ABILITY OF INDOMETHACIN TO ALTER PROSTAGLANDIN METABOLITE CONCENTRATIONS AND TO ENHANCE THE FUNCTION OF CORPORA LUTEA INDUCED IN POSTPARTUM SUCKLED BEEF COWS\textsuperscript{1,2,3}

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Summary

Fourteen anovulatory postpartum (38.0 \( \pm \) 1.9 d) beef cows that ovulated after an injection of 250 \( \mu \)g gonadotropin releasing hormone (GnRH) in saline were used to examine the influence of indomethacin on luteal function. Beginning the d after GnRH, 6 cows were given intrauterine infusions of indomethacin for 14 d and the other eight cows received vehicle. After GnRH treatment, concentrations of progesterone in serum were elevated longer (\( P<.01 \)) for indometacin-treated cows than for vehicle-treated cows. At the same time prostaglandin metabolite (PGFM) concentrations were lower (\( P<.01 \)) in indomethacin-treated cows than in vehicle-treated cows. In summary, indomethacin suppressed PGFM concentrations and enhanced function of corpora lutea induced in postpartum suckled beef cows.

(Key Words: Postpartum Suckled Beef Cows, Progesterone, 13,14-Dihydro-15-Keto-Prostaglandin \( F_2 \alpha \), Indomethacin, Gonadotropin Releasing Hormone.)

Introduction

Several treatments such as early weaning (Odde et al., 1980), limited nursing (Randel and Welker, 1976; Flood et al., 1979), gonadotropin releasing hormone (GnRH) treatment (Britt et al., 1975; Webb et al., 1977; Lishman et al., 1979; Fonseca et al., 1980; Kesler et al., 1980; Troxel et al., 1980) and a combination of sex steroids (Walters et al., 1977; Smith et al., 1979; Troxel et al., 1980) have been used to initiate ovarian cycles in anestrous suckled beef cows. The duration of the luteal phases after early weaning (Odde et al., 1980), limited nursing (Flood et al., 1979) and GnRH treatment (Webb et al., 1977; Lishman et al., 1979; Kesler et al., 1980), however, has been shown to be shorter and progesterone secretion is less than during the luteal phase of the normal estrous cycle. The incidence of short luteal phases in postpartum beef cows has been reported to be 70 to 80% after early weaning and GnRH treatment (Lishman et al., 1979; Kesler et al., 1980; Odde et al., 1980; Troxel et al., 1983) regardless of the interval postpartum when treated. These short luteal phases are generally 7 to 10 d as compared with a normal estrous cycle length of 20 to 21 d. Short luteal phases also occur spontaneously in postpartum beef cows and are most frequently observed between the first and second estrus detected after parturition (Odde et al., 1980). Because ova resulting from the induced ovulations appear to be capable of being fertilized, failure to sustain pregnancy may be due to early corpus luteum regression (Ramirez-Godinez et al., 1982; Troxel et al., 1983).

The exact cause of the short luteal phase in the postpartum cow is not clearly understood. One explanation may be the presence of a luteolytic factor such as prostaglandin \( F_2 \alpha \) (PGF\( _2 \)\( \alpha \)). Thatcher et al. (1980) reported elevated levels of 13,14-dihydro-15-prostaglandin \( F_2 \alpha \) (PGFM) during the early postpartum.

\textsuperscript{1}The prostaglandin metabolite assay and the validation of this assay were conducted under the supervision of Drs. J. E. Hixon and P. G. Weston in their laboratory. The authors appreciate their generosity.

\textsuperscript{2}Gonadotropin releasing hormone was generously supplied by Dr. M. Brown (CEVA Laboratories). The crystalline 13, 14-dihydro-15-keto-prostaglandin \( F_2 \alpha \) and the 13, 14-dihydro-15-keto-prostaglandin \( F_2 \alpha \) antisera were generously supplied by Dr. K. T. Kirkton (The Upjohn Co.). The progesterone antiserum was generously supplied by Dr. O. D. Sherwood (Univ. of Illinois).

\textsuperscript{3}This research was conducted as part of regional research project, NC-113 "Methods for Improvement of Fertility of Cows Postpartum."

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Received February 28, 1983.
Accepted January 17, 1984.
period of milked dairy cows and they reported that PGFM was produced by the uterus (Guilbault et al., 1981). Only when PGFM levels decreased, about 10 to 20 d postpartum, was there a detected rise in progesterone levels in milked dairy cows. Concentrations of PGFM, however, have not been determined in early postpartum suckled beef cows. Furthermore, the concentrations of PGFM have not been related to ovarian function in postpartum suckled beef cows.

The objectives of this experiment were to determine the effect of indomethacin, a prostaglandin synthesis inhibitor, on PGFM concentrations, and to determine if indomethacin would alter progesterone concentrations in postpartum suckled beef cows.

Materials and Methods

Experimental Animals. Thirty-two mature crossbred beef cows from the University of Illinois Dixon Springs Agricultural Center were administered 250 μg GnRH in saline im on d 24 to 56 (42.4 ± 2.2 d) postpartum. These cows were selected because previous data have demonstrated that the incidence of ovulation and short luteal phases after GnRH treatment in anovulatory suckled beef cows are not influenced by the interval postpartum (Troxel et al., 1983). Beginning the next day, 16 of the cows received indomethacin via intrauterine infusions (indomethacin group) for 14 d while the other 16 cows received intrauterine infusions of the indomethacin carrier (acetone-phosphate buffer solution) for 14 d (control group). Indomethacin (40 mg) was infused by a pipette via the cervix into each uterine horn two times a day at 12-h intervals. Therefore, each indomethacin treated cow received 160 mg of indomethacin/d. The indomethacin was dissolved in .4 ml acetone and diluted to a volume of 5 ml with .2 M phosphate buffer (Lewis and Warren, 1977). Based on serum progesterone concentrations on d 0 to 8 after GnRH treatment and on per rectum ovarian examinations on d 0, 18 of the cows were eliminated from the experiment because they were not anestrous at the onset of the experiment or did not ovulate in response to GnRH treatment. Cows were considered to be anestrous if no luteal structures were detected on the ovaries and if progesterone concentrations were less than .5 ng/ml on d 0. Cows were considered to have ovulated to GnRH treatment if progesterone concentrations increased to >1.0 ng/ml by 8 d after GnRH treatment. This has been demonstrated to be indicative of an ovulation induced by GnRH in anovulatory postpartum suckled beef cows (Kesler et al., 1981). Therefore, the final number of cows was eight and six for the control and indomethacin-treated groups. These 14 cows averaged 38.0 ± 1.9 d postpartum.

Blood samples were collected via jugular venipuncture into evacuated tubes before GnRH treatment (d 0) and before each infusion (twice a day for 14 d). All samples were assayed for progesterone concentrations and the two samples collected from each cow on d 0, 1, 3, 5, 7, 9, 11 and 13 were pooled and assayed for concentrations of PGFM.

General Procedures. Blood samples were placed in crushed ice immediately after collection and then placed in a cold room (4 C) for 24 h. The serum was then harvested and frozen until it was assayed for PGFM and progesterone concentrations.

During the winter, the cows had continuous access to fescue hay and were fed corn silage to appetite. Starting approximately 30 d before calving and continuing throughout the experimental period, the cows were fed .45 kg of soybean meal.

Assay of Hormones. Serum PGFM concentrations were quantified by radioimmunoassay. The PGFM was extracted from duplicate 500-μl aliquots of serum. After the addition of 50 μl of .5 N acetic acid and 4 ml of ethyl acetate, samples were shaken on a mechanical shaker for 15 min. The serum layer was frozen in a mixture of solid CO2 and methanol for 2 min before decanting the solvent fraction into disposable culture tubes. Extracts were dried under a stream of air at 37 C. Standard curves were established for each assay in quadruplet at concentrations of 0, 5, 10, 25, 50, 100, 200, 300, 400 and 500 pg/ml of crystalline PGFM dissolved in tris buffer (.1% gelatin, 1:10,000 merthiolate, pH 7.4).

Cross-reaction of PGFM antiserum has been reported to be .1% for PGF2α, 15.3% for 15-keto-PGF2α and 100.0% for 13,14-dihydro-15-keto-PGF2α (Cornette et al., 1974). The antiserum was used at a dilution of 1:5,000. When 5, 10, 25, 50 and 100 pg/ml (dissolved in

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100 µl of tris buffer) of exogenous 13,14-dihydro-15-keto-PGF₂α were added to 400 µl of plasma from a postpartum cow, the percentage recovery was 97, 99.5, 105.8, 101.4 and 89.1 respectively (X = 98.6; no. = 17). The least amount of PGFM which inhibited (P<.05) binding of [³H]PGFM to antibody, was 5 pg/ml (N = 6). Parallelism was determined by quantifying PGFM in different volumes of pooled early postpartum and pooled diestrous blood serum. When volumes of 250, 350, 500 and 650 µl of pooled diestrous serum were assayed, PGFM concentrations were 5.9 ± .6, 5.9 ± .9, 6.2 ± 1.4 and 6.2 ± .5 pg/ml, respectively. When volumes of 125, 250, 500 and 750 µl of pooled early postpartum serum were assayed, PGFM concentrations were 99.1 ± 18.1, 90.5 ± 9.2, 105.5 ± 7.9 and 94.2 ± 2.2 pg/ml, respectively. Extracts of both pools of serum were parallel (P<.01; r = .97) to the standard curve. Recovery aliquots were included by the addition of ~30,000 cpm of [³H]PGFM to pooled cow serum. The recovery rates were 74.2 ± 1.1.

The serum samples were assayed in one assay. Pooled serum was used for calculations of the intraassay coefficient of variation (11.0%) and the interassay coefficients of variation (16.7%). The mean of the pool sample was 48.3 ± 2.0 pg/ml.

Blood progesterone concentrations were determined by radioimmunoassay according to procedures previously described and validated in our laboratory (Troxel et al., 1980; Wiseman et al., 1983). Samples were assayed in duplicate and samples from one cow were determined in one assay. Pooled serum was used for calculations of intraassay (6.4%) and interassay coefficient of variation (16.7%). The mean of diestrous pool sample was 5.5 ± .4 ng/ml. All samples were adjusted for recovery (64.6 ± 3.5%).

Data Analysis. Duplicated estimates of hormone concentrations were averaged before analysis. Intraassay and interassay coefficients of variation were determined by the procedure of Rodbard (1971). The interval from GnRH treatment to the day when progesterone concentrations fell below 1.0 ng/ml was analyzed by analysis of variance (Steel and Torrie, 1960). The PGFM and progesterone concentrations during the 14-d period were analyzed by split-plot analysis of variance as described by Gill and Hafs (1971) due to repeated sampling of cows. Standard errors were determined from

Results and Discussion

Progesterone concentrations are shown in figure 1. The progesterone concentrations were different between treatment groups, and across the sampling period and the group x time interaction was also significant (all P<.01). During the 14-d sampling period, progesterone concentrations fell below 1.0 ng/ml earlier
(P<.01) for the control cows than for the indomethacin-treated cows (5.9 ± .4 vs 11.8 ± 1.3 d).

Concentrations of PGFM are shown in figure 2. Concentrations of PGFM were different between groups (P<.01). In addition, there was a change (P<.01) of PGFM concentrations across the sample period and the group x time interaction was significant (P<.01). This significant group x time interaction resulted from the increase of PGFM concentrations from d 0 through 7 in the control cows, while in the indomethacin-treated cows, PGFM concentrations did not change.

At approximately 7 d prepartum, PGFM concentrations gradually increase in the dairy cow (Thatcher et al., 1980), with a major increase between d 1 and 4 postpartum (Thatcher et al., 1980). After a peak of PGFM on d 4 postpartum, concentrations then declined linearly to d 15 postpartum in the milked dairy cow (Thatcher et al., 1980) and to d 22 postpartum in the suckled beef cow (Troxel, 1983). Guilbault et al. (1981) demonstrated that PGFM was produced by the postpartum uterus.

In the study by Thatcher et al. (1980) and by Troxel (1983) blood samples were collected on a daily basis. Kindahl et al. (1976) demonstrated that PGFM concentrations have daily transitory changes. Therefore, there would appear to be factors that will stimulate transitory increases in PGFM concentration. Oxytocin is one compound that has been shown to be capable of inducing transient increases in prostaglandin concentrations in cyclic cows (Newcomb et al., 1977; Milvae and Hansel, 1980). When oxytocin was administered to postpartum cows, a similar increase in PGFM concentrations was detected (Troxel, 1983). It, therefore, is possible that a transient increase in PGFM concentrations may occur after the oxytocin release associated with the suckling stimulus.

Several investigators (Webb et al., 1977; Lishman et al., 1979; Kesler et al., 1980) have demonstrated a high incidence (70 to 80%) of short luteal phases in anestrous postpartum suckled beef cows induced to ovulate with GnRH treatment. Kesler et al. (1981) demonstrated that corpora lutea developing from GnRH-induced ovulations did not become responsive to luteinizing hormone (LH) in vitro and did not continue to develop beyond day 5. When indomethacin was administered to postpartum suckled beef cows with corpora lutea induced by GnRH treatment, PGFM concentrations were suppressed and luteal life span was prolonged.

In summary, indomethacin enhanced the function and life span of corpora lutea induced in postpartum suckled beef cows. Although it is not practical to administered indomethacin in this manner, other routes of administration or other procedures could be developed to reduce the incidence of short luteal phases in postpartum suckled beef cows.

**Literature Cited**


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