62 ± 0.7 centimeters [cm][3]) had a decrease in the total volume of luteal tissue, whereas CON heifers (1.4 ± 0.7 cm^3) had an increase in the total volume of luteal tissue. No differences ($P > 0.10$) in concentrations of progesterone were present among treatments at day 7 or day 14. Low-dose aspirin consumption in beef heifers did not impact the proportion of females ovulating after synchronization or concentrations of progesterone during the subsequent luteal phase up to day 14.

Introduction

Aspirin is the most widely prescribed nonsteroidal anti-inflammatory drug (NSAID) in the world. It is composed of acetylsalicylic acid, which has analgesic, anti-inflammatory and antipyretic effects (Schisterman et al., 2009, James et al., 2008). The mode of action of aspirin and other NSAIDs is the nonselective inhibition of cyclooxygenase (COX), an enzyme that is responsible for the synthesis of prostaglandins.

Natural prostaglandin $F_{2\alpha}$ (PGF) release causes a female to return to estrus, so a reduction in PGF may allow for a longer period of embryo development and greater overall pregnancy rates. In addition, aspirin has the ability to increase systemic blood flow, which could impact the female reproductive tract and subsequently may impact follicle growth, corpus luteum (CL) formation and oocyte development.

In humans, low-dose aspirin therapy resulted in an increase of uterine blood flow that could improve the results of in vitro fertilization and intracytoplasmatic sperm injection (Haapsano et al., 2009). However, when a full dose of aspirin (100 mg/Kg of body weight) was administered to Brahman cows every 12 hours from day 7 to 13 postpartum, longer postpartum intervals, lower pregnancy rates, increased incidence of abnormal estrous cycle and reduced presence of CL after estrus were observed, compared with cows not fed aspirin (Stahringer et al., 1999).

One of the possible mechanisms of reduced reproductive performance, noted in other species, could be suppression of ovulation (Duffy et al., 2002). In contrast, greater pregnancy rates were obtained when using NSAIDs one hour before embryo transfer, compared with

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a placebo group in a study with heifers (Elli et al., 2001).

Perhaps the potential reproductive benefits of low-dose aspirin therapy observed in human medicine also could translate to improved reproduction in beef cattle. In a small study, Sanchez-Rodriguez et al., (2011) observed a tendency for improved pregnancy rates in cows administered low-dose aspirin therapy, compared with those not fed aspirin (pregnancy rates of 73.7 and 52.6 percent, respectively).

Before conducting large-scale research to evaluate whether feeding low-dose aspirin truly can improve pregnancy rates, determining the impacts of low-dose aspirin on ovulation, ovarian dynamics and concentrations of progesterone during the subsequent estrous cycle is imperative. Therefore, the objective of this experiment was to evaluate the administration of low-dose aspirin during estrous synchronization and the subsequent luteal phase on ovarian dynamics and concentrations of progesterone in beef heifers.

Material and Methods

Twenty-eight beef heifers (12 to 14 months of age and an average weight of 895 pounds) from the NDSU Beef Unit were housed in a two pens at the NDSU Beef Cattle Research Complex (BCRC). Heifers were adapted to the BCRC Insentec feeding system during a 16-day period. Heifers then were stratified by weight and randomly assigned to one of two treatments (Figure 1): 1) fed a diet to meet 100 percent of NRC requirements (CON, n =14) and 2) fed a diet to meet 100 percent of NRC requirements mixed with aspirin delivered at 6.5 mg/Kg of body weight (ASA, n = 14). Each treatment was represented evenly in each pen.

All heifers were synchronized using the five-day CO-Synch + CIDR synchronization protocol; heifers received 25 mg of prostaglandin $F_{2\alpha}$ (PGF; 5 milliliters [mL] Lutalyse, Zoetis Inc.), followed in three days by a CIDR insert (Easi-Breed CIDR, Zoetis) and 100 micrograms (µg) gonadotropin-releasing hormone (GnRH; 2 mL Factrel, Zoetis), followed in five days by CIDR removal and 50 mg PGF (two injections eight hours apart), followed in three days by 100 µg GnRH. In this experiment, the heifers were not bred.

Blood samples (10 mL per sample) were taken on days minus 21, minus 11, minus 8, minus 3, 0, 2, 7 and 14 relative to the time of a normal breeding (day of final GnRH administration in the five-day CIDR + CO-Synch protocol). Blood was analyzed for concentrations of progesterone (P4).

Transrectal ultrasonography was used to evaluate ovarian dynamics in response to treatments. Ovaries were evaluated for the presence of follicles and luteal structures and mapped on each day that blood samples were collected (excluding day minus 11). The purpose of each scan was to determine the stage of the estrus cycle at the project initiation (day minus 8); determine the presence of a dominant follicle, its growth, and the presence and size of corpora lutea (CL; days minus 3 and 0, respectively); confirm ovulation (day 2); and measure the size of CL (days 7 and 14).

The growth of the dominant follicle was obtained by the difference of diameter of the follicle that ovulated on day minus[3] and day 0. Similarly, differences in diameter were used to measure the CL growth, which was obtained by the difference of the diameter of the CL on days 7 and 14.

All data were analyzed using the GLM procedures of SAS (SAS Ins. Inc., Cary, N.C.) with a model including the effects of treatment (CON or ASA).

Results and Discussion

Effects of aspirin treatment during the synchronization protocol are presented in Table 1. On the day of CIDR removal (day minus 3), the diameter of the largest follicle for heifers fed aspirin tended ($P = 0.08$) to be greater than that of control heifers. However, no differences were observed ($P = 0.76$) in the volume of luteal tissue on day minus 3 between treatments.

The diameter of the largest

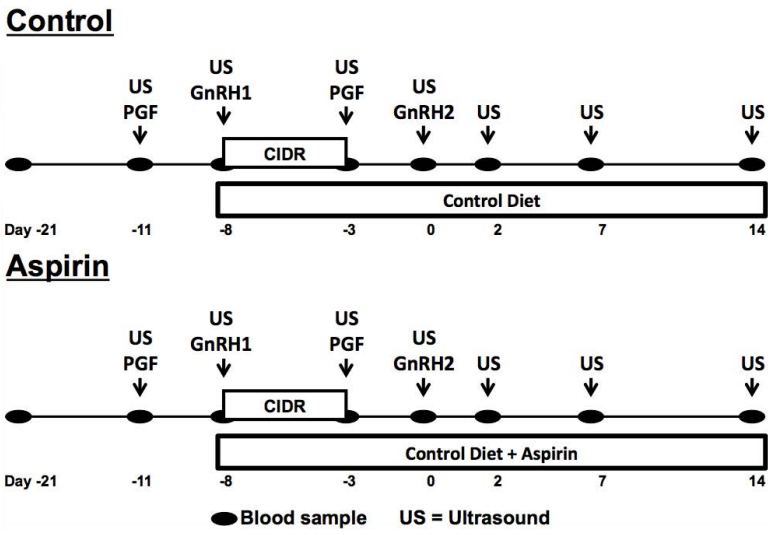

Figure 1. Schematic of aspirin and control treatments.

follicle at day 0 (last GnRH administration) and follicle growth from days minus 3 to 0 were not different ($P \geq 0.34$) between treatments. All heifers in both treatments ovulated in response to GnRH administration on day 0.

The diameter of the largest follicle and total volume of luteal tissue on day 7, as well as total volume of luteal tissue on day 14, were similar ($P \geq 0.12$) between treatments. However, a greater change ($P = 0.05$) in total luteal tissue was observed, with control heifers increasing and aspirin-treated heifers decreasing total luteal tissue from days 7 to 14. No differences ($P \geq 0.15$) in concentrations of progesterone were observed between treatments at the time of CIDR removal (day minus 3), normal breeding time (day 0), or seven and 14 days after normal breeding time (Figure 2).

The results of this preliminary effort indicate that ovulation was not impaired by low-dose aspirin administration in beef heifers. In addition, the size of the ovulatory follicles and volume of subsequent luteal tissue were not impacted. We did observe a difference in the change of luteal tissue volume from days 7 to 14 between treatments, but overall concentrations of progesterone were not different during the same interval. Taken together, we feel the next required step is to perform an additional experiment with a large group of females to determine whether any benefit is derived from low-dose aspirin feeding.

Table 1. Impact of low-dose aspirin feeding on ovarian dynamics in beef heifers.

Item	Aspirin	Control	SE	*P*-value
Largest follicle, mm.				
CIDR removal (day minus-3)	12.4	10.3	0.814	0.08
7 days after GnRH2 (day 7)	13.6	12.7	0.506	0.21
Ovulatory follicle (day 0, GnRH2)	14.2	13.7	0.63	0.59
Dominant follicle growth (day minus 3 to day 0, CIDR removal to GnRH2)	3.06	2.50	0.40	0.34
Total luteal tissue, mm^3				
CIDR removal (day minus 3)	3.4	3.75	0.73	0.76
7 days after GnRH2 (day 7)	5.08	4.09	0.58	0.24
14 days after GnRH2 (day 14)	4.55	5.48	0.41	0.12
Change in total luteal tissue (from days 7 to 14)	-0.62	1.40	0.71	0.05

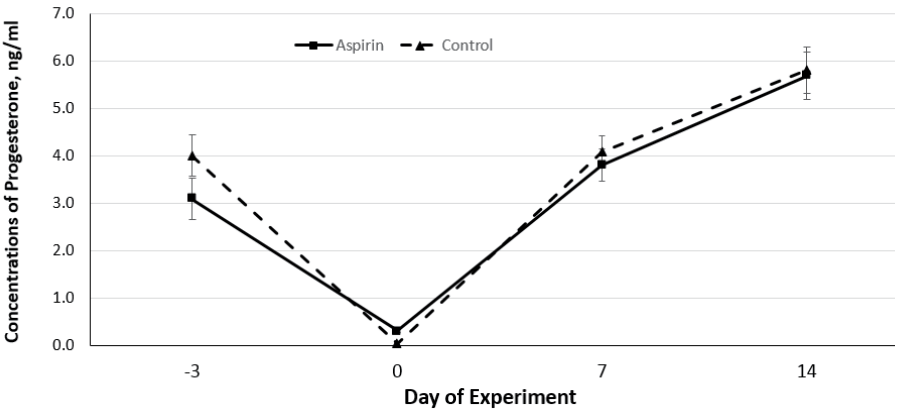

Figure 2. Concentrations of progesterone at CIDR removal (day minus -3), and normal breeding time (day 0), then seven and 14 days after normal breeding for heifers on aspirin and control treatments.

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