Estrogen in Plasma of Parturient Paretic and Normal Cows

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ABSTRACT

The endocrine factors associated with parturient paresis have not been defined totally. Estrogens stimulate uptake of calcium by bone. Since secretion of estrogen increases dramatically as parturition approaches, estrogen may be involved in homeostatic mechanisms regulating calcium metabolism. Plasma was collected for 30 days (-30) prepartum to 5 days (+5) postpartum from six Holstein and nine Jersey cows approaching three or more lactations. Of all cows, six Jerseys contracted parturient paresis. Estradiol and estrone were analyzed by radioimmunoassay, total calcium and total magnesium by atomic absorption spectrophotometry, and total phosphorus by colorimetry. Data were grouped into periods representing days -30 to -21, -20 to -11, -10 to -6, -5 to -4, -3 to -2, -1, 0 (parturition), +1, +2 to +3, and +4 to +5. Calcium in plasma was lower in parturient paresis cows on days +1 and +2 to +3, and magnesium was higher during the same periods but lower on days -4 to -5. Total phosphorus, estrone, and estradiol of normal cows and those with parturient paresis were not different. During the entire sampling period, phosphorus and estradiol were similar in both groups while magnesium was higher and calcium lower in cows with parturient paresis. Estrone was lower in cows with parturient paresis. Lower estrone in cows with parturient paresis may be predisposing for parturient paresis.

INTRODUCTION

At parturition the mammary gland begins to remove calcium from blood for milk synthesis which depresses calcium in plasma. If cows are unable to replenish calcium of blood, normally by bone resorption, parturient paresis (milk fever) ensues.

Calcium homeostasis in plasma is achieved, in part, through the action of parathyroid hormone (PTH) and calcitonin. The PTH elevates calcium in plasma by enhancing intestinal absorption, renal reabsorption, and bone mobilization of calcium whereas calcitonin inhibits resorption by bone (27). Estrogens stimulate uptake of calcium from plasma by bone (21, 22) and thus, are another factor imposed upon the mechanism of homeostasis. In addition, some work (23) suggested estrogens inhibited bone resorption.

Estrogens increase dramatically during late pregnancy in the plasma (25) and urine (10, 16, 17) of cows. As such, estrogens may increase accretion of bone and, therefore, depress calcium in plasma. The expected result is increased release of PTH which, in turn, enhances resorption of bone calcium. Thus, estrogens may increase turnover rate of calcium in bone. Under this situation, bone would be more sensitized to stimuli for resorption, and calcium in plasma could be replenished more readily. Conversely, animals with depressed estrogen in plasma may have less sensitized bone and may not be able to adapt as readily to depressed calcium in plasma. In addition, Gaverick et al. (11) have reported that cows suffering from parturient paresis had reduced excretion of estrogen. In contrast, Bargeloh et al. (3) reported that cows treated with estrogen had the same incidence of parturient paresis even though postpartum calcium in plasma was elevated in diseased animals compared to controls. Also, in contrast, Edqvist et al. (9) reported that prepartum estrone was the same in plasma of parturient paretic and

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normal cows. Based upon these data, the purpose of this study was to measure the estradiol and estrone by radioimmunoassay of plasma during the last 30 days of pregnancy in parturient paretic and normal cows and to define further the relationship of estrogens to calcium, phosphorus, and magnesium in plasma and, hence, to parturient paresis.

EXPERIMENTAL PROCEDURE

Animals

Six Holstein and nine Jersey cows of third lactation or more were in this study. Expected parturition date was designated as day zero (0). Blood was to be obtained on days -28, -21, -14, -11, -8, -5, -2, -1, 0, +1, +3, and +5 from parturition. Blood samples (30 ml) were collected by jugular vein puncture in heparinized evacuated tubes. Plasma was obtained by centrifugation at 1800 x g for 10 min and was stored at -10 C until analyzed.

Animals were group-fed a concentrate ration at .5 kg/100 kg body weight. The concentrate consisted of barley, 1045.5 kg; oats, 90.9 kg; wheat, 409.1 kg; beet pulp, 181.8 kg; soybean oil meal, 90.9 kg; sodium chloride, 18.2 kg, and sodium tripolyphosphate, 18.2 kg. Alfalfa hay and alfalfa silage were fed ad libitum. Based on average intake, animals consumed 156 g calcium and 41 g phosphorus for a calcium to phosphorus ratio of 3.8:1.

Estradiol-17β (E2) and estrone (E1) were measured by dextran-charcoal radioimmunoassay (28) utilizing [3H] E2 or [3H] E1 in the respective inhibition curves. The same antisera used in both curves was a gift of B. V. Caldwell and was the same lot (#029-14) used by Wu and Lundy (28). In addition, procedures and results were similar to those reported by Wu and Lundy (28) except that estrone and estradiol-17β were separated on .5 x 15 cm LH-20 columns rather than minicolumns. Cross-reactivity of the estrogens compared to estradiol were 42% for E1 and 24% for estriol. As such, the same antisera were used to assay estradiol-17β and estrone. Androstenedione, testosterone, dehydroepiandrosterone, pregnenolone, progesterone, cortisone, cortisol, and cholesterol all had relative cross-reactivities of less than .1%. Inhibition curves from chromatographed E2 and E1 of various dilutions of plasma were parallel to respective standard curves of E2 and E1 when the plasma contained greater than 19 pg E2 and 25 pg E1. These values were considered the limit of sensitivity of the assay of plasma even though standard curve values different (P<.05) from zero were less than 10 pg (the lowest value in each standard curve) for both estrogens. Exogenous E2 and E1 added to samples of plasma from a castrated cow (10 through 180 pg) could be recovered quantitatively (E2, slope = .99, y-intercept = 21.4, and r = .99; E1, slope = .86, y-intercept = 15.4, and r = .99). The y-intercepts approached values of the plasma blank of 16.8 ± 3.6 (SD) pg/ml for estradiol and 18.0 ± 1.7 pg/ml for estrone.

Plasma was analyzed for total calcium and total magnesium by atomic absorption spectrophotometry (Ziess PM Q11) (1). Total phosphorus was analyzed by the colorimetric methods of Rogers (24) with modifications outlined by Bloxham (4).

RESULTS

Six Jersey cows contracted parturient paresis while three Jersey and six Holstein cows did not. Age did not appear to be associated with incidence of disease. Ages ranged from 4 to 8.5 yr, and parturient paresis occurred in animals throughout this age range.

Since parturition could not be predicted, sampling days with respect to parturition did not fall on the same day. Therefore, all data were grouped into the following days peripartum: -30 to -21, -20 to -11, -10 to -6, -5 to -4, -3 to -2, -1, 0 (parturition), +1, +2 to +3, and +4 to +5.

Peripartum fluctuations in minerals of plasma by periods for normal and parturient paretic animals are in Figure 1. Calcium decreased in both groups at parturition while calcium of parturient paretic cows continued to decrease after calving. Least-squares analysis of variance (LSAOV) indicated significant differences (P<.05) between groups on days 1 and 2 to 3 following parturition whereas all other days were similar.

Total phosphorus remained stable at 10 to 15 mg/100 ml until parturition when it decreased nonsignificantly (P>.05) to 9 mg/100 ml. The difference during any period between the two animal groups was not significant (P>.05).
Magnesium increased nonsignificantly at parturition in both groups. However, it continued to increase in parturient paretic cows following parturition and was significantly different between groups on days 1 (P<.01) and 2 to 3 (P<.05) following parturition. It also differed (P<.01) on days 4 to 5 before parturition.

These mineral data also were analyzed for overall effect by LSAOV to determine if values differed between groups during the entire sampling period (30 days prepertum to 5 days postpartum). Phosphorus was again similar in both groups while magnesium (P<.01) and calcium (P<.01) were different in parturient paretic and normal cows.

Estrogen data were analyzed similarly and are in Figure 2. Both estrone and estradiol increased dramatically the last 20 days of pregnancy. Estradiol declined by the day following parturition while estrone began decreasing on the day of parturition. Estradiol of diseased and normal animals did not differ during any period or overall (P>.05).

Overall mean estrone, however, was lower in animals that suffered from parturient paresis and differed (P<.05) from the mean of normal animals. When estrone was analyzed by periods, there was no significant difference between groups.

Since Jersey cows were the only ones contracting parturient paresis, LSAOV was performed on the data within the Jersey Breed. Estrone was different (P>.01) between diseased and normal Jerseys (P<.01) not (P>.01) on days 5 to 4 before parturition. Estradiol did not differ between groups at any period while calcium and magnesium were different during periods one (P<.05) after parturition and 5 to 4 (P<.01) before parturition, respectively. Phosphorus was also different (P<.05) during period 5 to 4 before parturition.

When parturient paretic cows (six Jerseys) were excluded, there was no significant difference between breeds in estradiol, estrone, calcium, or phosphorus. Magnesium was different (P<.05) between breeds 10 to 6, 3 to 2, and 1 days before and 2 to 3 and 4 to 5 days following parturition. It was also different on the day of parturition.
Calcium of plasma dropped at parturition in normal and parturient paretic cows. Calcium decreased in cows with milk fever following parturition as in (14, 15, 18, 26). This was mainly due to removal of calcium from plasma for synthesis of milk (20). Animals suffering from milk fever cannot replace calcium of plasma rapidly enough to prevent paresis whereas those not contracting the disease have the capability of replacing calcium.

The LSAOV indicated there was no significant difference in phosphorus of plasma between diseased or normal animals. However, total, rather than inorganic, phosphorus was analyzed and could account for this fact. Some work (13) indicated inorganic phosphorus decreased at parturition but returned to basal amounts within 24 h.

Magnesium was different between parturient paretic and normal cows when Holsteins and Jerseys were analyzed together. These data agreed with (18, 20). Magnesium was lower when data from only the Jerseys were analyzed, and the difference between groups was significant.

These data substantiate (25) that estrone and estradiol of plasma increased dramatically during the last few days of pregnancy and then decreased sharply at parturition. It appeared that circulating estradiol was not associated with the incidence of parturient paresis as it was similar in normal and diseased animals. However, circulating estrone was lower in hypocalcemic (parturient paretic) animals than in normal animals during the entire period prepartum. Contrasting, some evidence indicated that high estrogen (7), and particularly estrone (21, 22), would stimulate bone accretion and, thus, would lower calcium in plasma. As such, parturient paretic cows might have been expected to have elevated estrogen. This was the case in nonparturient paresis which occurred at estrus (2) when estrogen of plasma was high (8). A possible explanation could be that the rise in estrogen at estrus was acute and, just as in estrone injection (22), bone accretion may have been stimulated resulting in acutely depressed calcium and subsequent paresis. However, in the parturient cow an alternate explanation for the role of estrone can be formulated. These cows had chronically lower (compared to nonparturient paretic cows) estrone during a few days period preceding parturition (Fig. 2). If estrone stimulated bone accretion, it would increase gradually during the latter stages of pregnancy. Accretion necessarily would drain away calcium in plasma at an increasing rate. Thus, physiological mechanisms for replenishment of calcium in plasma would be stimulated and perhaps sensitized. As such, these mechanisms would be prepared to replace the acutely depleted calcium that occurs at the initiation of new lactation. The end effect of estrogen stimulation might be to increase turnover of calcium in plasma and/or bone. The response of estrogens may be graded; that is, animals with higher estrogen, as in this study, may have a greater ability to replenish calcium in plasma. Thus, they would be protected from removal of calcium for lactation at parturition.

Muir et al. (19) have reported estradiol treatment (1 wk) resulted in a greater release of calcium from bone in response to EDTA treatment. This supports our hypothesis that chronically higher estrogen sensitized the animal, and probably bone to PTH stimulation, such that they were better able to adapt to lactational hypocalcemia.

Bargeloh et al. (3) suggested that exogenous estradiol-17β administered approximately the last week of pregnancy had no effect on the incidence of milk fever compared to nonestrogen treated controls. However, they had only one of five and none of five cases of milk fever in control and treated groups, respectively. Of importance, however, in their data is that calcium of plasma following parturition was higher in the estrogen treated group than the controls regardless of whether they contracted milk fever. This suggests that higher estrogen prior to parturition may protect against excessive hypocalcemia at parturition.

Edqvist et al. (9) did not find a difference in estrone between diseased and normal cows. Their data was analyzed for differences within periods. When our data were analyzed within periods, with both breeds included, there were no differences either. If Holsteins were excluded, estrone was lower at 5 to 4 days before parturition. When the current data were analyzed for differences in estrone during the entire prepartum period, values for the parturient paretic animals were different and lower (P<.05). This difference is readily observed in
Figure 2. Such a difference is not readily visible in Edqvist's et al. (9) data, and it is doubtful similar statistical analysis would suggest a difference in the prepartum estrone curves of diseased or normal animals. The contradiction between Edqvist's et al. (9) and these results are hard to resolve. It may relate to confounding of the former results due to animals being managed in three locations and in the latter results to two breeds.

Other work (22) has demonstrated that estrogens enhanced size of the parathyroids. Thus, estrogens may stimulate synthesis and release of PTH which would enhance the potential for bone resorption. In addition, deprivation of dietary calcium (which would inhibit intestinal uptake and effectively reduce calcium in plasma) would perhaps sensitize the homeostatic mechanism for calcium. Under such conditions (5, 6, 12), animals suffered lowered incidence of parturient paresis. In contrast, physiological situations that do not stimulate bone resorption but result in more nearly static calcium in plasma would effectively reduce calcium flux between body compartments. The end effect would be a reduction in the sensitivity of homeostatic mechanism for calcium. Thus, lactational draw on calcium in plasma would result in excessively depressed calcium of plasma since resorption was impaired.

Concepts discussed here suggesting that a deficiency in estrone may be a predisposing factor for parturient paresis may appear contrary to the generally accepted concept that estrogens depress calcium of plasma. We agree that estrogens depress calcium of plasma. However, we suggest this worked to the benefit of the animal in that regulatory mechanisms for calcium in plasma were sensitized more highly during late pregnancy, and, thus, they could adapt to excessive depression of calcium in plasma when lactation began.

REFERENCES
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