BIOLOGY OF REPRODUCTION **61,** 1317–1323 (1999)

Prostaglandin $F_{2\alpha}$ Concentrations, Fatty Acid Profiles, and Fertility in Lipid-Infused Postpartum Beef Heifers[1]

S.J. Filley,[3] H.A. Turner,[4] and F. Stormshak[2,3]

Department of Animal Sciences,[3] Eastern Oregon Agricultural Research Center, [4] Oregon State University, Corvallis, Oregon 97331

ABSTRACT

Effects of lipid infusion into postpartum (PP) beef heifers on plasma concentrations of linoleic acid and prostaglandin (PG) $F_{2\alpha}$ metabolite (PGFM), days to first estrus, and subsequent pregnancy rate were examined. Treatments (n = 5 per group) of 1 L intralipid (20% soybean oil; IL), 1 L 50% dextrose (DEXT; isocaloric to IL), 0.5 L intralipid (0.5 IL), and 1 L physiological saline (SAL) were infused i.v. over 4 h on each of Days 7 through 11 PP. Capacity of the uterus to produce PG was evaluated after i.v. injection of 150 IU of oxytocin (OT) to IL- and DEXT-treated heifers Day 12 PP. Change in plasma concentrations of PGFM from 0 to 4 h was greater for IL-treated heifers than for heifers given other treatments on Day 7 ($P = 0.04$) and on Day 11 ($P = 0.01$), but not on Day 9 ($P > 0.10$). Plasma linoleic acid on Day 11 and OT-induced release of PGFM on Day 12 were greater in IL-treated heifers compared with DEXT-treated heifers ($P < 0.06$ and $P = 0.01$, respectively). There were no significant differences among treatments for mean days to first estrus or pregnancy rate. Infusion of lipid increased systemic concentrations of linoleic acid and increased the capacity of PP heifers to produce uterine $PGF_{2\alpha}$ as indicated by plasma PGFM concentration after OT injection.

INTRODUCTION

The interval between calving and resumption of estrous cycles is 45–60 days in mature beef cows and 20–30 days longer in young heifers bred to calve as 2-yr-olds [1]. This difference in duration of the postpartum (PP) interval between mature cows and heifers is partially due to the stress of calving and first lactation, as well as to higher nutrient requirements for continued growth. Long anestrous periods result in decreased pregnancy rates and profits if well-defined breeding seasons are employed [1]; therefore, it is beneficial to shorten anestrous periods of primiparous beef heifers.

In the PP cow, fertility resumes after uterine involution and ovulation occur. Prostaglandin (PG) $F_{2\alpha}$ is an eicosanoid that is important for uterine involution, and its primary site of synthesis in the PP cow is the uterine caruncles, where it acts locally on the myometrium to cause contractions [2]. Duration of increased uterine synthesis of $PGF_{2\alpha}$ is negatively correlated with number of days to complete uterine involution and length of the interval between parturition and resumption of normal ovarian activity [3].

Prostaglandins are derived from the membrane phospholipid stores of arachidonic acid (C20:4), which are synthesized from dietary linoleic acid (C18:2), an essential fatty acid. There is some indication of immediate use of linoleate for PG production in lipid-infused estrous cycling heifers [4] and ewes [5]. Further, supplementation of primiparous beef heifers with fat high in oleic and linoleic acids during the last trimester of gestation significantly enhanced subsequent pregnancy rates [6]. It is not known whether intravenous administration of linoleic acid to heifers in the early PP period increases $PGF_{2\alpha}$ production or alters ovarian function.

Objectives of this experiment were to determine whether infusion of PP heifers with essential fatty acids (FA) would increase plasma linoleic acid and $PGF_{2\alpha}$ metabolite (PGFM) and promote increased PG production after oxytocin injection. Another aim was to examine the ability of exogenous essential FA to shorten the interval between parturition and onset of normal ovarian activity and to increase subsequent pregnancy rates.

MATERIALS AND METHODS

Animals, Treatments, and Samples

Twenty Hereford × Angus primiparous heifers, pregnant by artificial insemination to a single Hereford sire, were accustomed to handling, and body condition was scored (BCS; scale 1–9; 1 = emaciated, 9 = obese) immediately prior to calving and on Days 1, 30, and 150 PP. These heifers were assigned randomly to one of four treatments (n = 5 per treatment). Heifers and/or calves were individually weighed on Days 1, 30, and 150 PP and at weaning (Day 240 PP). All experimental procedures were performed in accordance with the institutional guidelines for the care and use of animals.

Treatments consisted of i.v. infusions of 1 L fat emulsion (IL; intralipid 20%; Pharmacia, Clayton, NJ; 20% soybean oil), 1 L 50% dextrose (DEXT; Phoenix Pharmaceutical, St. Joseph, MO; isocaloric to IL), 0.5 L intralipid (0.5 IL), or 1 L physiological saline (SAL; Phoenix) into the heifers on each of Days 7 through 11 PP (Day 0 = parturition; Fig. 1). IL consisted of 20% soybean oil (with FA components of 53% linoleic, 25% oleic, 11% palmitic, 8% linolenic, and 4% stearic acids). One to two days before infusion, the jugular vein on each side of the neck was catheterized using a 1.7-mm × 8.3-cm Angiocath catheter (Becton Dickinson Desert Medical, Sandy, UT) for convenient infusion of treatments and sampling of blood. Catheters were flushed with 1 ml of sodium heparin (40 U/ml) to keep them patent. Peristaltic pumps (Minipuls 2, model HP-4; Gilson Medical Electronics, Middleton, WI) were used to ensure continuous infusion of treatment over a 4-h period. Beginning on Day 7 PP, heifers were led into individual stalls, where they remained tethered and separated from their calves during infusion and blood-sampling periods. Blood samples were taken at Time 0, 4, and 8 h relative to initiation of infusion. Except as indicated below, all blood samples were obtained via the catheter opposite to the side of infusion and were collected into 10-ml heparinized tubes, immediately placed on ice, and within 1 h centrifuged at 2500 × *g* for 15 min at 4°C. Plasma was then removed and stored at −20°C until analyzed for FA and PGFM. Addi-

Accepted June 24, 1999.
Received December 22, 1998.
[1]Technical paper No. 11461, Oregon Agric. Exp. Sta.
[2]Correspondence: F. Stormshak, 112 Withycombe Hall, Corvallis, OR 97331. FAX: 541 737 4174; e-mail: fred.stormshak@orst.edu

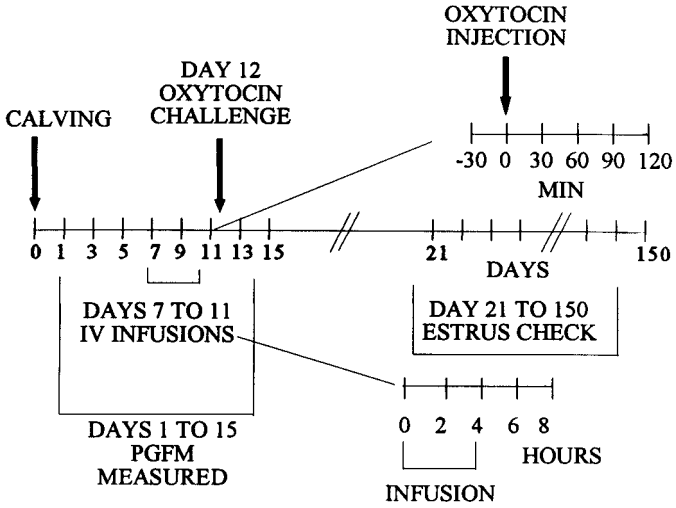

FIG. 1. Experimental timeline (0–150 days PP). Day 0 was time of calving; plasma PGFM was measured on alternate days (Days 1–15); treatments were infused on Days 7 through 11; and 150 IU OT was injected i.v. on Day 12. Heifers were bled at 0, 4, and 8 h relative to initiation of infusion (Time 0, Days 7, 9, and 11) and every 15 min from −30 to 120 min relative to OT injection (Time 0, Day 12). Return to estrus was determined by twice-daily observation for behavioral estrus and from plasma P_4 measured from blood collected twice weekly from Day 21 until 2 wk post-estrus. Estrus was defined on the basis of standing for the bull and confirmed by plasma $P_4 > 1$ ng/ml in two consecutive samples.

tionally, heifers were bled via venepuncture on alternate days (Days 1–15) to determine plasma PGFM profiles during the PP period.

After all infusions and blood samplings were completed for the day, heifers were moved to another stall and reunited with their calves. All heifers were then fed 2.3 kg alfalfa hay in individual feeders. After this hay was consumed, native flood meadow hay was fed ad libitum, hay refusals were measured, and 5-day feed intake was used to calculate mean daily intake as a percentage of metabolic body weight ($BW^{.75}$).

It was of interest whether lipid infusion would alter the ability of heifers to secrete $PGF_{2\alpha}$ independent of energy level; therefore only heifers subjected to isocaloric infusants (IL and DEXT) were included in an oxytocin (OT) challenge performed as follows: On Day 12 PP, heifers were separated from their calves, and 150 U of OT (Phoenix) was administered i.v. via catheters. Blood samples were taken at Time −30, −15, 0, 5, 15, 30, 45, 60, 75, 90, 105, and 120 min relative to OT injection, and plasma was obtained as described above.

Behavioral activity and plasma concentrations of progesterone (P_4) were used to determine number of days to first behavioral estrus. Sexual behavior of heifers (standing estrus) was observed twice daily from 21 to 150 days PP, initially by exposure of heifers to androgen-treated cows (personal communication, J. Reeves, Washington State University, Pullman, WA), and after the beginning of the breeding season (30–75 days PP) by use of intact bulls continually housed with heifers. Heifers were bled by jugular venepuncture twice weekly (Mondays and Thursdays) for plasma concentrations of P_4 beginning at 3 wk PP until 12 days after first behavioral estrus. First estrus with ovulation was defined as the time at which heifers stood for the bull and subsequent plasma P_4 was greater than 1 ng/ml for two consecutive samples. Pre-estrus luteal activity was determined by evaluating plasma concentrations of P_4 in samples taken during the 2 wk prior to first estrus.

PGFM Analysis

Concentration of PGFM (13,14-dihydro-15-keto $PGF_{2\alpha}$) in plasma samples (100 μl) from Days 1 to 15 (every other day), from samples collected during the infusion period (Time 0, 4, and 8 h on Days 7, 9, and 11), and from samples collected for the OT challenge was measured by RIA as described by Guilbault et al. [2] and established in our laboratory by Burke et al. [5]. Intra- and interassay coefficients of variation (CV) were 7.7% and 11.7%, respectively, and the sensitivity of the assay was 50 pg/ml. The PGFM antibody (#J-53 anti-PGFM) was a gift from William Thatcher (University of Florida, Gainesville, FL), while PGFM standards and competitor (13,14-dihydro-15-keto [5,6,8,9,11,12,14(n)-^{3}H] $PGF_{2\alpha}$; 177 Ci/mmol) were purchased from Cayman Chemical Company (Ann Arbor, MI) and Amersham (Arlington Heights, IL; now Amersham Pharmacia Biotech, Piscataway, NJ), respectively. PG-free plasma for use in standards was harvested from a cow treated twice (16 h apart) with an i.m. injection of a PG synthesis inhibitor, 20 ml of 50 mg/ml (1 g) flunixin meglumine (Banamine; Schering-Plough Animal Health Corp, Kenilworth, NJ). Blood was collected into evacuated heparinized flasks 4 h after the second injection, placed on ice, and centrifuged 2500 × *g* for 15 min at 4°C. Plasma was removed and stored at −20°C.

FA Analysis

FA were extracted from plasma for Day 7 (0, 4, and 8 h) and Day 11 (0 h) with methanol:chloroform (2:1 v:v) [7], methylated with boron trichloride and benzene, and then subjected to gas chromatography [8] for identification. Samples selected for FA analysis were aliquots of those in which plasma concentrations of PGFM were found to be altered. Values for selected FA are reported as weight percentage of total (unfractionated) plasma FA.

P_4 Analysis

Plasma P_4 was measured in duplicate (100 μl) by RIA as described by Koligian and Stormshak [9] using benzene: hexane (1:2 v:v) to extract plasma samples taken prior to and during the subsequent breeding season. Extraction efficiency was 80.3%; intra- and interassay CV were 9.3% and 14.8%, respectively; and sensitivity of the assay was 0.05 ng/ml. The antibody (#337 anti-progesterone-11-BSA) was a gift from Gordon Niswender (Colorado State University, Fort Collins, CO). Tritiated P_4 ([1,2,6,7-^{3}H]P_4; 115 Ci/mmol) was purchased from New England Nuclear (Boston, MA), and P_4 standards were purchased from Cayman.

Statistical Analysis

Data on plasma concentrations of PGFM during the infusion period were subjected to a repeated-measures ANOVA using Statistical Analysis Software [10]. Factors included in the full model were treatment (IL, DEXT, 0.5 IL, and SAL), heifer within treatment (T), hour (H), and day (D). Also included were the interaction effects of T × H, T × D, and T × H × D, and beginning PGFM concentration (Hour 0, Day 7) was included as a covariate. To further define treatment effects, linear and quadratic orthogonal regression contrasts were used for analysis of hour effects within treatment, and pair-wise contrasts at 0–4 h and 4–8 h relative to infusion were considered. Data for concentra-

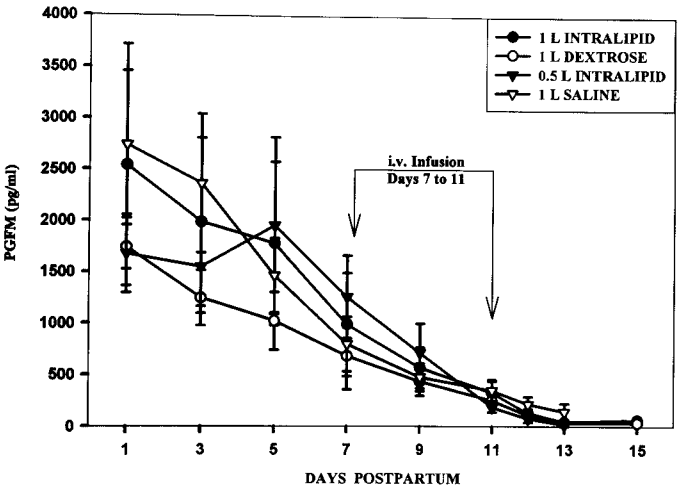

FIG. 2. Mean (± SE) PGFM concentration on Days 1 through 15 PP.

tions of PGFM on Days 1–15 and during the OT challenge (Day 12, Time −30 through 120 min) were also examined by repeated-measures ANOVA, with Hour 0 on Day 12 used as a covariate for the OT data. Data on concentrations of PGFM in response to the OT challenge were heterogeneous with respect to variance; therefore analysis was performed on log-transformed data.

Values for plasma weight percentage of FA during the infusion period on Day 7 were subjected to repeated-measures ANOVA and regression analysis. The model for ANOVA was T, heifer within T, H, and T × H. Repeated-measures ANOVA was also used to analyze for differences in plasma FA between Days 7 and 11 PP Time 0, with T, heifer within T, D, and T × D as factors in the model. Relationships between plasma weight percentage of FA and PGFM concentration were evaluated by correlation analysis.

ANOVA was used to analyze body weights (BW), BCS, 5-day feed intake, calf weights, number of days to first estrus with ovulation, and calving interval. Chi-square analysis was used to examine data for two levels of calving ease, no assistance and light pull. Heifers with difficult births were not used. Calf sex, reproductive status at 150 days PP (estrus or anestrus), and pregnancy rate the subsequent fall (pregnant or nonpregnant) were also analyzed by chi-square. Initial BW, BCS, calf weight, calf sex, and calving ease were measured and analyzed so data could be statistically adjusted in the event that animals were assigned to treatments in an unbalanced manner with respect to these characteristics.

RESULTS

Plasma concentrations of PGFM decreased over Days 1 to 15 PP ($P < 0.05$), and there was no significant T × D interaction (Fig. 2). Changes in plasma PGFM in response to infusion of IL, DEXT, 0.5 IL, and SAL on Days 7, 9, and 11 are depicted in Figure 3, with concentrations decreasing significantly over day. There was no significant T × H × D interaction; however, response to infusion was characterized by a T × D interaction ($P = 0.07$), i.e., differences among treatments depended upon day of infusion. Variation in plasma concentrations of PGFM was also different ($P < 0.01$) depending on day; therefore data for each day were analyzed separately.

There were no differences ($P > 0.10$) among treatments for Hour 0 plasma concentrations of PGFM on Day 7, 9, or 11. Therefore, Hour 0 on Day 7 was used as a covariate in ANOVA for PGFM at Hours 4 and 8 on Day 7 and at Hours 0, 4, and 8 on Days 9 and 11 to account for beginning PGFM concentration. Examination of data showed that IL caused the greatest changes in PG concentrations over time. Plasma concentrations of PGFM on Day 7, Hour 4 were significantly different among treatments. There was a dose response, heifers that had received IL having greater PGFM than DEXT-, 0.5 IL-, and SAL-treated heifers ($P < 0.04$). No additional differences among treatments were found for Day 7, Hour 4 or 8. Because of the extreme variation in response, no significant treatment differences were found by ANOVA for PGFM on Day 9 for any hour.

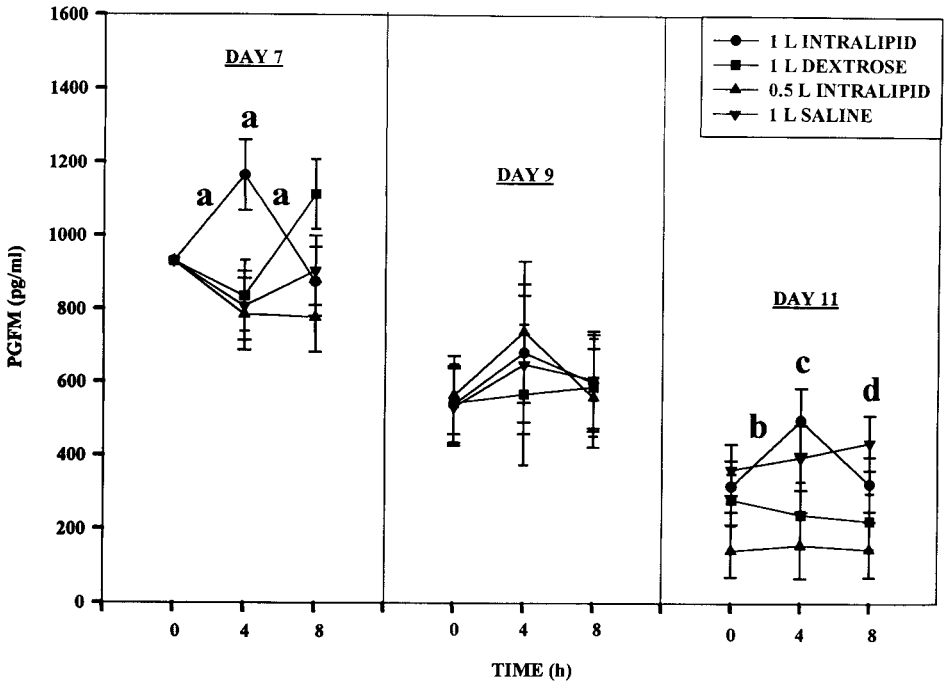

FIG. 3. Mean (± SE) PGFM concentration at Hours 0, 4, and 8 on Days 7, 9, and 11 PP. Data were adjusted for beginning plasma concentrations of PGFM (Hour 0, Day 7). [a]On Day 7, results for 1 L IL differed ($P = 0.04$) from those for other treatments for 0–4 h and 4–8 h and at 4 h. [b]On Day 11, results for 1 L IL differed ($P < 0.01$) from those for other treatments for 0–4 h. [c]Day 11, Hour 4: results for 1 L IL differed ($P = 0.06$) from those for 1 L DEXT and 0.5 L IL, but not from those for 1 L SAL ($P > 0.10$). [d]Day 11, Hour 8: results for 1 L SAL differed ($P = 0.07$) from those for 1 L DEXT and 0.5 L IL, but not from those for 1 L IL ($P > 0.10$).

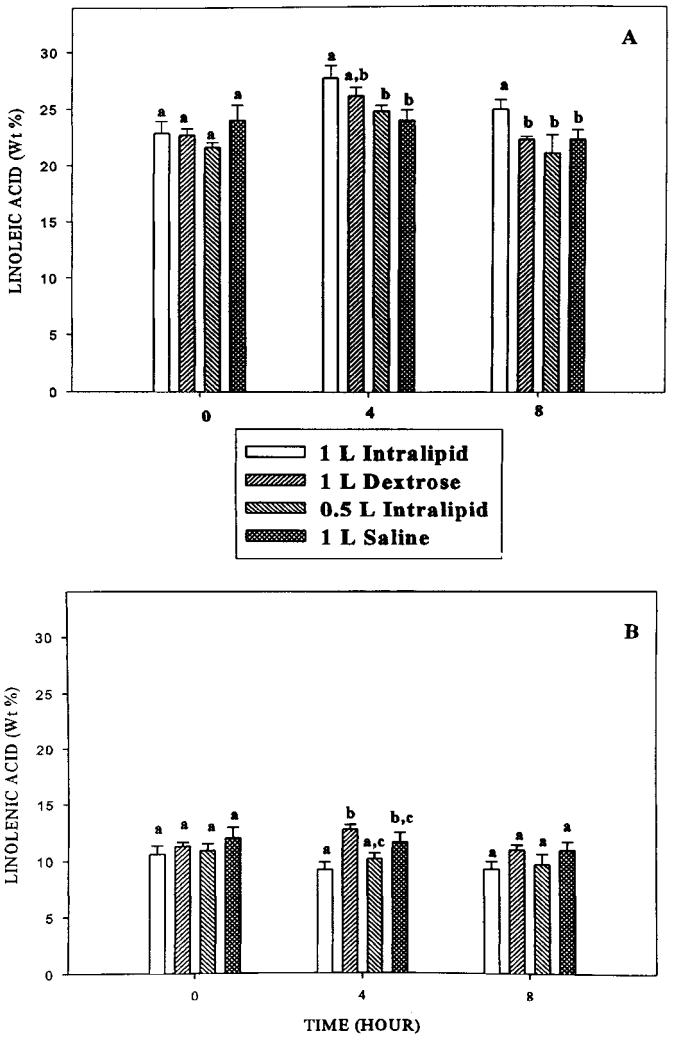

FIG. 4. Mean (± SE) weight percentage of plasma linoleic (**A**; C18:2) and linolenic (**B**; C18:3) acids in response to infusion on Day 7, Hours 0, 4, and 8. [a,b,c]Bars without a common letter within hour differ ($P < 0.05$).

However, on Day 11, plasma PGFM again differed among treatments for Hour 4, with heifers receiving IL having greater concentrations of PGFM than DEXT ($P < 0.06$)- and 0.5 IL-treated ($P < 0.02$), but not SAL-treated ($P > 0.10$) heifers; concentrations of PGFM from DEXT- and 0.5 IL-treated heifers were not different from each other; and SAL-treated heifers had concentrations of PGFM that were greater than those of 0.5 IL-treated heifers but not different from those of IL- and DEXT-treated heifers. The only significant difference found on Day 11, Hour 8 was that concentrations of PGFM for SAL-treated heifers were greater than those for DEXT- and 0.5 IL-treated heifers.

The above results of data reduction by ANOVA were supported by an overall regression analysis of the plasma concentrations of PGFM over Hours 0, 4, and 8, again with infusion day considered separately but without the covariate of Day 7, Hour 0. This analysis and additional sums of squares *F*-test confirmed a significant effect of treatment for Days 7 ($P = 0.05$) and 11 ($P = 0.05$), but not on Day 9 ($P > 0.10$). Administration of IL on Days 7 and 11 PP resulted in a quadratic response relative to the concentration of PGFM for 0, 4, and 8 h, which differed ($P < 0.01$) from the result with other treatments.

TABLE 1. Fatty acid profiles (mean ± SE) from whole plasma on Days 7 and 11 postpartum.

Fatty acid[a]	Weight (%) following 1 L Intralipid Day 7	1 L Intralipid Day 11	1 L Dextrose Day 7	1 L Dextrose Day 11
C16:0	14.3 ± 0.4	13.7 ± 0.5	14.1 ± 0.3	14.4 ± 0.5
C18:0	14.9 ± 0.2[b]	12.9 ± 0.3	13.8 ± 0.2[c]	12.3 ± 0.4
C18:1	13.9 ± 0.8	8.6 ± 0.7[d]	12.8 ± 0.5	12.3 ± 0.4[e]
C18:2	22.9 ± 1.1	40.5 ± 1.2[d]	22.7 ± 0.6	22.9 ± 0.7[e]
C18:3	10.6 ± 0.8	5.8 ± 0.4[d]	11.3 ± 0.4	11.7 ± 0.3[e]
C20:4	2.6 ± 0.2[f]	2.9 ± 0.1[g]	2.6 ± 0.1[f]	3.0 ± 0.3[g]
C20:5	1.7 ± 0.2	1.2 ± 0.2[d]	2.0 ± 0.1	2.3 ± 0.1[e]
C22:6	0.6 ± 0.1	0.7 ± 0.2[d]	0.5 ± 0.1	0.4 ± 0.0[e]

[a] C16:0, palmitic; C18:0, stearic; C18:1, oleic; C18:2, linoleic; C18:3, linolenic; C20:4, arachidonic; C20:5, eicosapentaenoic; C22:6, docosahexaenoic.
[b,c] Day 7 means within the same row with different superscripts differ ($P < 0.01$).
[d,e] Day 11 means within the same row with different superscripts differ ($P < 0.01$).
[f,g] Percentage of C20:4 differed ($P < 0.01$) between Days 7 and 11, but not among treatments ($P > 0.10$).

Analysis of data for plasma FA on Day 7 revealed that prior to infusion, the weight percentages of major FA in plasma were not different ($P > 0.10$) among treatments; however, significant differences existed at 4 and 8 h. Because linoleic acid (C18:2) is the dietary precursor of PG and because linolenic acid (C18:3) helps to modulate PG synthesis, profiles for these essential FA were most pertinent to this experiment; they are shown in Figure 4. Only samples for Day 7, when differences for 4-h concentrations of PGFM were the greatest, were analyzed for FA profile, and differences among treatments within the same hour are specified in the figure. Differences in C18:2 and C18:3 between hours were also present and depended on treatment and hour (T × H interactions, $P < 0.04$).

Regression analysis of these data revealed a significant quadratic response curve (i.e., increased from 0 to 4 h, then decreased from 4 to 8 h) for linoleic acid with all treatments except SAL. However, for linolenic acid, neither 0.5 IL nor SAL significantly altered this FA, and IL-treated heifers had a linear decrease ($P < 0.05$) in C18:3 over time, while the changes in C18:3 in DEXT-treated heifers resulted in a quadratic response curve ($P < 0.01$).

Other FA in plasma that increased or decreased in response to infusion of treatments on Day 7 are as follows (data not shown): By 4 h, plasma of heifers infused with IL had greater concentrations of palmitic acid (C16:0) and oleic acid (C18:1) than that of DEXT-, 0.5 IL-, and SAL-treated heifers ($P < 0.02$ and $P < 0.01$, respectively); while levels of stearic acid (C18:0) were greater than in DEXT-treated ($P < 0.06$) but less than in SAL-infused heifers ($P < 0.01$). By 8 h, plasma C16:0 did not differ ($P > 0.10$) among treatments; but IL-infused heifers had lower ($P < 0.01$) concentrations of C18:0 than those treated with SAL and greater ($P < 0.04$) C18:1 levels than those treated with DEXT, 0.5 IL, and SAL. No significant correlation was present for percentage of linoleic acid and PGFM concentration on Day 7, Hour 4.

On Day 7, FA elongation products were as follows: Plasma arachidonic acid (C20:4ω6) did not differ among treatments on Day 7 for samples examined during the infusion period (Table 1; data for 0.5 IL- and SAL-treated heifers not shown). By 4 h, plasma levels of eicosapentaenoic acid

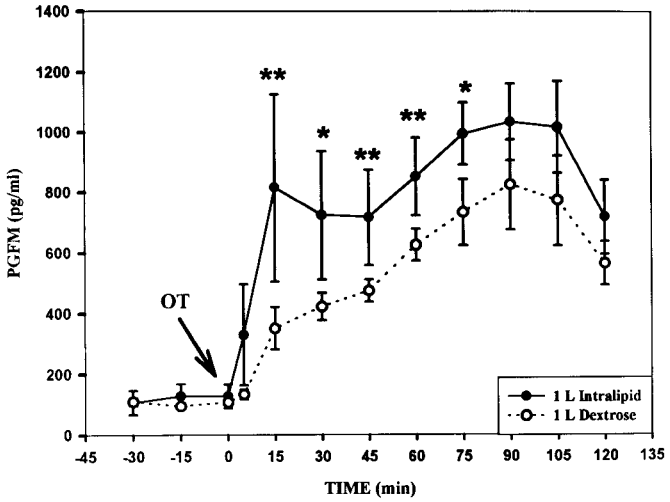

FIG. 5. Response of PGFM (mean ± SE) to OT challenge (150 IU i.v.; Time 0 h). *$P < 0.10$ and **$P < 0.05$ for differences between means.

(EPA; C20:5ω3) were lower ($P < 0.01$) for heifers receiving IL than for heifers subjected to other treatments. Plasma levels of docosahexaenoic acid (C22:6ω3) were greater for IL-treated heifers than for those treated with DEXT and SAL, but not 0.5 IL. By 8 h, EPA was lower ($P = 0.03$) for heifers given IL than for those receiving DEXT and 0.5 IL.

Results of FA plasma analyses revealed that initial plasma FA content did not differ significantly among treatments, with the exception that initial percentage of stearic acid in plasma was greater in IL-treated heifers than in heifers from other treatments (Table 1). Data from FA analysis for Day 11, Hour 0 plasma from IL- and DEXT-treated heifers were included in order to evaluate plasma FA profile (specifically, linoleic acid) prior to OT challenge. Weight percentage of C18:2 in Day 11 plasma was greater in heifers receiving IL ($P < 0.01$) than for DEXT-treated heifers.

OT-induced release of PGFM on Day 12 was greater ($P < 0.06$) for lipid-infused as compared with DEXT-infused heifers (Fig. 5; untransformed data), but no significant correlation between linoleic acid on Day 11 and PGFM response to OT injection on Day 12 was detected.

There were no differences ($P > 0.10$) among treatments for reproductive data (Table 2). According to the date of their second calving, all heifers diagnosed as pregnant conceived on their first estrus PP; 36% had no detected P_4 rise prior to first estrus, while 64% showed a transient (5.4 ± 0.8 days) increase in plasma P_4 (0.65 ± 0.25 ng/ml).

There were no differences ($P > 0.10$) among treatments for BW and condition scores (Table 3). Heifers lost BW ($P = 0.08$), and BCS decreased ($P < 0.01$) during the first 30 days PP. At 150 Days PP, heifer BW ($P = 0.40$) and BCS ($P = 0.53$) were not different from the values on Day 30 PP; however, BCS at Day 150 was significantly lower ($P < 0.01$) than for these heifers immediately prior to calving. No difference ($P > 0.10$) among treatments was found for calving assistance (75% unassisted), calf weights, calf sex (50% male), and mean daily intake of meadow and alfalfa hay as a percentage of metabolic body weight (9.4% of $BW^{.75}$).

DISCUSSION

Infusion with 1 L IL resulted in higher plasma linoleic acid and PGFM concentrations more consistently than other treatments, and both repeated-measures ANOVA and regression analysis were consistent with respect to this conclusion. These data are compatible with the hypotheses that infusion of lipid changes plasma FA profile and that increased plasma linoleic acid increases systemic $PGF_{2\alpha}$. However, administration of lipid only transiently increased plasma PGFM; no carryover effects on basal plasma concentrations of PGFM were detected on subsequent days. The decrease in PGFM over Days 1–15 in this experiment is similar to that previously reported, and it reflects the naturally declining plasma concentrations of $PGF_{2\alpha}$ in PP cows [2]. Inability of short-term infusion of essential FA (Days 7–11) to alter overall PP profile of PGFM may be due to factors other than substrate availability controlling this process.

Timing in delivery of exogenous lipid is apparently an important factor in stimulating PG production. This is exemplified in previous experiments with heifers [4] and ewes [5] in which response was dependent on day of the estrous cycle, and in this experiment with PP anestrous heifers, in which day of infusion affected PGFM response. Thus to obtain a greater response, a more appropriate regimen might have been to provide PP heifers with lipid earlier in the puerperium when uterine $PGF_{2\alpha}$ production is normally enhanced. The time factor may be a reflection of synthesis

TABLE 2. Mean number of days to first estrus, pregnancy rate, and calving interval.

	Treatment[a,b]				
Item	IL	DEXT	0.5IL	SAL	SE[c]
Days to first estrus[d]	129.5	126.3	120.0	133.2	6.0
Pregnancy rate[e] (%)	75	80	100	100	nd
Calving interval (days)	409.7	406.3	397.7	410.4	6.3

[a] IL, 1 L Intralipid (n = 4); DEXT, 1 L dextrose (n = 5); 0.5 IL, 0.5 L Intralipid (n = 5); SAL, 1 L saline (n = 5).
[b] No difference among treatments for any item ($P > 0.10$).
[c] Common estimate of the standard error; nd, not determined.
[d] For two heifers from DEXT, days to first estrus were estimated from calving date and plasma progesterone. For one heifer each from IL and DEXT, days to first estrus were estimated from calving date and behavioral estrus.
[e] Due to illness one IL-treated heifer was excluded from pregnancy rate data.

TABLE 3. Mean body weights, body condition scores, feed intake, and calf weights.

	Treatment[a,b]				
Item	IL	DEXT	0.5 IL	SAL	SE[c]
BW (kg)					
Day 1 PP	363.6	336.6	368.6	341.8	8.9
Day 30 PP	344.4	335.8	346.8	335.0	8.7
Day 150 PP	344.6	332.1	348.8	345.6	8.2
BCS					
Day 1 PP	5.4	5.3	5.3	5.1	1.0
Day 30 PP	4.3	4.0	3.9	3.4	1.0
Day 150 PP	3.9	3.7	3.5	3.8	0.7
Feed intake[d] (% $BW^{.75}$)	9.4	9.4	8.9	9.7	0.7
Day 1 PP	34.5	33.9	31.8	33.0	2.4
Day 150 PP	129.7	136.2	131.4	132.1	7.8
Day 240 PP	205.2	202.3	184.5	199.6	8.6

[a] IL, 1 L Intralipid; DEXT, 1 L dextrose; 0.5 IL, 0.5 L Intralipid; SAL, 1 L saline.
[b] There were no differences among treatments for any item ($P > 0.10$).
[c] Common estimate of the standard error.
[d] Daily intake of hay (alfalfa and meadow hay) expressed as a percentage of metabolic BW.

capability, for example, presence of enzymes, at various reproductive periods. In the guinea pig, an increase in PG synthase (PGS) protein, the enzyme required for synthesis of PG, increases both uterine PG synthesis toward the end of the estrous cycle [11] and ovarian PG synthesis just prior to ovulation [12]. Similar patterns of PGS presence in these tissues exist for the ovine uterus [13] and bovine preovulatory follicles [14]. Studies on the presence and regulation of PGS in the PP uterus are lacking, but it is known that expression of PGS is highly regulated in the rat uterus during pregnancy and parturition [15], and physiological stimulus for PG synthesis and release is regulated during luteolysis in the guinea pig [16].

Weight percentage of linoleic acid in plasma prior to treatment in this experiment is in agreement with values reported by Jenkins [17]. Changes in FA profiles during infusion were complex, but they reflect the content of lipids administered. A dose response in PGFM with increasing quantity of lipid infused (no lipid [SAL], 0.5 IL, and IL treatments) was observed at 4 h after infusion. It remains a possibility that further supplementation with lipid could increase secretion of PG. Budowski [18] demonstrated that production of PG in humans increased with increasing dietary linoleic acid up to a specific level, and then no further response was achieved. Filley et al. [19] demonstrated that plasma concentrations of linoleic acid and $PGF_{2\alpha}$ increase in response to feeding rumen-protected FA to PP beef heifers.

Infusion of lipid altered plasma FA profiles for at least 18 h postinfusion (time between last infusion on Day 10 and preinfusion sample obtained on Day 11). These changes were pronounced, and it is apparent that plasma and possibly tissue loading of essential FA occurred as reported with abomasal infusion of FA in sheep [20] and in cows fed or injected with fat [21]. During infusions the change in weight percentage of FA was small and sometimes nonsignificant. The volume of infusant and absolute quantity of individual FA infused were small relative to the volume of plasma in the animal and amount of FA in plasma. Some FA increased and some decreased during the infusion period, thus altering ratios of essential FA to nonessential FA and ratio of saturated to polyunsaturated FA. Mathias and Dupont [22] reported that PG production may be more sensitive to changes in ratios rather than absolute amount of FA.

In contrast to the results of the present and related in vivo research [19] involving lipid effects on $PGF_{2\alpha}$ production, it has been concluded from in vitro studies that linoleic acid suppresses the production of this eicosanoid [23–25]. Relationships between lipid administration and PG production must be examined closely because linoleic, oleic, and to a greater degree linolenic acid are known to have inhibitory effects on in vitro production of PG in rat stomach homogenates and ram seminal vesicles [23]. Danet-Desnoyers et al. [24] and Thatcher et al. [25] also reported inhibitory effects on $PGF_{2\alpha}$ production by cytosolic components from endometrial explants of pregnant cows. These components were initially associated with serum albumin and were later isolated and identified as including C16:0, C18:0, C18:1, C18:2, and C18:3 FA. Gan-Elepano et al. [26] described the intricacy of PG production that occurs within microsomal membranes and reported that membrane fluidity, and substrate, enzyme, and cytosolic protein concentrations, are difficult to replicate. Leikin et al. [27] also published information on a specific protein required for desaturation of C18:2ω6, which is apoprotein-like in nature and is not substituted for by albumin. Albumin in that study bound the linoleic acid and inhibited its desaturation. Although Burke et al. [5] considered a potential inhibitory effect of linoleic acid on $PGF_{2\alpha}$ production during the estrous cycle in ewes, no evidence for such an effect of this FA on production of $PGF_{2\alpha}$ was detected in the present study.

Infusion of lipid increased the capacity of PP heifers to synthesize and/or secrete $PGF_{2\alpha}$ as indicated by plasma concentration of PGFM after OT injection. OT causes the release of $PGF_{2\alpha}$ from the endometrium by binding to OT receptors in that tissue [28]. The pattern of plasma PGFM increase after OT injection is in agreement with a previous report by Del Vecchio et al. [29]. Low baseline PGFM concentrations prior to OT injection demonstrate that the immediate increase in PGFM after OT is not merely decreased clearance of eicosanoid hormone caused by lipid infusion. Also, heterogeneity of variance apparent in the production of PGFM after OT in the current study exemplifies the difference in capacity of individual heifers to respond to supplemental lipid. Differences in ability to respond to supplemental lipid in a manner favoring fertility have also been reported for cows with differing BCS [30, 31], different duration of supplement feeding [32, 33], and differing type of supplemental lipid [34, 35]. Lack of a significant correlation between plasma concentrations of linoleic acid on Day 11 PP and PGFM on Day 12 after OT injection is an intriguing result. However, it may reflect the use of tissue stores of PG precursors rather than plasma linoleic acid for $PGF_{2\alpha}$ production.

Although PGFM concentrations were transiently altered early in the PP period by infusion of lipid into heifers, no change in fertility was found. BCS and BW were unaffected by treatment and adequate at calving; however, nutritional status during the breeding season was undoubtedly a factor in the long PP interval of heifers in this experiment. Low BCS and BW are known to negatively affect efficiency of rebreeding [36], and cows gaining weight during the breeding season have increased conception rates compared with those losing weight [37]. Calf weights were also adequate and unaffected by treatment. These latter data suggest that calf survival supersedes reproductive function with respect to nutrient partitioning [38].

The extended PP anestrous period for the heifers in the current experiment may have made uterine effects less influential on ovarian activity as compared with those in cows that resume ovarian activity earlier in the PP period. This may provide an explanation for the lack of short cycles in these heifers and their high conception rate from breeding at first estrus after parturition. Cows returning to estrus after a period of PP anestrus frequently have estrous cycles of short duration (10–12 days) with plasma concentrations of P_4 greater than 1 ng/ml prior to resumption of estrous cycles of normal length [39]; however, these short cycles are not a prerequisite for normal cyclicity [40]. This P_4 ''priming'' of the system is thought to be important for supporting a pregnancy from subsequent ovulations by controlling uterine OT receptor populations and premature release of PG responsible for early demise of CL frequently observed in early PP cows [41, 42].

Initial evidence that infused lipid alters PGFM in the PP period is provided in this study, and further investigations on how the FA are distributed among plasma lipid fractions of cholesterol esters (CE), phospholipids (PL), non-esterified FA (NEFA), or triglycerides (TG) are now under way (D. Palmquist, personal communication). According to Palmquist, this information is important because in the ruminant, essential FA are selectively used for storage CE and are incorporated into PL that are used for essential functions, such as PG

production, rather than being incorporated into adipose tissue for energy stores as are TG and NEFA. Also, characterization of bovine uterine PGS in the PP period is needed, and further research to improve fertility in primiparous heifers using FA or other specific nutrients to favorably alter hormone concentrations is warranted.

In conclusion, infusion of IL into PP heifers alters plasma FA profile to favor linoleic acid content, transiently increases systemic PGFM, and increases the capacity of PP heifers to produce $PGF_{2\alpha}$ as indicated by plasma PGFM after OT injection. Reproductive response was not improved by short-term infusion of lipid in the early PP period.

ACKNOWLEDGMENTS

The authors gratefully acknowledge Dave Thomas (Department of Statistics, Oregon State University) for statistical assistance and Rosemary Wander (Department of Nutrition and Home Economics, Oregon State University) for use of the gas chromatograph.

REFERENCES

1. Wiltbank JN. Research needs in beef cattle reproduction. J Anim Sci 1970; 31:755–762.
2. Guilbault LA, Thatcher WW, Drost M, Hopkins SM. Source of F series prostaglandins during the early postpartum period in cattle. Biol Reprod 1984; 31:879–887.
3. Madej A, Kindahl H, Woyno W, Edqvist LE, Stupnicki R. Blood levels of 15-keto-13,14-dihydro-prostaglandin $F_{2\alpha}$ during the postpartum period in primiparous cows. Theriogenology 1984; 21:279–287.
4. Lucy MC, Gross TS, Thatcher WW. Effects of intravenous infusion of a soybean oil emulsion on plasma concentration of 15-keto-13,14-dihydro-prostaglandin $F_{2\alpha}$ and ovarian function in cycling Holstein heifers. In: Livestock Reproduction in Latin America. Vienna, Austria: International Atomic Energy Agency; 1990: 119–132.
5. Burke JM, Carroll DJ, Rowe KE, Thatcher WW, Stormshak F. Intravascular infusion of lipid into ewes stimulates production of progesterone and prostaglandin. Biol Reprod 1996; 55:169–175.
6. Lammoglia MA, Bellows RA, Grings EE, Bergman JW, Short RE, MacNeil MD. Effects of dietary fat composition and content, breed and calf sex on birth weight, dystocia, calf vigor and postpartum reproduction of first calf beef heifers. Proc West Sec Am Soc Anim Sci 1997; 48:81–84.
7. Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. Can J Biochem Physiol 1959; 37:911–917.
8. Song J, Wander RC. Effects of dietary selenium and fish oil (Max-EPA) on arachidonic acid metabolism and hemostatic function in rats. J Nutr 1991; 121:284–292.
9. Koligian KB, Stormshak F. Progesterone synthesis by ovine fetal cotyledons *in vitro*. J Anim Sci 1976; 42:439–443.
10. SAS. Version 6.10. Cary, NC: Statistical Analysis Systems Institute, Inc.; 1994.
11. Poyser NL, Leaver HA. Prostaglandin production by the uterus. Prostaglandins Reprod Physiol 1979; 91:195–204.
12. Poyser NL. Hormonal control of prostaglandin in reproductive tissues. In: Bakhle YS (ed.), Prostaglandins in Reproduction. Chichester, United Kingdom: Research Studies Press; 1981: 213–229.
13. Huslig RL, Fogwell RL, Smith WL. The prostaglandin forming cyclooxygenase of ovine uterus: relationship to luteal function. Biol Reprod 1979; 21:589–600.
14. Sirois J. Induction of prostaglandin endoperoxide synthase-2 by human chorionic gonadotropin in bovine preovulatory follicles *in vitro*. Endocrinology 1994; 135:841–848.
15. Sirois J, Hawkins HK. Upregulation of rat uterine prostaglandin endoperoxide synthase (isoform 1) at parturition. Biol Reprod 1992; 46(suppl 1):178.
16. Poyser NL. A possible explanation for the refractoriness of uterine prostaglandin production. J Reprod Fertil 1991; 91:371–384.
17. Jenkins TC. Butylsoyamide protects soybean oil from ruminal biohydrogenation: effects of butylsoyamide on plasma fatty acids and nutrient digestion in sheep. J Anim Sci 1995; 73:818–823.
18. Budowski P. Alpha-linolenic acid and the metabolism of arachidonic acid. In: Galli C, Simopoulos AP (eds.), Dietary ω3 and ω6 Fatty Acids. Biological Effects and Nutritional Essentiality. NATO ASI Series. Life Sci 1988; 171:97–110.
19. Filley SJ, Turner HA, Stormshak F. Plasma fatty acids, prostaglandin $F_{2\alpha}$, and fertility of postpartum heifers fed rumen by-pass fat. J Anim Sci 1998; 76(suppl 1):229.
20. Moore JH, Noble RC, Steele W. The incorporation of linolenic and linoleic acids into plasma lipids of sheep given intra-abomasal infusions of linseed oil, maize oil or linoleic acid. Br J Nutr 1969; 23: 141–152.
21. Tove SB, Mochrie RD. Effect of dietary and injected fat on the fatty acid composition of bovine depot fat and milk fat. J Dairy Sci 1963; 46:686–689.
22. Mathias MM, Dupont J. The relationship of dietary fats to prostaglandin biosynthesis. Lipids 1979; 14:247–252.
23. Pace-Asciak C, Wolfe LS. Inhibition of prostaglandin synthesis by oleic, linoleic and linolenic acids. Biochim Biophys Acta 1968; 152: 784–787.
24. Danet-Desnoyers G, Johnson JW, O'Keefe SF, Thatcher WW. Characterization of a bovine endometrial prostaglandin synthesis inhibitor (EPSI). Biol Reprod 1993; 48(suppl 1):115.
25. Thatcher WW, Staples CR, Danet-Desnoyers G, Oldick B, Schmitt EP. Embryo health and mortality in sheep and cattle. J Anim Sci 1994; 72(suppl 3):16–30.
26. Gan-Elepano M, Aeberhard E, Mead JF. On the mechanisms of fatty acid transformations in membranes. Lipids 1981; 16:790–795.
27. Leikin AI, Nervi AM, Brenner RR. Lipid binding properties of a factor necessary for linoleic acid desaturation. Lipids 1979; 14:1021–1026.
28. Roberts JS, McCracken JA, Gavagan JE, Soloff MS. Oxytocin-stimulated release of prostaglandin $F_{2\alpha}$ from ovine endometrium in vitro: correlation with estrous cycle and oxytocin-receptor binding. Endocrinology 1976; 99:1107–1114.
29. Del Vecchio RP, Chase CC Jr, Bastidas P, Randel RD. Oxytocin-induced changes in plasma 13,14 dihydro-15 keto prostaglandin $F_{2\alpha}$ concentrations on Days 10, 20 and 30 postpartum in the bovine. J Anim Sci 1990; 68:4261–4266.
30. Morgan AR, Williams GL. Effect of body condition and postpartum dietary lipid intake on lipid metabolism and GnRH-induced luteal function in postpartum beef cows. J Anim Sci 1989; 67(suppl 2):60.
31. Spoon RA, Morgan AR, Welsh TH, Williams GL. Interactions of postpartum dietary lipid intake and body condition on serum nonesterified fatty acid and insulin concentrations in multiparous beef cows. J Anim Sci 1990; 68(suppl 1):421.
32. Oss GM, Schutz DN, Odde KG. Effects of a high fat diet on reproductive performance in pre- and postpartum beef heifers. Proc West Sec Am Soc Anim Sci 1993; 44:44–47.
33. Lucy MC, Staples CR, Michel FM, Thatcher WW, Bolt DJ. Effect of feeding calcium soaps to early postpartum dairy cows on plasma prostaglandin $F_{2\alpha}$, luteinizing hormone, and follicular growth. J Dairy Sci 1991; 74:483–489.
34. Wehrman ME, Welsh TH Jr, Williams GL. Diet-induced hyperlipidemia in cattle modifies the intrafollicular cholesterol environment, modulates ovarian follicular dynamics, and hastens the onset of postpartum luteal activity. Biol Reprod 1991; 45:514–522.
35. Beam SW, Butler WR. Energy balance and ovarian follicle development prior to the first ovulation postpartum in dairy cows receiving three levels of dietary fat. Biol Reprod 1997; 56:133–142.
36. Randel RD. Nutrition and postpartum rebreeding in cattle. J Anim Sci 1990; 68:853–862.
37. Wiltbank JN, Rowden WW, Ingalls JE, Zimmerman DR. Influence of post-partum energy level on reproductive performance of Hereford cows restricted in energy intake prior to calving. J Anim Sci 1964; 23:1049–1053.
38. Short RE, Bellows RA, Staigmiller RB, Berardinelli JG, Custer EE. Physiological mechanisms controlling anestrus and infertility in postpartum beef cattle. J Anim Sci 1990; 68:799–816.
39. Odde KG, Ward HS, Kiracofe GH, McKee RM, Kittok RJ. Short estrous cycles and associated serum progesterone levels in beef cows. Theriogenology 1980; 14:105–112.
40. Peters AR, Lamming GE. Reproductive activity of the cow in the post-partum period. II. Endocrine patterns and induction of ovulation. Br Vet J 1984; 140:269–279.
41. Lishman AW, Inskeep EK. Deficiencies in luteal function during reinitiation of cyclic breeding activity in beef cows and in ewes. S Afr J Anim Sci 1991; 21:59–75.
42. Beard AP, Hunter MG. Effects of progesterone pretreatment on the oxytocin receptor concentration and the response to oxytocin during the simulated early luteal phase in the ovariectomized ewe. J Reprod Fertil 1994; 102:57–63.