Effect of Mastectomy on Milk Fever, Energy, and Vitamins A, E, and β-Carotene Status at Parturition

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ABSTRACT

The objective of this study was to compare blood profiles of intact and mastectomized periparturient cows to discriminate those metabolic changes associated with the act of parturition from the metabolic changes caused by lactation. Mastectomized and intact cows had similar increases in plasma estrogens and cortisol concentrations around the time of calving. Mastectomy eliminated hypocalcemia and the rise in 9,13-di-cis retinoic acid observed in intact cows. Mastectomy reduced but did not eliminate decreases in plasma phosphorus, α-tocopherol, and β-carotene associated with parturition in intact cows, suggesting the mammary gland is not the sole factor affecting plasma concentrations of these compounds. Dry matter intake was similar in both groups before calving. The day of calving, dry matter intake was lower in intact cows than in mastectomized cows, but after calving the mastectomized cows exhibited a pronounced decline in feed intake. Plasma nonesterified fatty acid (NEFA) concentrations rose rapidly in intact cows at calving and did not return to baseline level for >10 d. In contrast, NEFA concentrations in mastectomized cow plasma rose moderately at calving and returned to baseline level 1 to 2 d after calving. This study provides evidence that hypocalcemia in the cow is solely a result of the calcium drain of lactation. The act of parturition affects blood phosphorus, dry matter intake, and NEFA concentration independent of the effect of lactation.

(Key words: mastectomy, hypocalcemia, milk fever, retinoic acid)

Abbreviation key: RA = retinoic acid.

INTRODUCTION

The dairy cow commonly experiences an abrupt change in her metabolic status around the time she calves and begins lactating. These changes include negative energy, calcium, and protein balance as well as profound changes in circulating steroid hormone, mineral, and antioxidant vitamin profiles. These changes could be caused by the metabolic challenge of milk production, or could be caused by the act of parturition. A comparison of metabolic profiles in periparturient cows mastectomized in midgestation, so the metabolic demands of lactation are eliminated, but the changes associated with the act of parturition remain and, in periparturient intact cows, might allow resolution of the cause of these metabolic changes.

The typical dairy cow is in negative energy balance in early lactation. Removal of the udder of a dairy cow should remove the demand for energy and reduce body fat mobilization, without affecting hormone secretion associated with the act of parturition. If the hormonal events associated with parturition, especially the rise in plasma cortisol and estrogen concentrations, instigate the mobilization of body fat stores, body fat mobilization should occur even in the mastectomized cow.

Hypocalcemia is a common sequel of parturition in the dairy cow. The production of colostrum, with its high calcium content, is generally believed to remove calcium from the blood faster than it can be replaced from bone calcium stores or dietary calcium. However, Stott (1968) reported that older cows that had suffered milk fever in the past and that were then mastectomized, developed severe hypocalcemia at the next calving, suggesting that perhaps milk production was not the only cause of hypocalcemia in the periparturient dairy cow. In part, this experiment was designed to repeat this observation.

Plasma vitamins A and E concentrations decline around the time of calving (Goff and Stabel, 1990; Weiss et al., 1992). Colostrum contains substantial amounts of these two compounds. However, it is also possible that these two vitamins show substantial losses as a
result of increased utilization. If colostrum production were the only cause of the reduction in vitamins A and E observed at parturition, then the removal of the udder of cows should prevent any decline in blood concentrations of these vitamins at calving.

Vitamin A is the precursor to a variety of retinoic acid (RA) derivatives with varying biological roles in cell differentiation. The enzyme, 9-cis-Retinol-dehydrogenase, catalyzes the oxidation of 9-cis-retinol to 9-cis-retinaldehyde. This is the first enzymatic step needed for 9-cis-retinoic acid formation (el Akawi and Napoli, 1994). Mertz et al. (1997) surveyed several human tissues and found that 9-cis retinol-dehydrogenase mRNA expression was most abundant in the mammary gland. Interestingly, the major retinoic acid in plasma of periparturient cows was determined to be 9,13-di-cis-RA (Horst et al., 1995a). Plasma 9,13-di-cis-RA concentration increased markedly at the onset of lactation and remained elevated in early lactation. Mastectomy might eliminate a major in vivo source of 9-cis retinol-dehydrogenase and reduce production of 9-cis-retinoic acid and its metabolite, 9,13-di-cis-retinoic acid.

MATERIALS AND METHODS

Animals

Older Jersey cows (6 to 9 yr of age), in their third or greater pregnancy, were used in this study. The 10 cows chosen for mastectomy were all cows that would have been culled because they had had severe mastitis or had not become pregnant until late into lactation. Cows were nonlactating for at least 3 wk before surgery, and surgery was performed when the cows were 3 to 5 mo pregnant. This allowed a minimum of 3 mo for recovery from the surgery before calving. Eight intact cows of similar age to the mastectomized cows and expected to calve about the same time as the mastectomized cows were used as controls. The intact control cows were nonlactating for 8 to 9 wk before calving and were fed the same diets as the mastectomized cows for the last 8 wk of gestation. No sham surgery was performed on the intact animals. The intact cows were dry-treated for mastitis prevention and vaccinated against common infectious diseases and coliform mastitis during the dry period. All procedures performed on these cows were approved by the Animal Care and Use Committee of the National Animal Disease Center.

Surgery

Cows were placed in right lateral recumbency under general anesthesia throughout the surgery. An incision was made in the skin approximately half way between the teats and the abdominal wall from the caudal portion of the left side of the udder to the cleft between the anterior quarters of the udder, exposing the lateral ligament of the udder. The lateral ligament was incised, and blunt dissection exposed the left external pudendal artery and vein which were individually ligated. The left subcutaneous abdominal vein (milk vein) was similarly isolated and ligated. The left side of the udder was dissected free of the abdominal wall, revealing the medial suspensory ligament of the udder. This was incised along its length about 8 to 10 cm from the abdominal wall. Blunt dissection revealed the right external pudendal artery and vein and subcutaneous abdominal vein, which were ligated. The entire mammary gland could then be dissected free of the abdominal wall. The skin on the right side of the udder was incised so as to leave enough skin to cover the defect left after the udder was removed. A Penrose drain was placed the entire length of the incision and was removed 2 d after surgery. The skin edges were brought together and anchored to the underlying connective tissues. Supportive treatment included 20 L of physiological saline administered intravenously during surgery, prophylactic antibiotics for 7 d after surgery, and 1.1 mg of flunixin meglumine/kg of BW per day for 3 d after surgery for analgesia. In most cows, the skin edges healed, with a moderate degree of granulation within 6 wk. In three cows, the skin incision sutures became infected and required draining the wound, followed by granulation of the skin to close the defect. In these cows, complete closure of the skin wound required up to 12 wk. All cows were fully recovered at least 1 mo before parturition.

Diet

Cows were fed a poor quality alfalfa-grass hay ad libitum, with access to a mineral salt block until 3 wk before the expected date of parturition. At that time, cows were placed on a TMR consisting of alfalfa hay and a grain mix. The diet was purposely high in dietary cations (Table 1). The diet supplied 125,000 IU of supplemental retinyl palmitate and 2200 IU of supplemental DL-α-tocopheryl-acetate per day. The pre-calving diet was 15.8% crude protein, 1.52 Mcal NEL/kg, 37% NDF, 1.19% calcium, 0.4% phosphorus, and 0.5% magnesium. It had a cation-anion difference ([sodium + potassium] * (chloride + sulfur)] of + 443 mEq/kg diet DM. The intact cows were fed an additional 1.8 kg/day of a 17.2% protein corn-soybean meal-oats concentrate in the milking parlor after calving. The mastectomized cows remained on the same pre-calving ration following calving.

Blood Samples

Daily plasma samples for metabolic profile assays were collected from each cow beginning 2 wk before parturition of the left side of the udder to the cleft between the anterior quarters of the udder, exposing the lateral ligament of the udder. The lateral ligament was incised, and blunt dissection exposed the left external pudendal artery and vein which were individually ligated. The left subcutaneous abdominal vein (milk vein) was similarly isolated and ligated. The left side of the udder was dissected free of the abdominal wall, revealing the medial suspensory ligament of the udder. This was incised along its length about 8 to 10 cm from the abdominal wall. Blunt dissection revealed the right external pudendal artery and vein and subcutaneous abdominal vein, which were ligated. The entire mammary gland could then be dissected free of the abdominal wall. The skin on the right side of the udder was incised so as to leave enough skin to cover the defect left after the udder was removed. A Penrose drain was placed the entire length of the incision and was removed 2 d after surgery. The skin edges were brought together and anchored to the underlying connective tissues. Supportive treatment included 20 L of physiological saline administered intravenously during surgery, prophylactic antibiotics for 7 d after surgery, and 1.1 mg of flunixin meglumine/kg of BW per day for 3 d after surgery for analgesia. In most cows, the skin edges healed, with a moderate degree of granulation within 6 wk. In three cows, the skin incision sutures became infected and required draining the wound, followed by granulation of the skin to close the defect. In these cows, complete closure of the skin wound required up to 12 wk. All cows were fully recovered at least 1 mo before parturition.

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Table 1. Composition of diet fed to the cows during the last 3 wk before calving and for the first three weeks of lactation.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>% Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alfalfa hay (full bloom)</td>
<td>53</td>
</tr>
<tr>
<td>Beet pulp</td>
<td>15</td>
</tr>
<tr>
<td>Cracked corn</td>
<td>17</td>
</tr>
<tr>
<td>Outs</td>
<td>5</td>
</tr>
<tr>
<td>Soybean meal (44% protein)</td>
<td>5</td>
</tr>
<tr>
<td>Molasses</td>
<td>1.5</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>0.57</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>0.48</td>
</tr>
<tr>
<td>Sodium bicarbonate</td>
<td>1.5</td>
</tr>
<tr>
<td>Magnesium oxide</td>
<td>0.5</td>
</tr>
<tr>
<td>Trace mineral salt¹</td>
<td>0.2</td>
</tr>
<tr>
<td>Vitamins²</td>
<td>0.2</td>
</tr>
</tbody>
</table>

¹Trace mineral salt mix was 16.5% zinc, 16.5% manganese, 4.6% copper, 0.11% cobalt, and 0.1% selenium.
²Vitamin mixture supplemented diet with 125,000 IU of vitamin A, 25,000 IU of vitamin D, and 2,200 IU of vitamin E/d.

expected calving date and continuing for 2 wk into lactation. Not all blood samples from every day during the periparturient period were analyzed for a given blood component. Samples were chosen for analysis based on previous reports, suggesting the periods when physiological changes were most likely for a given blood component.

Assays

Plasma calcium and magnesium concentrations were determined by atomic absorption spectrophotometry (Perkin-Elmer, 1965). Plasma phosphorus (Parekh and Jung, 1970) and NEFA (Johnson and Peters, 1993) were determined colorimetrically. Plasma 1,25-dihydroxyvitamin D concentrations were determined by competitive binding assay following isolation and partial purification by liquid chromatography (Horst et al., 1990). Plasma concentrations of vitamin A (retinol), the retinoic acids, and vitamin E (tocopherol) were determined by absorption of UV light following separation from plasma samples by both liquid chromatography and HPLC (Horst et al., 1995a, 1995b).

Plasma samples from a subset of six randomly chosen mastectomized cows and six intact cows (chosen because they calved at about the same time as one of the mastectomized cows) were assayed to establish profiles of the reproductive hormones. Plasma progesterone, estradiol-17β, and estrone concentrations were determined by radioimmunoassay using commercial kits and established protocols (Progesterone DSL-3900, Estrone DSL-8700, Estradiol DSL-4400; Diagnostic System Laboratories, Webster, TX). Plasma cortisol concentrations were also determined by radioimmunoassay (Coat-A-Count Cortisol, Diagnostic Product Corp., Los Angeles, CA). All assays were validated for the bovine by assaying serially diluted samples of plasma known to have high concentrations of the steroid and demonstrating parallel responses to the standard curve for each steroid being assayed. The intraassay coefficient of variation for the progesterone, estrone, estradiol, and cortisol assays were 3.11, 3.63, 1.67, and 4.31%, respectively.

In all assays, samples from a control cow and a mastectomized cow were processed together so that interassay effects would be balanced across treatments.

Statistical Analysis

To investigate the impact of mastectomy on relevant variables we performed a split-plot analysis of variance, followed by a simple effect analysis (Duncan’s multiple comparisons; SAS, 1987). The statistical model included effects of treatment (intact vs. mastectomy), time (days relative to parturition), and the interaction of treatment and time (treatment × time). The mean square error for cow within treatment was used as the error term to evaluate the effect of treatment. Residual error (time × cow within treatment) was used to evaluate effects of the repeated measures factor, time, and its interaction with treatment (treatment × time). Determinations for the various variables on samples obtained 14 d before calving (10 d before calving for the steroid hormones and phosphorus) were considered baseline values for all variables examined. Results were considered significant when the possibility that the differences observed were due to chance was less than 5%. When greater than 5% we conclude that there was no change.

RESULTS AND DISCUSSION

General Observations

Two mastectomized cows gave birth to twins. All intact cows developed milk fever within 24 h after calving, and they were treated with intravenous infusion of calcium solution from one to three times after calving. Three intact cows also developed ketosis secondary to displaced abomasum within 1 wk of calving and these were treated by intravenous glucose infusion and abomasopexy with a single suture inserted through the right ventral abdomen while the cow was held in dorso recumbency.

Dry matter intake of mastectomized and intact cows was similar before parturition (Figure 1). Intact cows suffered a severe decline in DMI at parturition and on the first day of lactation compared to DMI during the period from 10 to 14 d before calving. A similar decrease in DMI was observed in the mastectomized cows during the periparturient period. After calving, DMI of the
mastectomized cows remained less than DMI during the period from 10 to 14 d before calving until the first 3 d of lactation.

Cows typically suffer about a 20% decline in DMI around calving (Bertics et al., 1992). The DMI the day of calving in the intact cows represented a 55% decrease from the average DMI during the period from 10 to 14 d before calving. This likely reflects an effect of milk fever on feed intake in these cows (Marquardt et al., 1977). More surprising is the decline in DMI observed in the mastectomized cows. They tended to eat less in late gestation than did the intact cows, though this difference was not significant. Two of the 10 mastectomized cows gave birth to twins. Twins may have reduced rumen volume and capacity beyond the space requirement of a single fetus. It is also possible the energy requirement of mastectomized cows was reduced because they had no mammary tissue to support or regenerate. The DMI the day of calving in the mastectomized cows represented a 92% decrease from their average DMI during the period from 10 to 14 d before calving.

Concentration of Hormones Associated with Parturition

Parturition is typically associated with a decrease in plasma progesterone concentration and an increase in plasma estrogens the week before calving, and a sudden increase in cortisol the day before calving. Consistent with previous observations, there were significant effects of time around parturition on the plasma concentrations of progesterone, estrogens, and cortisol in both the intact and mastectomized cows. Progesterone levels were between 6000 and 7000 pg/ml in both groups until 2 d before calving (Figure 2a). Progesterone concentrations decreased suddenly on the day before calving to between 1000 and 2000 pg/ml, reaching a nadir of 100 to 200 pg/ml within a day after calving. Plasma estradiol concentrations increased the final week of gestation, peaking the day before calving in intact cows (169 ± 23 pg/ml) and the day of calving in mastectomized cows (232 ± 45 pg/ml; Figure 2b). Plasma estradiol concentrations decreased precipitously after parturition. Although mastectomized cow estradiol values tended to be higher in late gestation than in the intact cows, there was no significant treatment effect (P = 0.134). Plasma estrone concentration also reached a zenith just before parturition and was higher in mastectomized cows (1516 ± 57 pg/ml) than in intact cows (806 ± 63 pg/ml; Figure 2c). Plasma cortisol concentrations became elevated the day before and peaked the day of calving in both intact and mastectomized cows, a profile typical for cows around the time of parturition (Horst and Jorgensen, 1982). Although the cortisol level tended to be higher in mastectomized cows at the beginning of the sampling period (from d -14 to d -7; Figure 2d), both groups had similar plasma cortisol concentration profiles, with a sudden increase at parturition and decrease after parturition. The peak plasma cortisol concentration was seen at calving in intact (19.4 ± 4.4 ng/ml) and in mastectomized cows (14.5 ± 4.6 ng/ml). The difference between the groups was not statistically significant (P = 0.11). Plasma cortisol concentration 1 d after calving remained elevated in intact cows but had essentially returned to basal levels in mastectomized cows.

These results suggest that removal of the udder of the cow did not alter the steroid hormone profiles associated with parturition. While the rise in estrone at calving was expected in both the intact and mastectomized cows, the significantly higher concentrations observed in the mastectomized cows was surprising. Our observation of a higher level of total estrogen in mastectomized cows is contradictory to observations made in mas-
EFFECT OF MASTECTOMY ON METABOLISM

Figure 2. Plasma progesterone (a), estradiol (b), estrone (c), and cortisol (d) concentrations in intact (n = 6; □) and mastectomized (n = 6; ▢) cows during the periparturient period.

tectomized goats (Peaker, 1995), in which the increase in estradiol concentrations that occurs near term in intact goats was completely obliterated. Our data also do not provide support for a report that estrogens are secreted by the mammary gland of cows (Walker et al., 1983). Because the mammary gland does have receptors for estrogens (Houdebine et al., 1985), the higher concentration of circulating plasma estrogens observed in our study of mastectomized cows may reflect a lack of uptake of estrogens from the circulation. The higher estrogen concentration in the mastectomized cows might also reflect the twin calvings in this group as higher estrogen production can be associated with the double placentas of twin calvings (Worsfold et al., 1989).

Plasma Minerals and 1,25-Dihydroxyvitamin D Concentrations

Plasma calcium concentration decreased significantly in the intact cows at the time of parturition, and all intact cows did develop clinical milk fever (Figure 3a). Mastectomy completely prevented the development of hypocalcemia in the cows, despite the high calcium and high dietary cation-anion difference diet. Plasma 1,25-dihydroxyvitamin D concentrations were greatly increased in intact cows beginning the day before parturition and for the first three days of lactation (Figure 3b). Mastectomized cows exhibited no changes in plasma 1,25-dihydroxyvitamin D concentration around the time of parturition. The production of 1,25-dihydroxyvitamin D is a response to hypocalcemia (Horst et al., 1978). Therefore, it is not surprising that the mastectomized cows did not increase production of 1,25-dihydroxyvitamin D.

Blood phosphorus concentration was decreased (Figure 3c) and blood magnesium concentration was increased (Figure 3d) in the intact cows around the time of parturition, results typical of cattle with hypocalcemia (Goff et al., 1986). Plasma phosphorus con-
Figure 3. Plasma calcium (a), 1,25(OH)2D3 (b), phosphorus (c), and magnesium (d) concentrations in intact (n = 8; ■) and mastectomized (n = 10; □) cows during the periparturient period.

Concentration did decrease significantly in mastectomized cows at calving, though the decrease was much less than in intact cows. The decline in blood phosphorus concentration observed in the typical periparturient cow has generally been attributed to the loss of phosphate to milk and the effects of parathyroid hormone released in response to the hypocalcemia. Parathyroid hormone increases the renal loss of phosphate and increases salivary secretion of phosphate (Wright et al., 1982). The salivary loss of phosphorus may be 30 to 90 g/d, which is more significant than the 1 to 10 g/d phosphorus typically excreted by the kidney (Reinhardt et al., 1988).

Because eliminating milk production and hypocalcemia failed to allow the cows to maintain normal blood phosphorus concentrations, it is clear that milk production and hypocalcemia are not the only factors responsible for the periparturient decline in blood phosphorus concentration. Horst and Jorgensen (1982) suggested that increased cortisol concentrations at calving might cause a redistribution of phosphorus from extracellular to intracellular stores resulting in the low plasma phosphorus concentrations observed in periparturient cows. In mastectomized cows, plasma magnesium concentrations remained relatively constant throughout the periparturient period. The rise in blood magnesium observed in periparturient cows has been observed to be inversely proportional to the decline in calcium at calving. One effect of parathyroid hormone released in response to hypocalcemia is an increase in renal tubule
reabsorption of magnesium, resulting in elevated blood magnesium concentration (Goff, 2000).

These results confirm the report of Niedermeier et al. (1949) that demonstrated no significant change in blood calcium or magnesium in mastectomized cows. It also confirms their observation that hypophosphatemia occurred at parturition even in mastectomized cows. In a review article written by Stott (1968), the statement is made that studies were under way in which 9 of 12 mastectomized cows developed milk fever at parturition. Unfortunately, that statement appears to be without substance. In both the current study and the study by Neidermeier et al. (1949), there is a perceptible but not statistically significant decline in blood calcium at calving in mastectomized cows. The values fall well within the normal limits for blood calcium of dairy cows, and we suggest that such small changes are normal day-to-day variation and are not physiologically significant.

**Plasma Vitamin Concentrations**

Plasma retinol, β-carotene, and α-tocopherol concentrations before calving were 168 ± 29 ng/ml, 1955 ± 705 ng/ml, and 1879 ± 240 ng/ml, respectively, in intact cows, and 207 ± 41 ng/ml, 1817 ± 34 ng/ml and 2367 ± 242 ng/ml, respectively, in mastectomized cows. Plasma concentrations of all three nutrients decreased in intact cows during the last days of gestation, reaching a nadir shortly after calving. The nadir plasma retinol, β-carotene, and α-tocopherol concentrations represented a decline of 67, 65, and 43%, respectively, from baseline concentrations in intact cows (Figure 4a, b, c). These dramatic decreases in plasma concentrations of retinol, β-carotene, and α-tocopherol are similar to earlier observations (Johnston and Chew, 1984; Goff and Stabel, 1990). In mastectomized cows, the nadir concentrations of plasma retinol, β-carotene, and α-tocopherol were 22, 22, and 25%, respectively, lower than baseline concentrations. The decrease in retinol was not statistically significant. Plasma concentrations of retinol, β-carotene, and α-tocopherol remained below baseline concentrations for the first 2 wk of lactation in intact cows. In mastectomized cows, only β-carotene concentration remained depressed in early lactation; plasma retinol and α-tocopherol concentration in early lactation returned to baseline or higher concentrations in mastectomized cows in early lactation.

Most biological effects of retinol are mediated by its retinoic acid metabolites. Two geometric isomers of retinoic acid; all trans-retinoic acid and 9-cis-retinoic acid regulate biological processes involved in cell differentiation and growth (Goss and McBirney, 1992). The major circulating retinoid in the cow is 9,13-di-cis-retinoic acid (Horst et al., 1995a). The 9,13-di-cis-retinoic acid can undergo interconversion to 9-cis-retinoic acid and thus 9,13-di-cis-retinoic acid may be serving as a labile store by which the body can regulate 9-cis-retinoic acid concentration in tissues. Horst et al. (1995b) have previously reported that the concentration of 9,13-di-cis retinoic acid increases at parturition and is relatively high in early lactation. These observations are corroborated in the present study (Figure 5). Plasma 9,13-di-cis-retinoic acid concentration increased three- to fourfold in the first week of lactation. However, mastectomy eliminated the rise in plasma 9,13-di-cis-retinoic acid concentration observed in the lactating cows. No significant differences were observed in plasma concentrations of all trans-retinoic acid and 9-cis-retinoic acid between intact cows and mastectomized cows (data not shown), and concentrations of these retinoids were not statistically altered by time around parturition.

The sudden decrease in plasma retinol, β-carotene, and α-tocopherol concentration observed at parturition
is generally attributed to loss of these vitamins to the mammary gland and colostrum. One milliliter of colostrum contains approximately 4300 ng of retinol, 200 ng of β-carotene, and 1900 ng of α-tocopherol, while 1 ml of milk has about 1800 ng of retinol, 40 ng of β-carotene, and 300 ng of α-tocopherol (Johnston and Chew, 1984; Hidiroglou, 1989). Theoretically, if no milk production occurred, there would be no decline in plasma retinol and α-tocopherol concentrations at calving. Statistically there was no decline in plasma retinol during the periparturient period in mastectomized cows, so it could be concluded that colostrum and milk account for the major portion of the decline in retinol observed in intact cows. While mastectomy did not affect the plasma concentrations of the retinoic acid metabolites known to be biologically active, it did essentially eliminate the rise in 9,13-di-cis-retinoic acid observed in intact cows. These data imply that the mammary gland is metabolizing relatively large amounts of 9-cis retinoic acid. We have not yet been able to determine how the metabolism of retinoic acids might be affecting the lactation process.

Removing the udder reduced but did not eliminate the reduction in plasma β-carotene and α-tocopherol concentrations observed in intact cows. Thus, uptake of β-carotene, and α-tocopherol by the mammary gland is not the only factor causing the precipitous decline in plasma β-carotene and α-tocopherol concentration. Because these nutrients are considered antioxidants, these results suggest that there is an increase in oxidation of these compounds at parturition beyond the oxidative stress imposed on the body at other stages of life. Another possibility is that the decline in DMI during the immediate periparturient period affects plasma β-carotene and α-tocopherol concentrations. However, the persistence of low β-carotene and α-tocopherol concentrations in early lactation, when DMI was similar or higher than it was prepartum, argues against a major role for declining intake of β-carotene and α-tocopherol as the cause of the blood changes observed.

**Plasma NEFA**

Plasma NEFA concentrations at 14 d before parturition were 0.19 ± 0.02 mEq in intact cows and 0.16 ± 0.02 mEq in mastectomized cows (Figure 6). These levels would be considered typical in cows with good but not excessive body condition (Cameron et al., 1998). In both intact and mastectomized cows, plasma NEFA concentration increased the day before calving. In intact cows, the plasma NEFA concentration peaked on d 1 of lactation at 1.25 ± 0.17 mM and remained elevated above baseline level for at least the first 10 d of lactation. These results suggest that the intact cows in this study were in severe negative energy balance in early lactation. The combination of the high incidence of milk fever and displaced abomasum in the intact cows likely contributed to the very high NEFA concentrations and the resulting high incidence of ketosis (three of eight cows) among the intact cows. In contrast, plasma NEFA concentrations in mastectomized cows peaked the day of calving at 0.50 ± 0.11 mM and had returned to baseline values the next day and were significantly lower than the NEFA concentrations observed in intact cows from 1 d before calving until the end of the study. These data suggest that a small portion of the rise in NEFA concentration is independent of milk production. It is likely that cortisol secreted to initiate parturition
caused mobilization of triglyceride in adipose tissue independent of any real need for energy in the mastectomized cows (Grummer, 1993). However, it is also clear that lactation and metabolic stresses imposed by lactation play a larger role in the mobilization of body fat.

**CONCLUSIONS**

In this study, we demonstrated that the mastectomized cow model does not affect the reproductive steroid hormone profiles normally associated with parturition. Both mastectomized and intact cows experience a sudden decline in progesterone concentrations and rise in estrogen and cortisol concentrations as parturition approaches. Mastectomy virtually eliminated hypocalcemia and the hypermagnesemia and increased production of 1,25-dihydroxyvitamin D that occur secondary to hypocalcemia. These results dispel earlier suggestions that milk fever might occur independently of the calcium drain imposed on the cow by milk synthesis. It reduced but did not eliminate the hypophosphatemia commonly observed in periparturient cows, suggesting that plasma phosphorus is declining at parturition independent of lactation due to some factor produced during the act of parturition. Mastectomy eliminated the rise in 9,13 di-cis-retinoic acid previously observed in periparturient lactating cows. Why the mammary gland would have such a profound effect on the formation of a metabolite of the active retinoid, 9-cis-retinoic acid is unknown. Mastectomy reduced but did not eliminate the rise in NEFA concentration previously observed in periparturient lactating cows. This small rise in NEFA concentration in plasma of mastectomized cows served in periparturient dairy cows. This small rise in NEFA concentration commonly observed in periparturient cows, suggesting that plasma phosphorus is declining at parturition independent of the energy drain imposed by lactation. However, it is clear that the intact cow mobilizes a much larger amount of body fat than does the mastectomized cow. The mastectomized cow model may prove a useful means of separating the effects of lactation from the effects of the act of parturition.

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