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EFFECTS OF CALVING-RELATED DISORDERS ON PROSTAGLANDIN, CALCIUM, OVARIAN ACTIVITY AND UTERINE INVOLUTION IN POSTPARTUM DAIRY COWS

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ABSTRACT

Postpartum ovarian activity, uterine involution and plasma concentrations of calcium and 15-keto-13,14 dihydro-prostaglandin F2α (PGFM) were assessed in dairy cows with retained fetal membranes (n=10) and milk fever (n=10) at parturition. In addition, calcium and PGFM were evaluated in dairy cows affected with uterine prolapse (n=10) and pyometra (n=14). Cows with retained fetal membrane averaged 24.2$\pm$3.7 d until their first postpartum ovulation, while controls averaged 29.0$\pm$3.7 d ($P>0.10$). In cows with retained fetal membranes, the difference in follicular activity between the contralateral and ipsilateral ovaries in relation to the previously gravid uterine horn was appreciably greater post partum when compared with that of the controls. Cows with milk fever had an average of 30.8$\pm$3.1 d until their first postpartum ovulation, while control cows had an average of 20.4$\pm$3.3 d ($P<0.05$). The mean diameter of the uterine horns in cows with milk fever was greater ($P<0.05$) compared with that of the controls between Days 15-32 post partum. Concentrations of plasma calcium were lower in cows with retained fetal membranes within 24 h after parturition and during the first week post partum than in the controls (6.27$\pm$0.18 vs 7.40$\pm$0.18 mg/100ml, $P<0.05$). Concentration of calcium was lower ($P<0.05$) in cows with milk fever on Day 1 prior to treatment (4.68$\pm$0.40 < 5.8$\pm$0.45 mg/100ml) than in control cows; however, the calcium (Ca) level was not different during the subsequent 7 d post partum after treatment. Cows with uterine prolapse had lower concentrations of Ca during the first 7 d post partum than the controls (6.10$\pm$0.15 vs 7.33$\pm$0.12mg/100ml;$P<0.01$). Cows with pyometra had higher ($P<0.05$) concentrations of plasma PGFM than the controls (208.$\pm$13.2>138.1$\pm$15.2).

Key words: Post partum, dairy cows, prostaglandin, calcium, calving-related disorders

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INTRODUCTION

The involuting postpartum uterus produces large amounts of F-series prostaglandins reflected by high secretion of prostaglandin $F_2\alpha$ and elevated peripheral concentrations of its metabolite 15 - keto -13, 14 dihydroprostaglandin $F_2\alpha$ (PGFM ; 9,10,16,24). The major increase in concentration of PGFM is around 4 d post partum, with concentrations returning to basal levels by 10 to 20 days post partum (9,10,46). A longer duration and higher magnitude of postpartum plasma concentration of PGFM have been associated with a faster rate of uterine involution (25,27); others, (18), however, have obtained normal uterine involution with reduced levels of $PGF_{2\alpha}$. Higher concentrations of $PGF_2\alpha$ early in the postpartum period have also been implicated in the early resumption of ovarian activity (18,19).

Cows with postpartum intrauterine infection have been reported to have longer periods of $PGF_{2\alpha}$ release than cows without uterine infection (3,26). Because uterine involution involves contractions and peristalsis as rhythmical waves of the uterine musculature (39), conditions that lower blood calcium content or production of $PGF_{2\alpha}$ from the uterus may reduce uterine involution and prolong intrauterine infection that may be present. Hypocalcemic-related disorders such as retained fetal membranes (RFM) and milk fever prolong the period of uterine involution and postpartum anestrus by predisposing cows to uterine infections (5,12,32). The relationships of RFM and milk fever to PGFM and calcium concentrations, uterine involution and ovarian activity are not consistent or well documented. In our experiment we examined the relationship between PGFM and calcium activity in dairy cows affected with postpartum disorders. Our objectives were 1) to assess postpartum uterine involution and ovarian activity in cows with retained fetal membranes and milk fever; 2) to relate these changes to the dynamics of plasma concentrations of PGFM, progesterone and calcium concentrations; and 3) to determine plasma concentrations of PGFM and calcium in cows with uterine prolapse and pyometra, conditions associated with reduced blood calcium and intrauterine infection, respectively (33,38).

MATERIALS AND METHODS

Eighty seven Holstein cows from 12 commercial dairy herds in Chino, California, were used in the study during the period of January 13, 1985, to May 6, 1986. Herds in this area are managed in drylot systems, and contained 300 to 1000 cows each. Veterinary health care was provided by the Chino Valley Veterinary Associates (C.A.Risco).

Ten cows that had retained fetal membranes (RFM) longer than 12 h after calving and that were free of signs of milk fever were paired with 10 control herdmates that calved normally and expelled their membranes on the same day. Similarly, 10 paretic cows with signs of milk fever but without RFM were paired with 10 herdmates that underwent normal parturition on the same day. All cohorts were matched for parity, milk production $\pm$ 250 kg and occurrence of dystocia. A blood sample was collected 24 h after calving and then daily for the next 13 d in cows with RFM and milk fever and a

matched control group of cows. Thereafter, blood was collected 3 times a week until Day 60 post partum. Blood samples from cows with milk fever were collected prior to calcium therapy. Ten milliliters of jugular blood were collected from each cow into heparinized vacutainers and placed immediately in an ice bath. Blood was taken to the laboratory within 6 h of collection and centrifuged at 2000 x g for 30 min. Plasma was then harvested and stored at -20^0 C. Concentrations of PGFM (through d 18 post partum), calcium (Ca; through d 7 post partum) and progesterone (P_4; 3 times a week) were determined in plasma for cows in the RFM and milk fever matched control groups.

Beginning 7 d post partum, the reproductive tract of each cow in the RFM and milk fever groups was examined per rectum after blood samples had been collected on each Monday, Wednesday and Friday until 60 days post partum. The cervical diameter and diameter of each uterine horn at the external bifurcation were recorded at each palpation. The previously gravid uterine horn was identified as the one with the larger diameter during the first 30 d after calving. Cows were scored for body condition at parturition and weekly thereafter to evaluate body condition changes using the method described by Wildman et al.(50). The diameter of the largest palpable follicle and presence of a CL on each ovary were recorded at palpation. Large follicles were diagnosed as vesicular structures possessing a smooth outline with fluctuation below the surface. The presence of a CL was determined by ovarian shape, distortion of ovary and the presence of a line of demarcation in the ovary between the CL and ovarian tissue as described by Zemjanis (52). Similar to reports by Guilbault et al. (18,19) the presence of either a follicle or CL beginning on Day 7 through Day 60 post partum was reported as an estimate of ovarian activity. Days from calving to ovulation (resumption of cyclicity) was defined as the first day when progesterone concentration exceeded 1 ng/ml and reached normal luteal levels in 3 consecutive samples (5 to 6 d) in the absence of a cystic structure. If a cow did not resume cyclicity within the prescribed 60-day postpartum period, 60 d was assigned arbitrarily for resumption of cyclicity. The duration of the luteal phase after the first ovulation was defined as the number of days post ovulation that the P_4 concentration was greater than 1ng/ml. The number of estrous cycles was defined as the total number of luteal phases that occurred during the 60-d postpartum period.

Ovarian cystic degeneration (OCD) was diagnosed according to the following criteria: a follicle-like structure greater than 25 mm in either ovary that persisted for at least 10 d in the absence of a palpable corpus luteum. Ovarian cysts were classified as either being follicular or luteal according to the above criteria and to the progesterone concentration in the plasma as described by Kessler et al. (22). Cows diagnosed clinically as cystic but with a progesterone concentration of less than 1 ng/ml were considered to posses follicular cysts. Conversely, cows with progesterone concentrations between 1 to 2 ng/ml without evidence of uterine pathology or discharge were considered to posses luteal cysts.

Cows in the RFM and milk fever groups along with their corresponding controls were obtained from 2 dairies with comparable nutritional management programs. Treatment for these conditions was requested on a routine basis. Cows with milk fever received a 23% calcium borogluconate solution intravenously within 30 min after the

condition was diagnosed. The first blood samples from cows with milk fever were collected prior to calcium administration and only 1 treatment was required. Cows with RFM were treated at the time of diagnosis with intrauterine boluses containing 6 g of oxytetracycline on alternate days until the membranes were expelled.

Cows with uterine prolapse and pyometra and their matched control groups were obtained from 12 dairies. Ten cows affected with uterine prolapse were paired with 10 control herdmates that calved normally. Likewise 14 cows with pyometra (retention of purulent exudate in the uterine lumen and a corpus luteum present), detected after 60 d post partum and with a history of being anestrous were paired with 13 herdmates without pathological changes in the uterus but with a corpus luteum.

Blood samples from cows with a uterine prolapse were collected within 3 h after calving and then daily for the next 14 d post partum, while cows with pyometra and their matched controls had blood samples collected for 7 consecutive days after the diagnosis of pyometra or the selection of control cows,respectively. Blood plasma was analyzed for PGFM (through d 14 post partum) and calcium (through 7 d post partum) concentrations in cows with uterine prolapse and in their matched controls. Progesterone, calcium and PGFM concentrations were determined in all 7 samples of cows with pyometra and their matched control herd mates. Techniques for blood collection and handling were similar to those previously described for cows with RFM and milk fever.

Hormone Assay

Concentrations of progesterone in plasma were determined by a competitive binding radioimmunoassay validated for our laboratory (23). The sensitivity was 0.15 ng/ml, and the intra-/and inter-assay coefficients of variation were 9.2 and 12.0%, respectively. Unextracted plasma samples were assayed for PGFM using a polyethylene glycol radioimmunoassay system previously validated in our laboratory by Guilbault et al. (16). The intra- and inter-assay coefficients of variation for a reference sample of 1000 pg/ml were 7.0 and 13.5%, respectively. Total plasma calcium concentrations were measured utilizing a Calcette 4009 unit (Precision Systems, Inc., Sudbury, MA). The system uses ethylene glycol-bis-N-N'tetraacetic acid (EGTA) for fluorometric titration of calcium (1).

Statistical Analysis

Data were analyzed by method of least squares analysis of variance using the General Linear Model procedures of the Statistical Analysis System (41). Plasma concentrations of PGFM and calcium, the body condition score, the diameter of the cervix and uterine horns were evaluated by polynomial regression analysis. The mathematical model included pair, treatment, their interactions, time up to the third (cubic) order of polynomial regression, and the interactions of treatment by time. Thus a regression curve was established for each treatment as well as a single pooled within treatment-pair curve. This made possible the test for parallelism (e.g., homogeneity of regression). This test has been demonstrated by Snedecor (42), Damron and Harvey (8),

Wilcox et al. (49), and Guilbault et al. (15). Because the ovarian activity of postpartum cows is not synchronized and timing varies markedly among cows, plasma concentrations of progesterone and ovarian responses (diameter of largest follicle and presence of CL) were transformed by additively accumulating the experimental response over the 60-day period. This transformation provides an overall postpartum response of the cow which is then integrated into a single, continuous postpartum response function for the entire 60 day period (19,20). For example, the dynamics of individual progesterone profiles (occurrence of a progesterone rise, magnitude of the rise, and number of rises) contribute to the overall postpartum response. The accumulated response curves are analyzed as described above. However, ovarian palpation responses also included the effect of ovary (contralateral or ipsilateral to the previous pregnant uterine horn) and higher order interactions (ovary by day, treatment by day and treatment by ovary by day) in the mathematical model.

RESULTS

Expulsion of fetal membranes occurred within 3 d in all RFM-group cows. Only 1 cow with RFM developed septic metritis by Day 8 post partum and was treated with penicillin (3000 units/kg i.m. for 5 d). Her temperature and appetite were normal by Day 14; however, the inability to completely retract the uterus into the pelvis persisted beyond 25 d post partum.

The number of days postpartum to the first ovulation, interval (days) to the second ovulation, the number of estrous cycles and the number of cows affected with OCD for the RFM and milk fever groups are presented in Table 1. There was no difference ($P>0.10$) in the number of days to the first postpartum ovulation between RFM and control cows (24.2 ± 3.7 vs 29.0 ± 3.7 d). One cow in the control group did not ovulate or cycle during the 60 d postpartum period. Duration of the first luteal phase following ovulation was not different ($P>0.05$) in RFM cows compared with that of the control cows (17.0 ± 1.9 vs 13.2 ± 2 d). There was no difference ($P=0.54$) in the number of estrous cycles or the frequency of occurrence of OCD during the 60-d postpartum period between RFM (2/10) and control (2/10) cows. In the RFM group there were 2 follicular cysts, whereas in the control group 1 cow had a follicular cyst and 1 had a luteal cyst. All the cystic cows in the RFM and control groups recovered spontaneously and resumed normal ovarian function by Day 40 post partum. All 4 cystic structures were located on the ovary contralateral to the previously gravid uterine horn.

Cows with milk fever experienced longer ($P<0.05$) intervals to the first postpartum ovulation than control cows (30.8 ± 3.1 vs 20.4 ± 3.3 d, respectively). One cow with milk fever and 1 control group cow did not ovulate or cycle during the 60-day postpartum period. Duration of the first luteal phase following ovulation was longer ($P<0.05$) in cows with milk fever than in control cows (19.2 ± 1.7 vs 13.8 ± 1.8 d). There was no difference ($P=0.10$) in the number of estrous cycles during the 60-d postpartum period between milk fever and control cows (1.5 ± 0.2 vs 2.2 ± 0.3). There was only 1 incidence of OCD in the milk fever group and no incidence in the control group. In the milk fever group, the follicular OCD was diagnosed on the ovary

contralateral to the previously gravid horn, with spontaneous recovery occurring by Day 45.

Table 1. Least squares means and standard error for days to first ovulation, duration of first luteal phase, number of estrous cycles and number of cows affected with ovarian cystic degeneration (OCD) in cows with retained fetal membrane (RFM), milk fever (MF) and in their respective matched control (C) herd mates

Condition	Days to first ovulation	Length of first luteal phase	No. of cycles	Frequency of OCD occurrence
RFM (n=10)	24.2±3.7	17.0±1.9	1.8±0.23	2
C (n=10)	29.0±3.7	13.2±2.1	2.0±0.23	2
MF (n=10)	30.8±3.1*	19.2±1.7*	1.5±0.28	1
C (n=9)	20.4±3.3*	13.8±1.8*	2.3±0.30	0

* significant difference ($P<0.05$) within columns.

Mean concentrations of plasma PGFM for the entire postpartum sampling period did not differ between RFM (0.731±0.048 ng/ml) and control cows (0.755±0.048 ng/ml). Postpartum regression curves for PGFM were not parallel ($P<0.01$); cows in the RFM group had higher PGFM concentrations during the initial 3-d sampling period than control group (Figure 1, Table 2). Thereafter, changes in concentration of PGFM were similar in both groups of cows, returning to basal levels by 14 d post partum. Concentrations of plasma calcium were lower ($P<0.05$) in RFM cows (6.27±0.18 < 7.40±0.18 mg/100ml) than in control cows within 24 h after parturition and throughout the first 8 d postpartum, when compared to controls as indicated by first order regression equations (Table 2) and as depicted in Figure 2.

Regression curves for postpartum accumulated plasma P4 concentrations did not differ ($P>0.10$) between RFM and control cows. However, accumulated diameters (mm) of the largest palpable follicles were affected by the relationship of the ovary to the previously gravid uterine horn and by whether or not fetal membranes were retained (Table 2, Figure 3) In both RFM and control cows follicular activity increased by approximately Day 20 post partum, and follicular activity was greater on the ovary contralateral to the uterine horn of the previous pregnancy. In cows with a RFM, the difference in follicular activity between the contralateral and ipsilateral ovary was appreciably greater post partum compared with that in the control cows (treatment by ovary by day; $P<0.01$). This difference between RFM and control cows was sustained throughout the 60-d period. Although Figure 3 data do not include responses of cows with OCD, responses were similar when cows with OCD were examined.

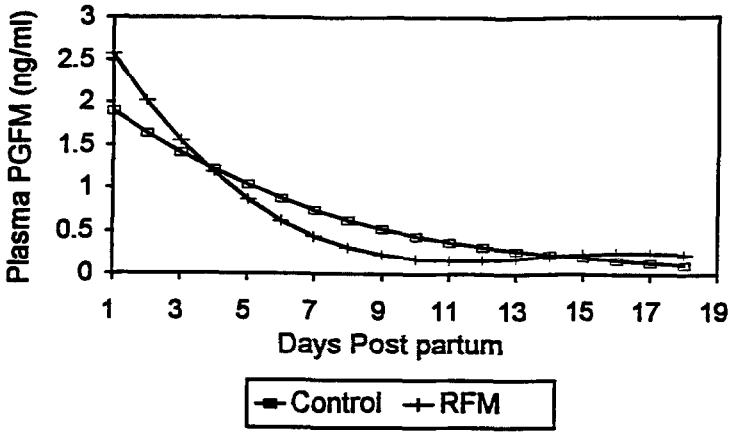

Figure 1. Mean concentration of daily plasma PGFM (ng/ml) between cows with retained fetal membranes (RFM) and control cows. Overall standard error of the mean for daily plasma PGFM estimated at mean day post partum is 0.047 ng/ml.

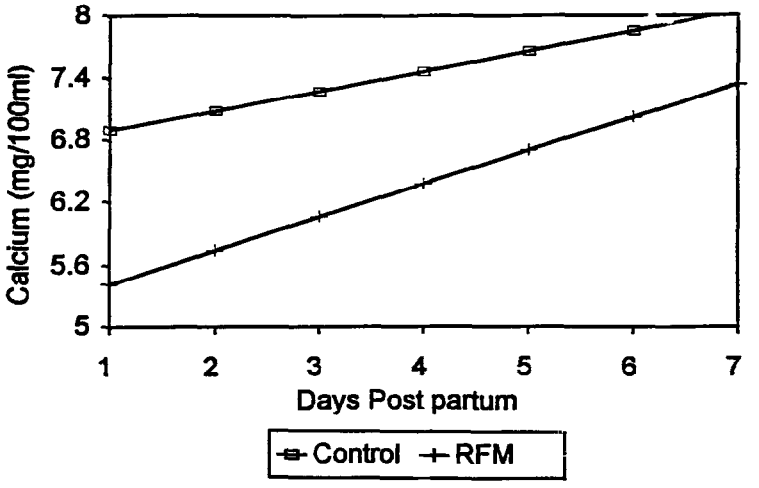

Figure 2. Mean concentration of daily plasma calcium (mg/100ml) between cows with retained fetal membranes (RFM) and control cows. Overall standard error of mean for daily calcium plasma concentration estimated at mean day postpartum is 0.19 mg/ml.

Table 2. Least squares polynomial regression coefficients for postpartum dependent variables.

Response	Group	Side	B0	B1	B2	B3	Total no. of observations	R^2
Sum of largest follicle[a]	Control	Ipsilateral	-21.357	1.6017X ±3.2587	0.00790X^2 ±0.10431	0.000005X^3 ±0.001006	706	0.55
		Contralateral	-52.272	7.7875X ±2.6424	0.15018X^2 ±0.08845	0.001006 ±0.000878		
	Milk fever	Ipsilateral	-80.312	9.5238X ±3.9308	-0.24041X^2 ±0.12304	0.002218X^3 ±0.001171		
		Contralateral	-111.227	9.6469X ±3.4211	-0.13731X^2 ±0.10826	0.000704X^3 ±0.001036		
	Control	Ipsilateral	-44.422 ±4.612	3.1520X ±4.6120	-0.02870X^2 ±0.14248	0.000361X^3 ±0.00135	686	0.53
		Contralateral	-55.905	5.8335X ±3.8788	-0.06797X^2 ±0.12713	0.000214X^3 ±0.001246		
	Retained fetal membranes	Ipsilateral	-112.324	8.3370X ±4.316	-0.12212X^2 ±0.13552	0.000709X^3 ±0.001285		
		Contralateral	-123.808	11.8626X ±3.6627	-0.18523X^2 ±0.11921	0.001173X^3 ±0.001158		
Prostaglandin metabolite	Control		2634.532	-276.067X ±33.7778	7.31572X^2 ±1.61893		320	0.63
	Milk fever		2738.252	-332.0246X ±31.9599	9.6794X^2 ±1.5341			
	Control		2193.488	-304.4110X ±78.1777	16.10032X^2 ±9.06940	-0.312200X^3 ±0.293614	333	0.62
	Retained fetal membranes		3232.94	-704.0724X ±80.3948	52.43306X^2 ±8.94516	-1.254539X^3 ±0.280898		

Response	Group	Side	B0	B1	B2	B3	Total no. of observations	R^2
Calcium	Control		5.736	0.3571X ±0.1007			129	0.43
	Milk fever		5.011	0.4228X ±0.0996				
	Control		6.698	0.1927X ±0.0928			140	0.41
	Retained fetal membranes		5.099	0.3201X ±0.0882				
Body condition score	Control		3.353	-0.0244X ±0.0042	$0.00031X^2$ ±0.00006		449	.7
	Milk fever		3.620	-0.0298X ±0.0040	$0.00025X^2$ ±0.00006			
Uterine horns diameter	Control		113.084	-7.2724X ±0.252	$0.18510X^2$ ±0.0085	$-0.001505X^3$ ±0.00008	878	0.88
	Milk fever		113.433	-6.5499X ±0.2494	$0.15767X^2$ ±0.00838	$-0.001237X^3$ ±0.000083		

[a] Determined in cows without ovarian cystic degeneration.

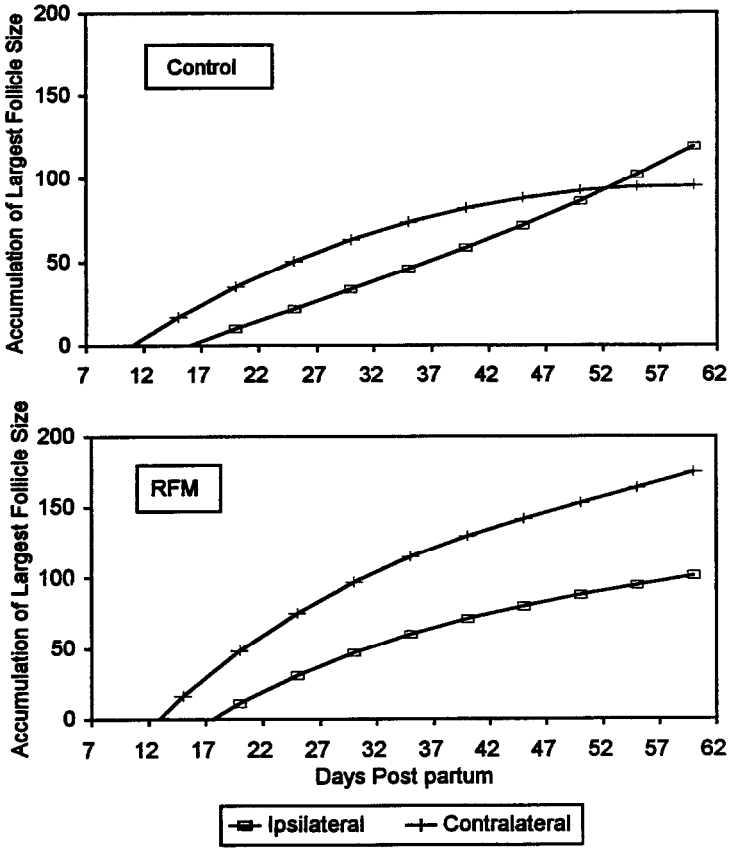

Figure 3. Comparison of accumulated diameter (mm) of largest palpable follicle between cows with retained fetal membranes (RFM) and control cows without ovarian cystic degeneration. Overall standard error of mean for accumulation of largest follicle size estimated at mean day postpartum is 4 mm.

Reduction in the diameter of the cervix was similar in both RFM and control group cows; by Day 40 post partum cervical size remained constant. The previously gravid uterine horn was larger than the nongravid uterine horn in both groups through

40 d post partum. However, no difference was detected ($P > 0.10$) in the uterine diameter between cows with and without RFM throughout the postpartum period.

There was no difference in body condition score at parturition between RFM and control cows; furthermore, the sustained loss in body condition post partum was comparable between the 2 groups.

Mean concentrations of plasma PGFM were not different ($P > 0.10$) between cows with milk fever (0.921 ± 0.049 ng/ml) and control (0.970 ± 0.054 ng/ml) cows during the first 14 d post partum. Moreover, the postpartum regression curves of plasma PGFM were not different between groups described by second order regression equations (Table 2) and shown in Figure 4. By 14 d post partum cows with milk fever and control cows exhibited basal concentrations of PGFM. The concentration of Ca was lower ($P < 0.05$) in milk fever cows on Day 1 prior to clinical treatment than in control cows ($4.68 \pm 0.4 < 5.8 \pm 0.45$ mg/100ml.). However, concentrations of Ca were not significantly different between MF (6.49 ± 0.19 mg/100ml) and controls cow ($7.01 \pm .20$ mg/100ml) during the subsequent 8 d postpartum period following treatment.

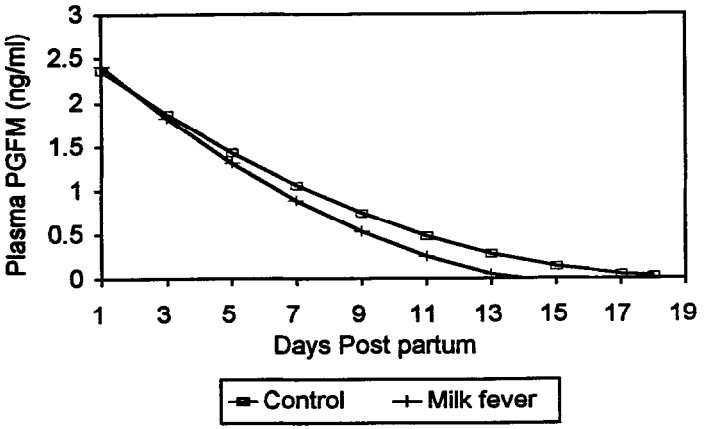

Figure 4. Mean concentration of daily plasma PGFM (ng/ml) in cows with milk fever and control cows. Overall standard error of mean for daily plasma PGFM (ng/ml) estimated at mean day postpartum is 0.052 ng/ml.

Regression curves for accumulated postpartum plasma P4 concentrations were not different ($P > 0.10$) between milk fever and control cows. A clear difference of enhanced follicular activity in the ovary contralateral to the previous pregnant horn was detected in control cows as described by third order polynomial regression equations (Table 2) and shown in Figure 5. However, in milk fever cows this local difference in follicular

activity between ovaries was not as apparent (treatment by ovary by day; $P<0.01$; Figure 5).

Follicular development on the ovary contralateral to the previously pregnant uterine horn was suppressed in milk fever cows and was comparable to development on the ipsilateral ovary. Not until 27 days post partum was follicular development stimulated on the ovary contralateral to the previously pregnant uterine horn in milk fever cows. The cow with OCD was excluded from the analysis of accumulated diameters of the largest palpable follicle.

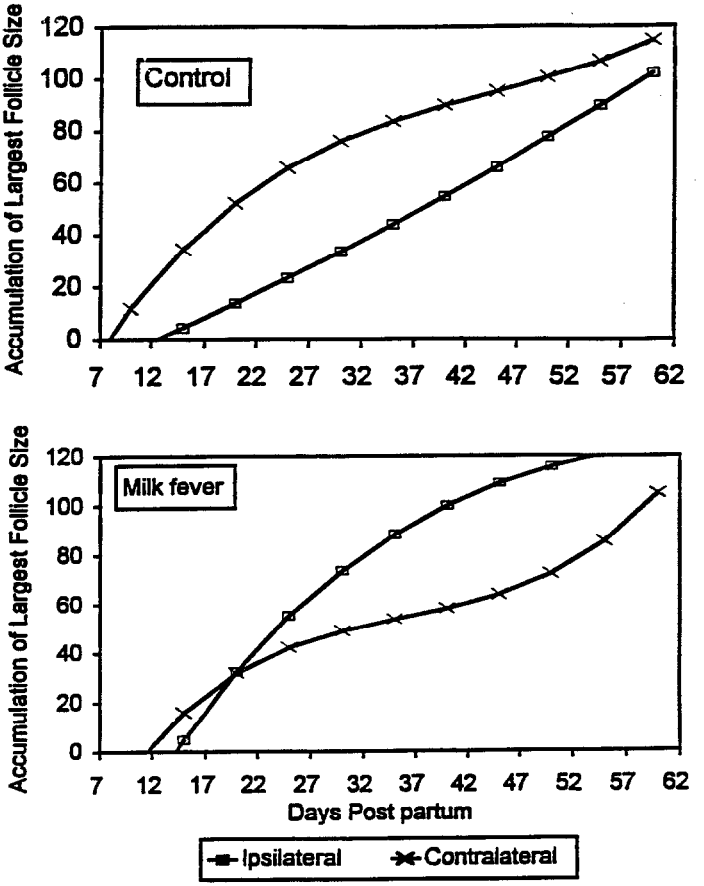

Figure 5. Comparison of accumulated diameter (mm) of largest palpable follicle between milk fever cows and control cows without ovarian cystic degeneration. Overall standard error of mean for accumulation of largest follicle size estimated at mean day postpartum is 3 mm.

The decline in cervical diameter between milk fever and control cows occurred at similar rates, and there was no difference in cervical diameter between the 2 groups at the initial palpation on Day 7 post partum. The diameter of the previously gravid uterine horn was greater than the nongravid uterine horn for both groups through 40 d post partum. However, regression curves for the mean diameter of both uterine horns (y = uterine horn diameter [mm], x = days post partum) between cows with milk fever (Y_{mf} = 113.433 - 6.5499x + 0.1576x^2 - 0.0012x^3) and control (Y_c= 113.084 - 7.2724 x 0.1851x^2.0015x^3) were not the same (Figure 6). The mean diameter of both uterine horns was greater ($P<0.05$) in milk fever cows than in control cows between Days 15 to 32 post partum as shown by third order polynomial regression equations (Table 2, Figure 6).

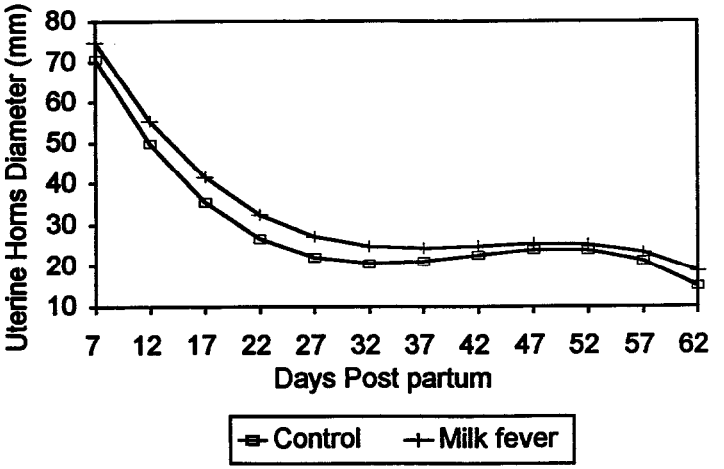

Figure 6. Comparison of average diameters (mm) of both uterine horns for cows with milk fever and control cows over the 60 day postpartum period. Overall standard error of mean for uterine horn diameter estimated at mean day post partum is 0.3 mm.

Cows with milk fever had a higher body condition score at parturition than the control cows as described by second order regression equations (Table 2) and shown in Figure 7. Body condition decreased soon after parturition in both groups; however, milk fever cows had the greater sustained body condition loss. By Day 30 post partum, control cows had begun to regain body condition, whereas cows with milk fever did not begin to regain body condition until after Day 60 post partum.

Cows affected with uterine prolapse had lower ($P<0.01$) calcium concentrations than control cows ($6.10\pm0.15 < 7.33\pm0.12$ mg/100ml). This was evident when examining the different ($P<0.01$) regression equations for the 7-d sampling period:

uterine prolapse cows (Y_{up}=5.9304+0.0425x), and control cows (Y_c=6.3604+0.2463x); $\hat{Y}$= plasma Ca [mg/100ml], x=day post partum. Concentrations of PGFM were not different (P>0.10) between cows with uterine prolapse (514.8±50.9 pg/ml) and control (666.4±58.8 pg/ml) cows. Because they expressed clinical signs of hypocalcemia, 2 of the 10 cows with uterine prolapse were treated intravenously with a calcium borogluconate product.

Mean concentrations of PGFM, calcium and progesterone during a 7-d period for control cows and those with pyometra are presented in Table 3. Cows with pyometra had higher (P<0.05) plasma concentrations of PGFM and progesterone in their plasma than controls. The control cows had a lower mean progesterone level because 7 of 13 cows underwent CL regression during the 7-d sampling period. In contrast, none of the cows with pyometra underwent CL regression. Calcium levels did not differ between the 2 groups.

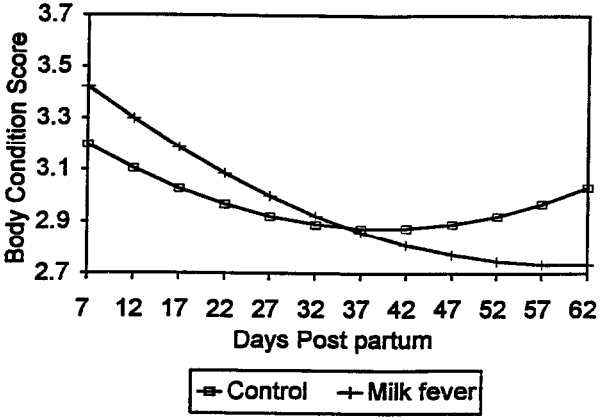

Figure 7. Body condition score (BCS) means for cows with milk fever and for control cows during the 60-day postpartum period. (The loss of body condition was more pronounced in milk fever cows. Overall standard error of mean for BCS estimated at mean day post partum is 0.02).

Table 3. Least squares mean and standard error for plasma concentration of PGFM, calcium and progesterone in postpartum cows with and without pyometra during the 7- day sampling period

Group	n	PGFM (pg/ml)	Calcium (mg/100ml)	Progesterone (ng/ml)
Control	13	138.1 ± 15.2	8.43 ± 0.13	7.73 ± 0.36*
Pyometra	14	208.6 ± 13.2*	8.32 ± 0.11	9.90 ± 0.31*

*Significant difference (P<.05) within columns.

DISCUSSION

Elevated concentrations of PGFM occurred in the peripheral plasma of all postpartum cows. An immediate and rapid decrease within the first 8 to 10 d occurred, with PGFM concentration becoming basal by 14 d post partum. This pattern of PGFM concentrations is similar to that of previous reports (9,10,16,24,46). However, the latter studies (9,10,16,24,46) showed an increase of PGFM the first 2 d post partum followed by a gradual decline with basal levels reached by Days 12 to 14 post partum. This difference can be attributed to the different scheme in blood sampling frequency, which occurred daily beginning at 14 d prepartum. In contrast, the first blood sample for PGFM determination in the present study occurred at 24 h after calving and daily thereafter. Caruncular tissue is more active in the synthesis and metabolism of PGF2α than myometrium and intercaruncular endometrium (16,17). The degree of caruncular development and subsequent amount of postpartum necrosis may determine the extent of uterine PGF2α production (45). In cattle with an indeciduate placenta, PGFM concentrations become basal at the same time that caruncular tissue is lost completely from the uterus around Day 14 post partum.

Higher plasma concentrations of PGFM were evident in RFM than in control cows during Days 1 to 3 post partum. This agrees with a previous report were higher values of PGFM were present 1 to 3 d after parturition in RFM than in control cows (4). Early activation of $PGF_2\alpha$ release from the uterus has been associated with certain placental prepartal events involved in the etiology of retained fetal membranes (37). Bjorkman and Sollen (2) demonstrated pathological conditions of the placenta which appear to have been present in RFM cows before parturition. Whether or not treatment with oxytetracycline boluses caused endometrial irritation and contributed to the initial increase in PGFM concentration in this study cannot be ruled out.

Cows with RFM also have a greater rate of uterine contractions (30). A known property of $PGF_2\alpha$ is its ability to stimulate smooth muscle contraction. Myometrial contraction is of paramount importance in the involutionary process of the postpartum uterus. The correlation between higher endogenous PGFM concentrations and a faster rate of uterine involution has been reported in some studies (24,25,27), while in others (18) normal uterine involution was obtained with reduced levels of PGFM. Uterine involution is complete when the uterus returns to its nongravid position in the pelvis and the asymmetry between uterine horns diameter is minimal (6). Uterine involution was complete by approximately 40 d post partum in both the control and RFM cows. Similar temporal relationships in reduction of uterine size during the postpartum period have been made by others (10,24,29,40).

By measuring the accumulation of occurrences of the largest follicles, follicular activity was evident in the present study by Days 7 to 15 post partum in all cows. Similar to that of a previous report (40), both the control and RFM cows in the present study had larger palpable follicles in the contralateral ovary throughout the postpartum period, suggesting a suppression of follicular development on the ipsilateral ovary to the previously pregnant uterine horn. In most cows the first postpartum ovulation occurs in

the ovary contralateral to the previously gravid horn (28,40). Ovarian cystic degeneration did not bias the results obtained in days to ovulation, since there was no difference in the incidence of OCD between RFM and control cows. In addition, spontaneous recovery and resumption of cyclicity occurred prior to 60 d post partum in affected cows. As many as 60 % of cows in which OCD develops prior to the first postpartum ovulation develop normal estrous cycles spontaneously (51).

In contrast to the findings reported here, previous studies have shown that RFM tend to delay uterine involution and ovarian cyclicity, this difference was partly due to the fact that RFM cows had completed 1.2 more lactations than control cows (5,12,32). Involution of the uterus is delayed in older cows (2). In the present study, all cows were matched for parity. A moderate to severe negative energy balance, as a result of too much weight loss early in the postpartum period, can also delay uterine involution and ovarian function (48). By evaluating body condition scores, the inferred rate of weight loss between RFM and control cows was similar. Body condition scores or energy balance status was not reported in the studies cited above (5,12,32). Most studies of retained fetal membranes have found that this disorder is a predisposing factor for metritis (11,12). Generally, cows with retained fetal membranes subsequently develop metritis and have a poorly involuting uterus. A heavy leukocyte count in the uterus of metritic cows tends to delay involution (14). In the present study, uterine involution was not affected in RFM cows.

Cows with retained fetal membranes had a significantly lower plasma calcium content during the first 7 d post partum than the controls. A cause and effect relationship between the reduced calcium content and RFM cannot be made in the present study because it was not known whether the lower calcium levels were present prior to parturition. However, the association between reduced calcium content near or prior to parturition and RFM has been made (7,21,36).

In cows with milk fever, calcium levels were significantly lower only in the sample just prior to therapy with a calcium preparation. After a single treatment, clinical signs regressed in all cases, indicating that calcium administration was sufficient to restore plasma calcium levels. This rebound in calcium concentration has been described by others and supports clinical experience that most cases of milk fever respond to a single treatment. The values for control cows without signs of milk fever (paresis) appeared low (5.80 ± 0.45 mg/100 ml) in the present investigation. In another Southern California study, we reported that control cows without paresis had mean calcium concentration above 6.53 ± 0.34 mg/100 ml (38). In a study involving 39 hypocalcemic parturient cows, it was observed that paresis was associated with plasma calcium levels below 5 mg/100 ml. It was concluded that the degree of hypocalcemia appeared to be more critical for the development of paresis than did the duration of hypocalcemia (31).

Delays in uterine involution and resumption of ovarian cyclicity in cows with milk fever have been reported previously by (5,12,32). In the present study, mean uterine horn involution curves were not parallel for milk fever and control cows; between Days 15 and 32 mean uterine horn diameter was larger for cows with MF. Statistically it

appeared that uterine involution was less efficient in milk fever than in control cows. Whether this small difference is of biological importance is not known.

Body condition scores represent overall fitness of the animal and indirectly monitor changes in energy balance. Therefore, consecutive decreases in numerical score indicate that a cow is losing weight and is in a greater negative energy balance. Cows in the milk fever group had a higher numerical score assigned to them at parturition than the controls. Indeed, heavier cows are at increased risk for milk fever and ketosis (13). Milk fever cows had a more pronounced loss in body condition score, which stabilized later in the postpartum period, compared with that of the control cows. This loss of body condition was likely associated with a greater negative energy balance in the milk fever cows and could have contributed to the depressed follicular activity seen early post partum and the increase in days to first ovulation. There was a numerical difference for milk fever cows to have fewer estrous cycles (1.5 vs 2.2) during the postpartum period studied than the control cows. An inverse relationship between energy balance during early lactation and days to first ovulation has been documented; dairy cows in severe negative energy balance experienced longer postpartum anestrous periods (44).

The length of the first post partum luteal phase following ovulation appears to be dependent on the interval from calving to ovulation (6,32). In the study by Madej et. al (27), first ovulation followed by a normal luteal phase length occurred at around 30.9$\pm$ 8.9 d post partum, similar to the of milk fever cows in the present study. The interval from parturition to the occurrence of first ovulation followed by a normal luteal phase length was correlated positively to the time required for completion of uterine involution (27). Uterine involution in milk fever cows was complete at around day 32 post partum in our study, coincidently with their first post partum ovulation.

Cows affected with uterine prolapse in this study had significantly lower calcium levels soon after parturition. This finding is similar to those of previous reports (35,38). The lower calcium concentrations also were evident during the first 7 d post partum, were comparable to that of RFM cows. Prolapse of the uterus did not have an effect on plasma concentrations of PGFM during the early postpartum period in this study. In contrast, PGFM levels decreased significantly in cows with a uterus that was prolapsed manually within 8 h after calving and re-inserted (16).

During the 7-d period of blood collection, cows with pyometra had higher progesterone levels than control cows. This difference was attributed to the constant presence of the CL in cows with pyometra. In contrast, 7 of the 13 control cows had regressed CL during the period of blood collection, as evidenced by signs of estrus and reduction of plasma progesterone concentration to below 1ng/ml. Similar to a previous report (34), cows affected with pyometra had higher concentrations of PGFM in their blood than the controls. The higher concentration of PGFM seen in the pyometra group suggests a constant irritation to the endometrium from bacterial agents or their products. Inflammatory reactions within the uterus can initiate endometrial PGFα release (43). In cows with pyometra, the prolonged diestrus has been attributed to a lack of luteolysis. Thus our results indicate a paradox occurring in pyometra cows; a high concentration of

PGFM in a condition of sustained CL function. It would appear reasonable to conclude that the synthesis and release of prostaglandin in these cows is insidious over a prolonged period of time. The PGFM secretory pattern associated with luteolysis in cattle is episodic, with approximately 2 well-defined surges of PGFM occurring at 10-h intervals just prior to luteolysis and 2 additional pulses after luteolysis (47). Although, daily concentrations of PGFM were elevated in the present study, large concentrations associated with luteolytic peaks (e.g., 323 pg/ml;55) were not detected. However, serial samples within a 24-h period were not collected to determine if cows with pyometra have an altered secretory pattern of PGFM secretion.

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