Parathyroid Hormone and Calcium Interactions in the Periparturient Mare

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(ABSTRACT)

The initiation of lactation involves an increased flow of Ca into mammary secretions, which leads to responses of serum concentrations of Ca and parathyroid hormone (PTH) that may be influenced by dietary Ca. Eight light mares from Farm A and eight Thoroughbred mares from Farm B were bled and milked 10 d pre-foaling, and eight mares (four from each farm) were bled and milked 5 d post-foaling. Milk Ca was measured by two commercial tests, one for [Ca + Mg] and the other [Ca]. Serum PTH and total Ca were measured in 16 mares, and ionized Ca in four mares. Parturition was induced in all mares with fenoprostalene on Farm A, and in four mares with oxytocin on Farm B; no significant difference was found between induction methods or between induced and spontaneous foaling mares. Dietary Ca was .34% DM on Farm A and .79% on Farm B. Mean serum total Ca concentrations decreased from 12.5 mg/dl to a nadir of 11 mg/dl on d 2 post-partum, and mean PTH increased from 46 pg/ml to a peak of 186 pg/ml on d 2 post-partum. Mean serum PTH concentrations were lower (P = .03) and total Ca concentrations were higher (P = .01) on Farm B in comparison to Farm A, probably reflecting the difference in Ca

intake. The nadir in mean ionized Ca and total Ca concentrations was reached on d 2 post-partum, 1 day later than has been observed previously in the dairy cow. Milk Ca concentrations increased from 50 ppm 7 d pre-foaling to 350 ppm on the day of foaling, with no difference between farms. The [Ca + Mg] test reached a critical level of 200 ppm 4.5 d pre-foaling, the [Ca] test 2 d pre-foaling. The [Ca + Mg] and [Ca] tests reached 250 ppm 2.5 and 1 d pre-partum, respectively. In short, serum Ca and PTH concentrations showed periparturient changes which reflected dietary Ca pre-partum. Foaling date was more closely associated with milk [Ca] than with [Ca + Mg] and by a critical level of 250 ppm than by 200 ppm.

(Key Words: mare, parathyroid hormone, calcium, milk, periparturient)

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Introduction

The initiation of lactation introduces a substantial flow of Ca from the blood into mammary secretions. Associated changes in serum concentrations of Ca and parathyroid hormone (PTH) have been found to reflect dietary Ca in the dairy cow (Ramberg et al., 1984) and may be similarly useful in the mare. They also may relate to the increase in Ca concentration in mammary secretions pre-partum, which have been used to predict foaling (Ley, 1994a).

The approach of foaling has been predicted traditionally from physiological changes that include mammary development, relaxation of the perineal area, and cervical relaxation; other signs are increased heart rate and sweating. More recently, mammary secretions have been tested for total Ca and found to be a valuable indicator within 24 to 48 h of impending parturition. Foaling occurs in most cases 24 to 48 h after the mare reaches a milk Ca concentration of 200 ppm (Ley et al., 1994a, 1994b). Increases in milk Ca and other electrolytes have been observed up to one month prior to parturition (Leadon et al., 1984). In addition, the Ca test has been an indicator of maturity and viability of the foal (Cash et al., 1985; Ousey et al., 1989; Ley et al., 1989a and 1993).

Previous research in the mare during the last trimester of pregnancy has described endocrine changes in progesterone, estrogen, prostaglandin $F_{2\alpha}$, oxytocin, relaxin, and prolactin, the exception being the PTH. Most research on PTH in horses has concerned its possible suppression by hypercalcemia and hypophosphatemia during chronic renal failure (Brobst et al., 1978; Tennant et

al., 1982; Elfers et al., 1986; Mathews et al., 1993). In most other species, chronic renal failure involves hyperphosphatemia, hypocalcemia and an increase in PTH (Zeller, 1987). Equids are unique because they tend to absorb most dietary Ca and P then regulate the excretion via the kidney (Schryver et al., 1970, 1974). In contrast, the ruminant species tend to regulate Ca and P through the digestive tract (Kronfeld et al., 1976).

The relationship of PTH to serum Ca has received much attention in the dairy cow, which is predisposed to parturient hypocalcemia and paresis (milk fever). This condition occurs when the Ca demand for milk synthesis exceeds immediate replenishment from the digestive tract and from bone stores (Kronfeld et al., 1976; DeLuca, 1979; Green et al., 1981; Kichura et al., 1982). Parathyroid hormone is secreted in response to hypocalcemia and, in turn, stimulates absorption of Ca from the digestive tract, reabsorption of Ca by renal tubules, and release of Ca from bone stores (Hollis et al., 1981; Goff et al., 1986). Parathyroid hormone and Ca work through a negative feedback system; as blood Ca decreases PTH increases and vise versa (Habener et al., 1984).

Unlike the dairy cow, the mare is not prone to parturient hypocalcemia. The mammary drain of Ca is less in the mare than in the dairy cow (Akers, 1990), because of the considerably smaller volume of milk production in the mare. Therefore, it is reasonable to assume that the degree of hypocalcemia would be less in the mare than in the dairy cow. Nevertheless, the degree of hypocalcemia may reflect dietary Ca in the mare as in the dairy cow, and therefore be important in formulating the diet of the periparturient mare. In addition, the PTH status of periparturient mares may be correlated to serum ionized Ca, total Ca and milk calcium during the onset of lactation. The

objective of this study was to determine if there was an interaction between PTH and Ca prior to parturition, as well as during the days following birth, and to evaluate the predictive value of two commercial milk Ca tests for parturition and the onset of lactation.

Review of Literature

Parathyroid Hormone

Parathyroid hormone is an 84 amino acid polypeptide involved with calcium homeostasis. Its secretion and release by the parathyroid gland is regulated by blood Ca (Markowitz et al., 1988). A small decrease in blood ionized Ca will cause an increase in PTH concentrations, likewise, an increase in ionized Ca will suppress PTH concentrations in the circulation. Parathyroid hormone responds to a decrease in ionized Ca using three different mechanisms (Edmonson and Li, 1976): 1) it will directly mobilize calcium from skeletal stores, 2) it will stimulate calcium reabsorption in the distal tubules of the kidney, and 3) it will, indirectly, increase the rate of intestinal Ca absorption.

Synthesis. The precursor for PTH is synthesized in the rough endoplasmic reticulum of the chief cells in the parathyroid glands as pre-pro-PTH (115 amino acids), which is then processed to pro-PTH (90 amino acids) (Segre et al.,1974; Slatopolsky et al., 1982). Next the pro hexapeptide is removed from pro-PTH in the Golgi apparatus by proteolytic cleavage, and the remainder of the peptide is the bioactive PTH (84 amino acids).

Secretion. Calcium is the principal regulator of PTH secretion, and the rate of secretion is dependent on the concentration of extracellular Ca ion. Parathyroid hormone increases the concentration of Ca in the extracellular fluid through its effect on bone, kidney, and gut. Negative feedback inhibition of the parathyroid gland contributes to the regulation of Ca concentrations within narrow limits. Adenylate cyclase and its product, adenosine 3' 5' -

monophosphate (cAMP), are intermediates in the control by Ca of PTH secretion. Intracellular levels of cAMP change in parallel with changes in PTH secretion. In addition, the changes in PTH may be due to other secretagogues, such as, epinephrine, isoproterenol, dopamine, secretin, prostaglandin E_2, Mg, as well as hypocalcemia (Habener et al., 1984). Also, there are agents that suppress the secretion of PTH and decrease intracellular levels of cAMP, such as α-adrenergic agonists or prostaglandin $F_{2\alpha}$.

The exact role of adenylate cyclase and the way in which cAMP is formed during the secretory events involved in PTH release remains unknown. *In vitro* studies have shown that cAMP is released concomitantly with the release of PTH in response to hypocalcemia, suggesting that the formation of cAMP is linked to a process of exocytosis (Habener et al., 1984). Biochemically, it may be that cAMP is involved in the phosphorylation of a substrate protein in the parathyroid gland. The substrate may be a membrane protein involved in the fusion of the secretory granule with the plasma membrane, resulting in the discharge of hormone from the granule into the extracellular space.

There are two cellular storage pools of PTH available for secretion, an older storage pool and a newly synthesized pool. Ionized Ca affects the secretion of hormone from both pools; however, agents that influence cAMP affect the secretion of the hormone only from the storage pools (Morrissey and Cohn, 1979; Slatopolsky et al., 1982).

Metabolism. The liver is the principal site of PTH metabolism. Intact PTH is cleaved by the liver into smaller fragments, which are in peripheral circulation. The rate of metabolism of PTH is dependent on the extracellular Ca

levels (Habener et al., 1984). Metabolism is decreased by high concentrations of Ca and is increased by low concentrations of Ca.

The kidney is another site for PTH metabolism. Carboxy-terminal (C-terminal) fragments are derived from parathyroid gland secretion and metabolism of intact PTH by the liver and kidney. The C-terminal fragments are removed from circulation by the kidney through glomerular filtration. Amino-terminal (N-terminal) PTH fragments, which are probably produced by the liver or kidney, may mediate the affect on bone. N-terminal fragments are removed by the kidneys through glomerular filtration and peritubular uptake (Segre et al., 1974; Slatopolsky et al., 1982).

Circadian rhythm

Serum PTH concentrations follow a circadian pattern (Jubiz et al., 1972; Sinha et al., 1975; Markowitz et al., 1981; Halloran et al., 1985), but Ca concentrations remain constant (Wong and Klein, 1984). In dogs, the bone contribution to blood Ca fluctuates in a reciprocal manner with dietary Ca intake, but blood Ca remains constant. In humans, the nadir for serum total Ca occurs between midnight and early morning, while the nadir for ionized Ca occurs in the late afternoon or evening (Jubiz et al., 1972; Sinha et al., 1975). Serum PTH increases nocturnally, which coincides with the decrease in total Ca. Fluctuations in ionized Ca precede inverse changes in PTH concentrations (Markowitz et al., 1988).

Chronic Renal Failure in Equines

Equids tend to absorb most dietary Ca and P then regulate the excretion via the kidney (Schryver et al., 1970, 1974). In contrast, the bovine and other species tend to regulate Ca and P through the digestive tract (Kronfeld et al., 1976). Chronic renal failure is associated with hyperphosphatemia, hypocalcemia, and hyperparathyroidism in most species (Melick et al., 1969; Osborne et al., 1976; Zeller, 1987). In comparison the horse develops hypophosphatemia and hypercalcemia, and shows no indication of hyperparathyroidism (Brobst et al., 1977 and 1982; Tennant et al., 1982; Elfers et al., 1986). In nephrectomized ponies, serum Ca concentrations rose to abnormally high levels while P remained low (Tennant et al., 1981). On the other hand, nephrectomized dogs and cats develop hyperphosphatemia and hypo- or normocalcemia (Osborne et al., 1976). Basically, there are three possible reasons for hypercalcemia in the horse: 1) increasing Ca absorption through high dietary intake of Ca, in combination with decreasing urinary excretion of Ca; 2) increasing Ca resorption from bone; 3) increasing PTH secretion and/or decreasing PTH degradation (Elfers et al., 1986).

Ponies fed a grass hay high in Ca exhibited hypercalcemia after bilateral nephrectomy, but ponies fed a low Ca diet did not (Schryver et al., 1970, 1974; Tennant et al., 1981). Intact PTH is cleaved by the kidney and liver into an active N-terminal fragment and an inactive C-terminal fragment (Elfers et al., 1986). The N-terminal fragment is bound to receptors and cleared by the blood while the C-terminal fragment requires adequate renal function to be cleared from circulation. Hypercalcemia may be due to the reduced urinary excretion of

Ca during chronic renal failure, or may be secondary to a reduced rate of PTH breakdown by the diseased kidneys.

Endocrine changes during pregnancy and at parturition

Progesterone changes during the onset of pregnancy directly reflect maternal ovarian function, specifically the corpus luteum (Pashen, 1984). After day 35 of pregnancy, the accessory corpora lutea contribute to the progesterone pool. The placenta secretes progesterone after day 50 of gestation, and is the sole source of progesterone that will maintain the pregnancy after day 150. The mare has a relatively low circulating progesterone concentration during mid-gestation in comparison to the fetus which has higher progesterone levels (Barnes et al., 1975). At about 30 to 60 d prior to parturition, progesterone concentrations increase until birth when they decline to a baseline concentration. Concomitantly, there is an increase in the number of progestagens. These have been identified as 5α pregnane metabolites of progesterone, 5α-pregane-3, 20-dione, 3β-hydroxy-5 α-pregnane-20-one (3β-ol), 20α-hydroxy-5α-pregnane-3-one (20α-ol) (Seamons, et al., 1979), 20α-dihydroprogesterone, and 17α-hydroxyprogesterone (Barnes et al., 1975). The 5α-pregane-3, 20-dione is formed mainly by the placenta and a maternal source, 20α-ol is mainly maternal in origin, 3β-ol is produced almost entirely by the fetus, and 20α-dihydroprogesterone is presumed to be fetal in origin. The biological function of these metabolites is not known, but their gradual increase in circulation during the preparturient period suggests that a general alteration in progesterone metabolism occurs near term (Pashen, 1984).

Concentrations of estrogens are high in the blood and the urine of mares during the second half of gestation. Peak levels occur during the 7 and 8 mo of pregnancy followed by a gradual decrease until parturition.

There are two main groups of estrogens present in the equine during pregnancy; the common phenolic estrogens, estrone and estradiol 17β, and ring B unsaturated estrogens equilin and equilenin, which are specific to the equine (Pashen, 1984). The biosynthetic pathways for the production of the phenolic estrogens involve the fetal gonads. The gonads increase in size in parallel to maternal estrogen concentrations and supply precursors which are converted by the placenta into estrogens. The gonads produce dehydroepiandrosterone, which is metabolized by the placenta into estrone and estradiol 17β. It is still unclear whether or not dehydoepiandrosterone is a precursor of equilin. Thus, a true feto-placental unit exits in the mare for estrogen production.

Prolactin concentrations rise markedly during the last week of gestation, remain high until 1 to 2 mo after parturition, and are higher in the morning than in the evening (Ginther, 1992). The role of prolactin in mammary development is presumed on the basis of studies done with mares and other species. The interrelationships between equine prolactin and other hormones around parturition are not known.

Relaxin is present in increasing concentrations in the serum of mares beginning around day 80 and continuing until parturition (Stewart et al., 1982). The pattern of relaxin production is similar to estrogen, although no link has been established. It has been suggested that relaxin works synergistically with progesterone to enhance uterine quiescence.

In sheep the increase in fetal cortisol concentrations induce activation of placental enzymes which permit progesterone to be converted to estrogen, and as a result cause a drop in progesterone concentrations and a rise in estrogen (Flint et al., 1974). The rise in estrogen stimulates a rise in prostaglandin F 2α production at the onset of parturition.

In mares, fetal adrenal cortical hormones play a role in the initiation of parturition; however, the mare has the ability to delay parturition (Bazer and First, 1983; Ginther, 1992). This is part of the mares natural survival instinct. Neither estrogen nor progesterone play a crucial role in the initiation of parturition. Progestins favor a uterine quiescence before parturition. Also, an increase in the estrogen:progestin ratio may play a role in the production of prostaglandin $F_{2\alpha}$.

Prostaglandin $F_{2\alpha}$ stimulates myometrial contractions. Both prostaglandin E_2 and prostaglandin $F_{2\alpha}$ have been detected in allantoic fluid (Ginther, 1992). In other species prostaglandin E_2 is important for cervical ripening. In this regard, exogenous prostaglandin E_2 could be used locally to soften the equine cervix.

Oxytocin is the final hormone in the cascade of endocrine changes during parturition. Oxytocin stimulates both myometrial contractions and milk let down (Ginther, 1992). Oxytocin receptors in the uterus of the mare do not increase near term, therefore, the amount of oxytocin needed for initial stimulation of the myometrium is minimal. Oxytocin receptor formation is induced by changes in the estrogen:progestin ratio and increased prostaglandin $F_{2\alpha}$ production.

Parathyroid hormone has been found in the placenta and amniotic fluid of the human. Concentrations in human amniotic fluid were about 80% of the concentration in blood serum (Brotherton, 1991). The placenta contains a complete PTH-dihydroxyvitamin D_3 system that allows the absorption of more Ca from the intestine for fetal needs during pregnancy.

Induction of parturition

Induction of parturition in the horse has been practiced for managerial or clinical reasons, for teaching, and for research. The criteria for inducing a mare to foal are as follows: colostrum or milk present in the teats, adequate mammary development, pre-foaling milk Ca concentration of 200 ppm, gestation length $\geq$ 330 d, relaxed perineum and sacrosciatic ligaments, and a dilated cervix (Ley et al., 1994).

Equine parturition has been induced using various methods. Oxytocin has been commonly used, alone or in combination with estrogen (Jeffcott and Rossdale, 1977). Oxytocin is released from the posterior pituitary gland and stimulates the stretching of the cervix and vagina. In addition, oxytocin acts on the myometrium to cause muscle contractions, which are enhanced by prostaglandin $F_{2\alpha}$ that is released in response to oxytocin (Pashen, 1980). A low dose of oxytocin is reported to be a safe procedure for inducing a mare to foal (Pashen, 1980); however, high doses oxytocin may cause perineal tears, uterine rupture, premature placental separation, and fetal hypoxia.

Another method to induce foaling is to use a synthetic prostaglandin, such as fenprostalene or fluprostenol. This method provided a frequency of dystocia, foal survival rate, effective colostral transfer to foals, and post-

parturient neonatal vitality that was similar to spontaneous foaling mares (Ley et al., 1994).

The benefit of using oxytocin or a synthetic prostaglandin is that the time of delivery in comparison to the initial injection is 1 to 2 h. In contrast, delivery after the final injection of dexamethasone, which has also been used to induce parturition, is approximately 5 d later (Ley et al., 1994).

Lactogenic hormones

A cascade of events occurs in the endocrine system during the third trimester of gestation that prepares the mammary gland for the secretion of milk. These events are closely integrated with the hormonal control of parturition. *In vivo* studies in laboratory and farm species have defined hormones from the ovary (estrogen and progesterone), adrenals (glucocorticoids), pituitary (prolactin and growth hormone), thyroid, and during pregnancy the placenta (prostaglandin and placental lactogen) as important controlling factors in mammary development (Forsyth, 1991). Synergistic actions of steroid and peptide hormones are necessary for mammary growth and function.

The lactating gland has at least three types of epithelial cells non-secretory epithelial cells lining ducts, secretory epithelial cells in terminal ducts and in lobules of alveoli, and myoepithelial cells which surround alveoli and small ducts within the basal lamina (Forsyth, 1991). At birth in the human a branched tubular gland is present consisting of ducts and primitive ductolobular structures in which epithelial and myoepithelial cells can be recognized. Puberty marks the next phase in mammary development. Estrogen causes development of the stromal tissue, growth of the extensive ductile system, and

deposition of fat into the mammary gland (Guyton, 1991). Progesterone promotes development of the lobules and alveoli of the mammary gland, causing the alveolar cells to proliferate, to enlarge, and become secretory in nature. Maximum development occurs during pregnancy with full lobuloalveolar growth and the attainment of secretory activity during mid-gestation (Forsyth, 1991).

The initiation of lactation has been defined in two stages: 1) the alveolar cells differentiate cytologically and enzymatically during the last third of pregnancy in most species, and 2) copious secretion of milk which usually occurs 1 to 4 d before to 1 to 3 d after parturition (Tucker, 1994). The minimal hormonal requirement for lactation involves increased secretion of prolactin, glucocorticoids, and estradiol-17β coupled with a decrease in progesterone. In rabbits, the mammary tissue is more sensitive to prolactin, especially in terms of inducing synthesis of the primary milk protein casein, than in comparison to other species. For example, prolactin in the rat is luteotrophic, thereby increasing synthesis of progesterone, an inhibitor of lactogenesis. Concentrations of prolactin in serum do not increase during gestation until the second stage of lactogenesis during the periparturient period. In contrast, serum prolactin in humans does increase during gestation, coincident with the increasing secretion of estrogens. The high circulating concentrations of estrogen and progesterone suppress the number of prolactin binding sites in the mammary tissue, which delays lactogenesis until the concentrations of these steroids decline during the periparturient period.

Placental lactogens are proteins with potent growth hormone-like and prolactin-like activities (Akers, 1983). Placental lactogens have been identified in

cows, goats, and sheep. Intraductal injection of human placental lactogen into rabbit mammary glands initiates secretion of milk and synthesis of casein (Tucker, 1994). Ovine placental lactogen is lactogenic in cultures of rabbit and ovine mammary explants, but the lactogenic activity is less than that for prolactin. Action of placental lactogen is mediated via the prolactin receptor, but placental lactogen binding to this receptor is low. Physiological concentrations of placental lactogen are minimal and are probably not lactogenic, especially in the presence of high concentrations of progesterone.

The role of growth hormone in lactogenesis is not well defined in many species. A combination of growth hormone, glucocorticoid, and triiodothyronine is moderately lactogenic in hypophysectomized goats (Tucker, 1994). Bovine growth hormone has no effect on the initiation of casein synthesis in goat mammary tissue cultures. In contrast, human growth hormone readily induces lactogenesis. These effects may be mediated through the prolactin receptor, because human growth hormone binds to prolactin binding sites on membranes of ovine and bovine mammary tissue.

Cortisol induces differentiation of the rough endoplasmic reticulum and Golgi apparatus of mammary tissue explant cultures *in vitro* (Tucker 1994). This is essential to permit induction of casein synthesis by prolactin. Adrenal steroids are an important part of the lactogenic mechanism, because adrenalectomy inhibits ovariectomy-induced synthesis of casein-like proteins in pregnant rats. Also, cortisol is essential for prolactin to stimulate casein gene expression in cultured mouse mammary glands. Thus, cortisol is required to synergize with prolactin to initiate the lactation process.

Prostaglandins may play a role in lactogenesis. Prostaglandin $F_{2\alpha}$ administered to pregnant rats induced lactation, which was preceded by abortion (Tucker, 1994). Prostaglandin $F_{2\alpha}$ is luteolytic in most species that depend on the corpus luteum for progesterone secretion. Concentrations of prostaglandin F in plasma increase coincidentally with the second stage of lactogenesis and the shifts in progesterone, prolactin, and glucocorticoids. Therefore, prostaglandins may play a role in lactogenesis by stimulating prolactin and glucocorticoid secretion.

Lactogenic hormones in mares have not been researched as they have been in other species, except for prolactin (Ginther, 1992). Further investigations of lactogenic hormones in mares may allow a better understanding of the physiological changes that occur with mammary development and the onset of lactation.

Calcium secretion into milk

Calcium homeostasis is regulated mainly by PTH and calcitonin and these are somewhat dependent on dietary Ca intake and bone Ca turnover (Sherwood et al., 1968). Parathyroid hormone increases when plasma Ca concentration decreases at parturition (Mayer et al., 1969), and, conversely, PTH decreases as plasma Ca concentrations return to normal levels post-partum.

A multicompartmental model of the Ca exchange pool has been developed to describe the regulatory function of Ca (Ramberg et al., 1970, 1984; Kronfeld et al., 1976). There are four compartments which are divided into plasma equivalent spaces of 10, 20, 40, and 80% of the body weight. The fourth compartment, being the largest, represents the Ca residing in bone. Therefore,

Ca balance is represented by the difference in the rate of Ca deposition into bone and the rate of Ca removal from bone.

In-flows of Ca from the gut and from bone enter the first compartment, and all excretory losses occur in this compartment. Excretory losses of Ca include feces, urine, and milk. In addition, changes in departmental masses and Ca transport between compartments occur during pregnancy, parturition, and lactation (Ramberg et al., 1970). The fourth and third compartments tend to become depleted during the onset of lactation compared to compartments one and two.

Calcium secretion into milk occurs through bi-directional fluxes between blood plasma and milk (Kronfeld et al., 1971). It appears that Ca secretion from blood plasma to milk is through an active transport mechanism, and there is a 3 to 6 h delay in radiocalcium transfer from the blood to milk, which suggests that the mammary gland contains a calcium reservoir or pool (Visek et al., 1953). Calcium transport in blood is linearly related to milk production (Kronfeld et al., 1971). At the onset of lactation there is a sudden increase in Ca absorption and decreases in the rates of Ca outflows to bone and feces, but there is no immediate change in the removal of Ca from bone Furthermore, there is a two week delay before the rate of Ca removal from bone increases and the rate of Ca absorption subsides, which is mainly due to a decrease in the efficiency of absorption (Ramberg et al., 1970).

Periparturient hypocalcemia in the lactating dairy cow

The beginning of lactation in the dairy cow creates a sudden demand for large quantities of Ca for milk production. The first milk the cow produces is

colostrum, which is rich in Ca as compared to the milk secreted 3 days later. Both the need for Ca in the colostrum, and the large amount of milk being produced predisposes the dairy cow to parturient hypocalcemia (Littledike, 1976). In contrast, the mare is not predisposed to this condition, but its relevance is important in understanding calcium homeostasis during the onset of lactation.

Parturient hypocalcemia is a metabolic disorder that effects mature, high producing dairy cows during the first 2 to 3 days of lactation (Kronfeld et al., 1971. The concentration of Ca in the blood decreases because of the Ca removal for milk synthesis exceeds Ca replenishment from the intestine, bone and kidney at parturition (Goings et al., 1974; Wiggers et al., 1975). Parturient hypocalcemia develops despite adequate function of the parathyroid gland and the vitamin D endocrine systems (Mayer et al., 1969; Horst et al., 1978; Hove, 1985). Plasma Ca returns to normal within 48 h post-calving in most cases. The decline in peak plasma levels of 1, 25-dihydroxyvitamin D [1, 25-$(OH)_2$ D] and PTH 2 to 4 h after calving is consistent with a stabilization of Ca metabolism early post-partum in normal cows (Hove, 1985). Usually at this point, constant Ca concentrations after parturient hypocalcemia indicates that Ca metabolism has adapted to the demands of lactation.

The failure to maintain Ca homeostasis is not completely controlled by a decrease in PTH, because PTH levels in paretic cows have been observed to be equal or greater than that of non-paretic cows (Mayer et al., 1969; Horst et al., 1978). Much research has been done on feeding low Ca diets pre-partum to attenuate hypocalcemia and prevent paresis. Both heifers and cows fed high Ca diets pre-partum had higher serum Ca concentrations due to the increased intestinal absorption of the dietary Ca. Cows fed high Ca diets had decreased

PTH levels and lowered calcitonin levels prior to parturition (Horst et al., 1978; Kichura et al., 1982). Cows fed high Ca diets pre-partum had a higher incidence of hypocalcemia despite the fact that PTH concentrations in these cows were similar to normocalcemic cows (Shappell et al., 1986).

Cows that had received low Ca diets pre-partum had decreased intestinal absorption of Ca, decreased serum Ca concentrations, and increased PTH concentrations (Shappell et al., 1986). The cows fed low Ca diets had produced more total milk and had higher colostral Ca concentrations compared to cows fed high Ca diets. Therefore, feeding low Ca diets to dairy cows stimulated PTH pre-partum and the increased PTH, by mobilizing Ca from bone and reducing urinary Ca excretion, attenuated the decrease in serum Ca concentrations during the onset of lactation.

Parturient hypocalcemia in dairy cows has been associated with low serum Ca concentrations and reciprocally high PTH concentrations (Mayer et al., 1969). The failure of these paretic cows to resorb Ca from bone has not been associated with an inability to synthesize PTH, 1, 25-$(OH)_2$ D, or calcitonin. However, hypocalcemia has been associated with increased levels of estrogen, a known inhibitor of bone resorption, in pre-partum cows. In paretic cows, when the inhibitory effect of estrogen was removed, the bone cells became responsive to PTH and 1, 25-$(OH)_2$ D in circulation and active bone resorption continued in these cows (Hollis et al., 1981). Hypocalcemia, also, develops occasionally in nonparturient cows during estrus (Kronfeld et al., 1976; Hollis et al., 1981). Estrogen inhibited bone resorption of Ca, and decreased the responsiveness of bone to PTH in osteoporotic women (Riggs et al., 1976).

Milk Calcium in Mares

Changes in mammary gland development and secretions involved in lactogenesis have been extensively studied in dairy cows (Johnson and VanJonack, 1975). This work has now been extended to the equine species, because prediction of parturition would minimize the time needed for foal watch and ensure availability of assistance for a mare having difficulties. Various constituents, including electrolytes, such as Ca and Mg, in pre-colostral secretions in the mare change characteristically during the onset of milk secretion (Forsyth et al., 1975; Peaker et al., 1979; Leadon et al., 1984).

The foal controls the time of parturition according to its *in utero* maturation (Rossdale and Silver, 1982). This accounts for the inconsistency in gestational length between mares. The pre-foaling mammary secretions correlate with fetal maturation and the mare's readiness for birth (Leadon et al., 1982, 1984). By studying these changes in the milk, foaling can be predicted within 24 to 48 h of impending parturition (Ousey et al., 1984, 1989; Cash et al., 1985; Ley et al., 1989a; Ley et al., 1989b).

Water hardness test strips like the Merckoquant Water Hardness Test Strip (Merck, Darmstradt, West Virginia) have been used to predict parturition in mares (Cash et al., 1985; Ousey et al., 1989). The determination of water hardness is based on the sequestration of Ca and Mg ions with disodium ethylenedinitrilo tetra acetate, which causes a color change from green to red-violet in the test strip zones. The test strip consists of four zones of decreasing sensitivity to Ca and Mg, the greater the number of zones with a color change, the greater the concentrations of Ca and Mg. This test has revealed that milk Ca and Mg increase progressively prior to parturition. It has been used to predict

foaling. If the mare's milk sample had three zones change two days in a row she would usually foal on the third day. If the mare had all four zones change color she would usually foal with 24 to 48 h. The results with this strip test method were somewhat inconsistent between horses, so needed to be interpreted along with mammary gland size, gestational age, and waxing to predict the time of parturition.

Another water hardness strip test is the Sofchek Water Hardness Test Strip (Environmental Test Systems, Elkhart, IN). The Sofchek test estimates Ca and Mg similarly to the Merckoquant Water Hardness Test, but it only has one zone. A low Ca and Mg level turns the zone a green or brown color, and a high Ca and Mg level turns it orange or read. This test is able to predict readiness for birth more consistently than the previously discussed strip test (Ley et al., 1989a). However, both strip test methods measure Mg which is relatively high in mammary secretions prior to the sharp rise in Ca pre-partum. Therefore, the Sofchek Water Hardness test, as well as the Merckoquant Water Hardness Test, may indicate an earlier readiness for birth than a test specific for Ca (Ley et al., 1989a, 1989b).

The Titret Calcium Hardness Test Kit (CHEMetrics, Inc., Calverton, VA) has been used more recently as an indicator of impending parturition in the mare (Ley et al., 1989a and 1993). It measures Ca only and is therefore not altered by high levels of Mg in early mammary secretions. A Ca level of 200 ppm using the Titret test has been a reliable and consistent indicator of fetal readiness for birth and parturition within 24 to 48 h.

These various mammary secretion tests have been used to predict spontaneous foaling and to determine when a mare is ready to have an induced

foaling. There is a correlation between mammary electrolyte secretions and fetal maturity as well as maternal readiness for birth. Thus milk Ca indicates that both the mare and foal are ready for birth concurrently (Ousey et al., 1984). The Titret test and the Sofchek test have indicated fetal and maternal readiness for birth, and have been successfully used with induction procedures (Ley et al., 1989a, 1989b).

Objectives

The general objective of this study was to begin an examination of the responses of the Ca system to parturition and the initiation of lactation in the mare. Such responses have been demonstrated to be important in other species, especially the dairy cow, so it was predicted that they might also provide useful information in the mare.

There were two specific aims:

1. To examine the interactions of serum concentrations of total Ca, ionized Ca, and PTH in the periparturient mare, and to identify any possible influences of dietary Ca.

2. To evaluate lactation, and to see if serum Ca and PTH concentration would improve the prediction of foaling.

PARATHYROID HORMONE AND CALCIUM INTERACTIONS IN THE PERIPARTURIENT MARE

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(ABSTRACT)

The initiation of lactation involves an increased flow of Ca into mammary secretions, which leads to responses of serum concentrations of Ca and parathyroid hormone (PTH) that may be influenced by dietary Ca. Milk and blood samples were taken from eight mares on Farm A and eight mares on Farm B for 10 d pre-foaling, and four on each farm for 5 d post-foaling. Milk Ca was measured by two commercial tests. Serum samples were analyzed for PTH and total Ca in 16 mares and for ionized Ca in four mares. Parturition was induced with fenoprostalene on Farm A, and with oxytocin on Farm B; no significant difference was found between induction methods or between induced and spontaneous foaling. Dietary Ca was .34% DM on Farm A and .79% on Farm B. Mean serum total Ca concentrations decreased from 12.5 mg/dl to a nadir of 11 mg/dl on d 2 post-partum, and mean PTH increased from 46 pg/ml to a peak of 186 pg/ml on d 2 post-foaling. Serum PTH concentrations were lower (P = .03) and total Ca concentrations were higher (P = .01) on Farm B in comparison to Farm A, probably reflecting the difference in Ca intake. The nadir in the mean ionized and total Ca concentrations was on d 2 post-partum, 1 d later than

reported previously in the dairy cow. Milk Ca concentrations increased from 50 ppm 7 d pre-foaling to 350 ppm on the day of foaling. The [Ca + Mg] test reached a critical level of 200 ppm 4.5 d pre-foaling, the [Ca] test 2 d pre-foaling. The [Ca + Mg] and the [Ca] tests reached 250 ppm 2.5 and 1 d pre-partum, respectively. In short, serum Ca and PTH concentrations showed periparturient changes which reflected dietary Ca pre-partum. Foaling date was associated more closely with milk [Ca] than with milk [Ca + Mg] and by a critical level of 250 ppm than by 200 ppm.

(Key Words: mare, parathyroid hormone, calcium, milk, periparturient)

Introduction

Foaling has been predicted from physiological changes that include mammary development, relaxation of the perineal area, and cervical relaxation; other symptoms are increased heart rate and sweating. More recently, mammary secretions have been tested for total Ca, and foaling has begun in most cases 24 to 48 h after the milk Ca concentration reached 200 ppm (Ley et al., 1994a, 1994b). The mammary transfer of Ca might influence serum Ca and parathyroid hormone (PTH), so that these variables might contribute to the prediction of foaling.

The relationship of PTH to serum Ca has received much attention in the dairy cow, which is predisposed to parturient hypocalcemia (Kronfeld et al., 1976). This condition occurs when the Ca demand for milk synthesis exceeds immediate replenishment from the digestive tract (Ramberg et al., 1984) Dietary Ca influences the responses of serum Ca and PTH to the initiation of lactation in the dairy cow (Shappell et al., 1986), so it might be important in the mare. The objective of this study was to determine the interactions of serum PTH and Ca in the periparturient mare, and to evaluate the predictive value of milk Ca tests for parturition.

Experimental Procedures

Mares

One Thoroughbred, one Hanovarian, and six Quarter horse mares (510 ± 90 kg BW) between the ages of seven to fourteen were from Farm A (the Smithfield Equine Research Center), and eight Thoroughbred mares (590 ± 60 kg BW) between the ages of eight to fifteen were from Farm B (the Middleburg Agricultural Research Extension Center).

Diets

Farm A. The nutrient contents of the concentrate (TABLE 1) were calculated from its formula: 83.5% corn, 10% soy bean meal-44, 6% molasses, and 5% limestone (NRC, 1989). The forage analysis was conducted by the Dairy Herd Improvement Association Forage Testing Laboratory at Virginia Tech (TABLE 1).

Farm B. The two concentrates and two forages were analyzed by the Dairy Herd Improvement Association Forage Testing Laboratory at Virginia Tech (TABLE 1).

Daily intake. Digestible energy requirement for mares in the 11 mo of gestation is 19.7 Mcal DE/d, that is, 20% over the maintenance level (NRC, 1989). On Farm A the intake of concentrate was 5.45 kg/d or 18.1 Mcal DE/d, so intake of forage was only 1.6 Mcal/d by difference, the Ca intake was 19.8 gm/d, and dietary Ca was .34% DM basis (TABLE 2). On Farm B the intake of concentrate was 3.2 kg/d or 10.7 Mcal DE/d, so the intake of forage was 9.0 Mcal DE/d by difference, the Ca intake was 45.2 gm/d, and dietary Ca was .79% (TABLE 2).

The NRC recommendations for Ca in 11 mo pregnant mares are 38 gm/d for a 510 kg BW (Farm A), and 43 gm/d for a 590 kg BW (Farm B). Thus, mares on Farm A were not receiving sufficient Ca (NRC, 1989).

Another estimate of energy intake during the 11 mo of gestation is 24.6 Mcal DE/d, or 50% over maintenance (Donoghue et al., 1990). Accordingly, on Farm A the intake of forage was 6.5 Mcal DE/d by difference, the Ca intake was 19.4 gm/d, and on Farm B the intake of forage was 13.8 Mcal DE/d by difference, the Ca intake was 53.3 gm/d (TABLE 2).

Induction of parturition

Eight mares were induced to foal on Farm A and four mares were induced to foal on Farm B. The time of induction was determined by general criteria: gestation length >330 d, physical parameters (such as relaxation of the perineal area), milk presence in teats, mammary development, and milk Ca levels. Mares on both farms were not induced until their milk Ca levels were >200 ppm using the Titret Water Hardness Test (Ley et al., 1994).

To induce the mares on Farm A, fenoprostalene .5 to 1.0 mg was administered s.q. at time 0 h and repeated at time 2 h (Ley, 1994a). On Farm B, 10 IU of oxytocin was administered i.m. every 15 min until the mare delivered the foal. There was no difference between induced and spontaneous foaling mares and between fenoprostalene and oxytocin induced mares, so the results were pooled.

Sample collection and handling

All mares were bled and milked daily for 5 to 10 d prior parturition. Four mares from each farm were bled for 3 to 5 d post-partum. All sampling was done between 0700 and 0900 h to minimize the effects of PTH being released on a circadian rhythm. Two 20 ml Vacutainer tubes (Becton Dickenson, Rutherford, NJ) were used to collect blood by jugular venipuncture. Blood samples were allowed to clot for 18 to 24 h at 4°C. Samples were centrifuged for 18 min, and serum was harvested and stored at -20°C until thawed for assay.

Milk calcium

Six ml of milk were collected and tested for [Ca + Mg] using the Sofchek Water Hardness Test (Environmental Test Systems, Elkhart, IN) and for [Ca] using the Titret Calcium Hardness Test (CHEMetrics, Inc., Calverton, VA). One ml of milk was diluted with 6 ml of distilled water. The Sofchek strip was dipped into the solution and read after 15 s. If the [Ca + Mg] was greater than 120 ppm, the Titret test was applied to estimate [Ca] (Ley et al., 1989a, 1993).

Serum calcium

Total Ca concentration [TCa] was determined on all mare serum samples using the Colorimetric Determination of Calcium in Serum (Procedure No. 587, Sigma Diagnostics, St. Louis, MO). This kit uses o-cresolphthalein complexone, which complexes with Ca in an alkaline medium to form a red colored complex at a pH 10 with an absorbance maximum at 575 nm (Kessler and Wolfman, 1964). Interference from Mg ions is prevented by 8-hydroxyquinoline (Teitz, 1976).

Ionized Ca

Blood samples from six mares on Farm A were collected and analyzed for ionized Ca concentration [ICa]. Serum [ICa] was determined using a Corning 288 Blood Gas Machine (# 4019) immediately after collection.

An electrode membrane that is selectively permeable to ICa. The selective permeability establishes an electrical potential as the charge associated with the ion leaves its counter-ion behind in solution. The magnitude of this electrical potential is determined by the concentration difference between the two sides of the membrane (Teitz, 1976).

Serum PTH assay

The Allegro Intact-PTH Immunoradiometric Assay (Kit No. 40-2170, Nichols Institute, San Juan Capistrano, CA) was designed for use in humans, and has been validated for use in equines (Wilson et al., submitted 1994). A standard sample was used to ensure the consistency of calibration, and a Beckman 5500 Gamma Counter was used to measure PTH concentrations [PTH].

The Intact PTH assay system is based on a two-site immunoradiometric assay methodology (Al-Shaw et al., 1981). Two affinity purified antisera are used: mid-region C-terminal specific antisera and the N-terminal specific antisera. The C-terminal antisera recognizes several different epitopes within the 39-84 region of PTH which are then adsorbed to polystyrene beads. These beads serve to capture all forms of immunoreactive PTH, including intact PTH and hormonal fragments. The affinity purified N-terminal antisera is radiolabeled with ^{125}I. It binds to the complex as a function of the concentration

of the intact hormone captured by the immobilized mid-region C-terminal antisera. The concentration of the intact PTH is determined from the amount of ^{125}I-labelled N-terminal antisera retained on the polystyrene bead. Both the N-terminal and C-terminal epitopes must be present within the same molecule for the ^{125}I-labelled N-terminal antisera to bind directly to the bead; therefore, the assay only measures intact PTH (Nussbaum et al., 1987).

Statistical analysis

Data were summarized as means and standard errors unless otherwise stated. The data were examined for differences in time, site, and time-site interactions using analysis of variance with repeated measures (SAS, 1990). Orthogonal polynomial contrasts were used to test for trends with time. Linear and quadratic relationships of dependent variables with time were also evaluated by a curve-fitting program (SWW2, 1994).

Results

Serum [PTH] and [TCa]

Mean [TCa] in eight mares (four Farm A and four Farm B) began decreasing 3 d pre-foaling and continued decreasing until 2 d post-foaling (FIGURE 1a). The change with time was curvilinear:

$$[TCa] = 11.95 - .28\ (T) - .03\ (T)^2$$

$$r = .8581\ P = .0014,$$

where (T) is time (days).

Conversely, [PTH] began increasing 3 d pre-foaling and continued increasing until 2 d post-foaling (FIGURE 1b):

$$[PTH] = 124 + 20.5\ (T) + 1.71\ (T)^2$$

$$r = .8434,\ P = .0021$$

After the nadir at d 2 post-foaling, [TCa] increased (FIGURE 1a):

$$[TCa] = 9.3 + .99\ (T) - .079\ (T)^2$$

$$r = .9934,\ P = .0066$$

A reciprocal relationship was found between [PTH] and [TCa] from d -7 to +5 in periparturient mares and from d 2 to 5 in post-partum mares (FIGURE 2). The linear relationships between [PTH] and [TCa] were significant (FIGURE 2a, b), but the quadratic curves achieved better fits (FIGURE 2c, d).

In general, [TCa] was lower (P = .01) and [PTH] was higher (P = .03) in mares on Farm A than on Farm B (FIGURE 3). Serum [TCa] began decreasing in mares on Farm A at 7 d pre-foaling and continued decreasing until 2 d post-foaling (FIGURE 3a):

$$[TCa] = 11.17 - .35\ (T) - .02\ (T)^2$$

$$r = .8193,\ P = .0037$$

The change in [TCa] in mares on Farm B was curvilinear (FIGURE 3a):

$$[TCa] = 12 - .22\ (T) - .03\ (T)^2$$

$$r = .8232,\ P = .0034$$

After d 2 post-foaling, [TCa] increased in mares on Farm A, while [TCa] in mares on Farm B remained constant (FIGURE 3a).

Serum PTH concentrations began increasing in mares on Farm A from 7 d pre-foaling until 2 d post-foaling (FIGURE 3b):

$$[PTH] = 179 + 28\ (T) + 1.79\ (T)^2$$

$$r = .8163,\ P = .0039$$

The increase in [PTH] in mares on Farm B was curvilinear (FIGURE 3b):

$$[PTH] = 69 + 13\ (T) + 1.62\ (T)^2$$

$$r = .607,\ P = .0627$$

After d 2 post foaling, [PTH] decreased linearly in mares on Farm B (FIGURE 3b).

Serum [TCa], [ICa], and [PTH]

The nadir in [ICa] was on d 2 post-partum, but was less pronounced than [TCa] (FIGURE 4). Mean serum [ICa] was correlated with mean serum [TCa] (FIGURE 5), and in three out of four individual mares (FIGURE 6). A reciprocal relationship was found between mean serum [PTH] and [ICa] concentrations (FIGURE 7). Serum PTH and ionized Ca concentrations showed an inverse relationship (FIGURES 7), but in only one of four individual mares (FIGURE 8).

Milk [Ca] pre-partum.

Quadratic curves were fit to least squares means for both commercial milk tests (FIGURE 9). A critical level of milk Ca concentration of 200 ppm has been proposed previously to predict foaling within 24 to 48 h (Ley et al., 1993, 1994a, b); it is shown by a dashed line in FIGURE 9a. It was reached by the [Ca + Mg] test at -4.5 d pre-foaling and by the [Ca] test at -2 d (FIGURE 9a):

$$[Ca + Mg] = 367 + 54\ (T) + 4\ .18\ (T)^2$$

$$r = .9369,\ P = .0005$$

$$[Ca] = 337 + 82\ (T) + 7.28\ (T)^2$$

$$r = .9657,\ P = .000098$$

If the critical level of milk Ca concentration was raised to 250 ppm, as shown by a dashed line (FIGURE 9b), it was reached by the [Ca + Mg] test at -2.5 d pre-foaling and by the [Ca] test at -1 d (FIGURE 9b).

Discussion

The present findings in the mare reveal certain similarities and differences compared to previous results in the dairy cow. Both serum [TCa] and [ICa] reached a nadir and serum [PTH] a peak during the periparturient period, but changes are less pronounced and occurred 1 or 2 d later in the mare (FIGURES 1, 2, and 4) than in the cow (Mayer et al., 1969; Shappell et al., 1986). The degree of hypocalcemia in the cow has been attributed mainly to the increasing rate and magnitude of the mammary drain of Ca at the initiation of lactation (Kronfeld et al., 1976; Ramberg et al., 1984). This mammary flow of Ca is likely to be much less in a mare that has one foal than in a dairy cow producing a quanity of milk that would be adequate for several calves (Akers, 1990). Moreover, foals usually start to suckle strongly about 12 to 24 h after birth (Forsyth, 1975), and this delay would contribute to the nadir in serum [TCa] and [ICa] developing later in the mare than in the dairy cow.

The degree of change in serum [TCa] and [PTH] was attenuated by a higher Ca diet in the mare (FIGURE 3), as shown previously in the dairy cow (Shappell et al., 1986). Dietary Ca pre-partum has assumed great importance in the dairy cow, because of its influence on the degree of parturient hypocalcemia and the incidence of parturient paresis (Ramberg et al., 1976). Although the mare is not prone to parturient paresis, the present results suggest that an optimal range of Ca intake, perhaps at least the minimum recommendation of .45% (NRC, 1989) and including the .79% used on Farm B, would be prudent for pre-partum mares.

Serum [ICa] and [TCa] were positively correlated with one another (FIGURE 5) and negatively with serum [PTH] (FIGURE 7). Again, these results resemble previous findings in the dairy cow (Mayer et al., 1969; Blum et al., 1972; Ballantine and Herbein, 1991). Serum [ICa] was consistently between 40 and 50% of [TCa] in these periparturient mares, as it has been in periparturient dairy cows (Blum et al., 1972; Ballantine and Herbein, 1991).

The mean slope for the regression of serum [PTH] on [TCa], expressed as a fraction of the intercept, was .072 for eight periparturient mares (FIGURE 2a) and .082 for 20 periparturient dairy cows (Mayer et al., 1969). This slope has been regarded as an index of the parathyroid response to hypocalcemia and, as such, would suggest that the responsiveness of the parathyroid gland to hypocalcemia is similar in the mare and the cow.

The serum [PTH] and [TCa] responses reached extreme values on d 2 post-partum and failed to confer any advantage on milk [Ca + Mg] and [Ca] as predictors of foaling. Our results showed that the [Ca] test reached a critical level closer to foaling than does the [Ca + Mg] test, which concurred with previous research done by Ley (1989a, 1993). Raising the critical level from 200 to 250 ppm predicted the foaling more closely at -2.5 d and -1 d for the [Ca + Mg] and [Ca] tests (FIGURE 9).

These comparisons showed that the [Ca] test was more accurate for determining milk Ca concentrations in comparison to the [Ca + Mg] test (Ley et al., 1989, 1993). The preferred critical milk Ca level was 250 ppm, because it predicted foaling within 1 d using the [Ca] test in comparison with 2.5 d. This difference will be important when determining the appropriate time to induce mares to foal or to engage in a foal watch.

Temporal relationships suggest that changes in serum [TCa], [ICa], or [PTH] had little or no influence on the rise in milk [Ca] pre-partum in mares. These negative findings shift attention to other hormones involved in parturition and lactogenesis that also affect Ca metabolism, such as cortisone and estrogen. Estrogen tends to reduce Ca removal from bone (Muir et al., 1972; Hollis et al., 1981), and its decline pre-partum would tend to maintain serum Ca in opposition to the increasing mammary drain (Horvath and Kutas, 1959; Pashen, 1984). Cortisone tends to facilitate the transfer of Ca from blood into adipose tissue during stress (Moseley and Axford, 1973). The possibility that cortisol may promote Ca transfer from blood into mammary secretions pre-partum warrants further investigation. A review of the literature reveals that little is known about lactogenic hormones, except prolactin, in the mare (Akers, 1985; Ginther, 1992; Tucker, 1994).

Implications

The practical importance of dietary Ca for mares during the last month of pregnancy has been reinforced by our findings. Dietary Ca was less on Farm A (.34% DM) and more on Farm B (.79% DM) than the .45% minimum recommended by the NRC (1989). Less extreme perturbations of serum [TCa], [ICa], and [PTH] were observed on Farm B than on Farm A, and these results, by analogy with similar previous studies in dairy cows, suggest that .79% is closer than .34% to an optimal level of dietary Ca for pre-partum mares.

Foaling time was predicted more closely by the milk test for [Ca] than by the test for [Ca + Mg], and by a critical level of 250 ppm than by 200 ppm. Further studies on the sensitivity and specificity of these tests are needed. The rise in milk [Ca] pre-partum does not appear to be influenced by serum [PTH] and attention should turn to identifying other causal and conditioning factors, which may also be useful in predicting foaling.

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Table 1.

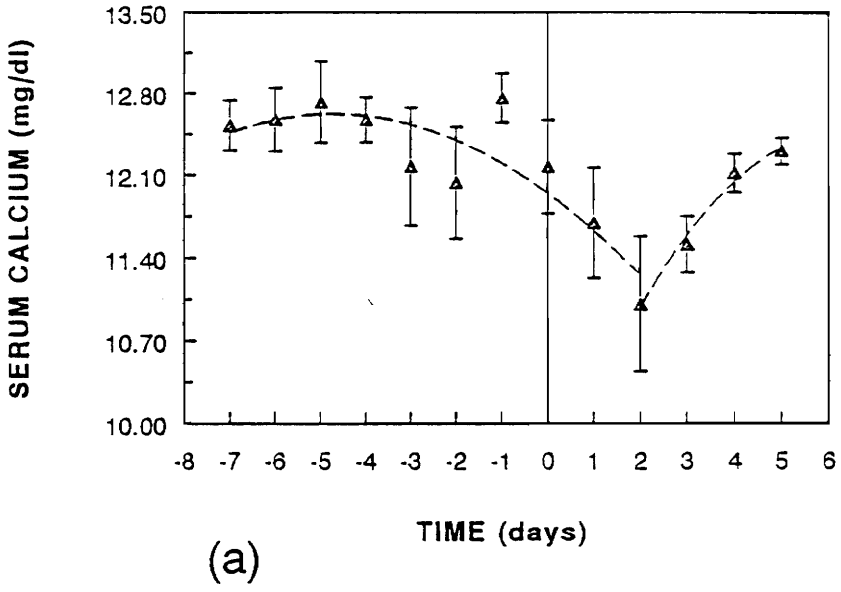
Dietary calcium and phosphorus concentrations in the forages and the concentrate from both farms compared with the NRC (1989).

	FARM A		FARM B		
% Dry Matter	Forage	Concentrate	Forage	Concentrate	NRC
Calcium %	.4	.34	.28	1.06	.45
Phosphorus %	.28	.34	.28	.63	.34

Table 2.

Two estimated daily intakes of 11 mo pregnant mares, assuming daily intakes of DE proposed by NRC (1989) or Donoghue (1990).

	NRC, 1989		Donoghue et al., 1990	
	Farm A	Farm B	Farm A	Farm B
Total DE (Mcal/d)	19.7	19.7	24.6	24.6
Concentrate (Mcal/d)	18.1	10.7	18.1	10.7
Forage (Mcal/d)	1.6	9.0	6.5	13.9
Calcium (gm/d)	19.8	45.2	19.4	53.3
Calcium (% DM)	.34	.79	.36	.74



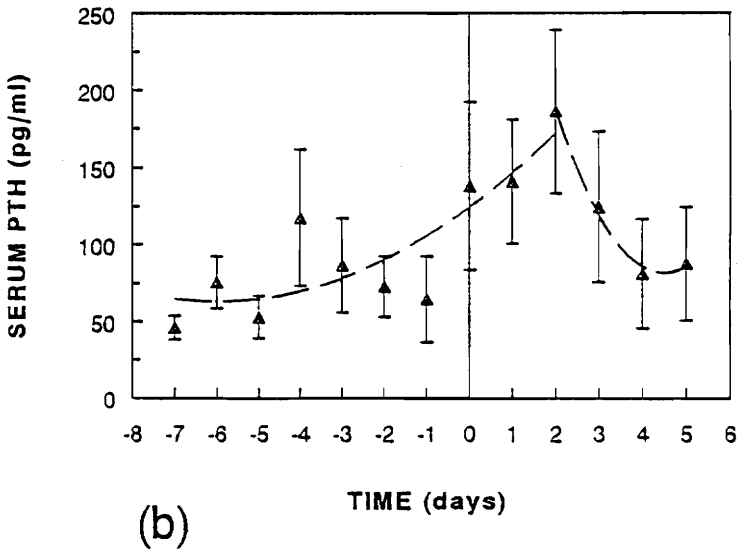


Figure 1. Serum [TCa] (a) and [PTH] (b) concentrations in eight periparturient mares. Values are mean ± SEM. Quadratic curves were fit to the data from 7 d pre-partum to 2 d post-partum and from 2 to 5 d post-partum.

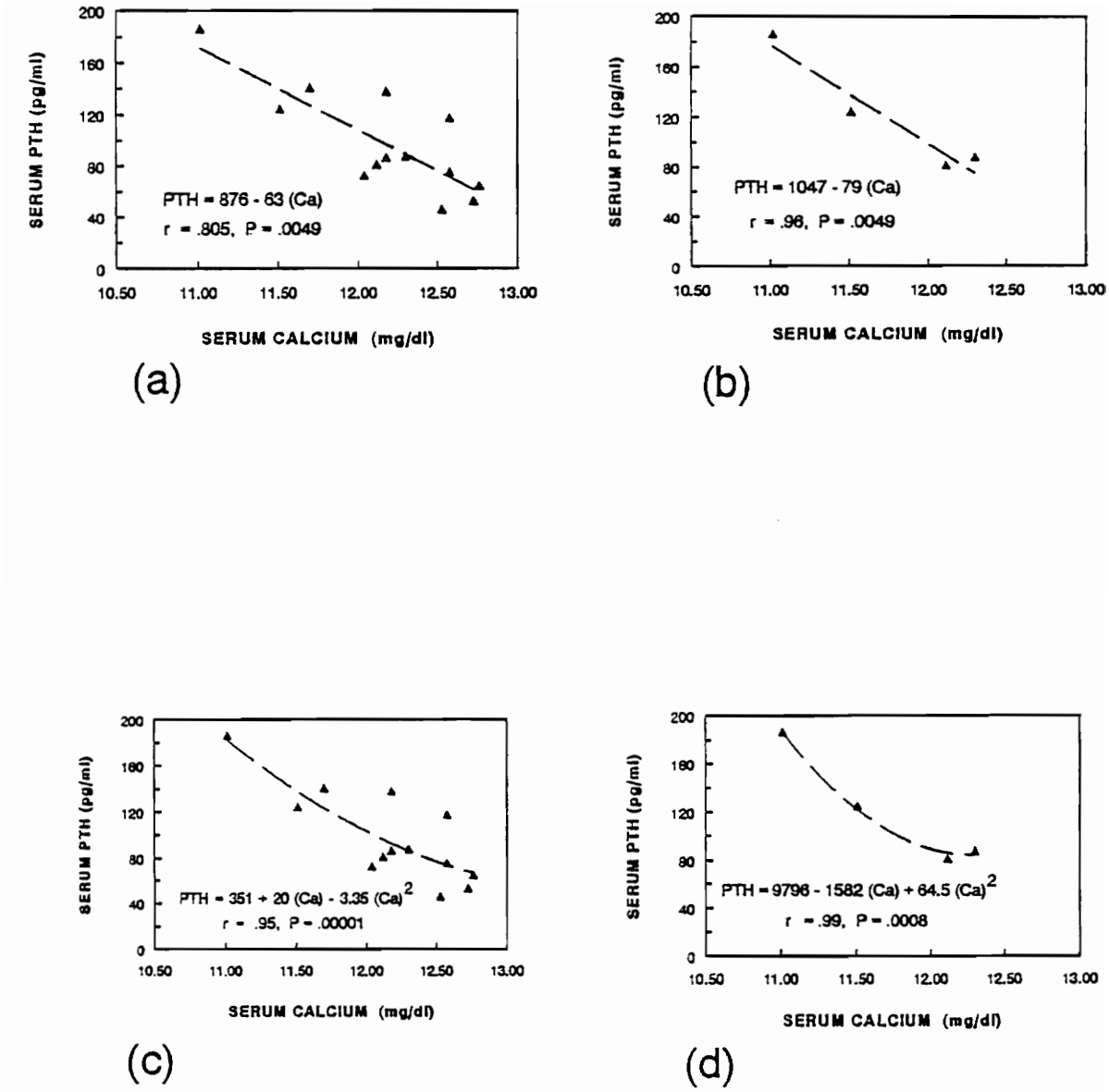


Figure 2. Linear and quadratic relationships of mean serum [PTH] and [TCa] from d 7 pre-partum to d 5 post-partum (a and c) and from d 2 to d 5 post-partum (b and d).

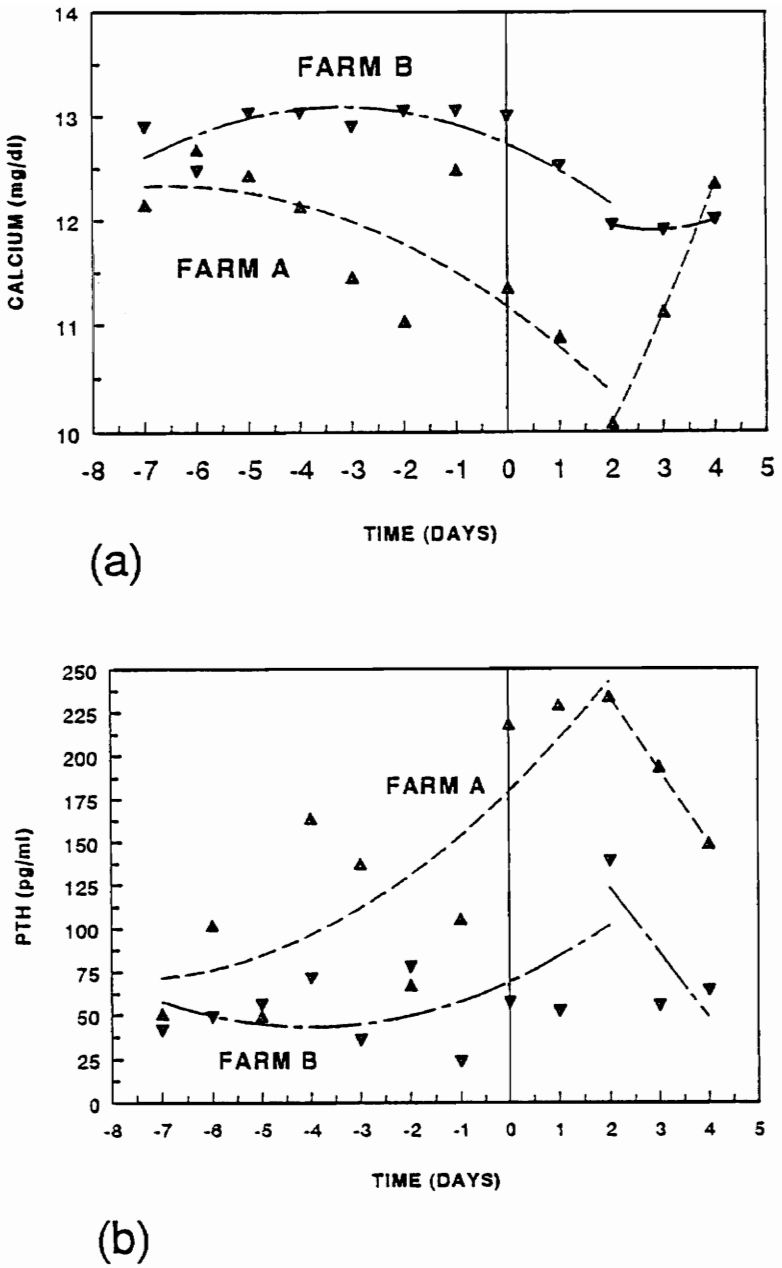


Figure 3. Mean serum [TCa] (a) and [PTH] (b) in eight periparturient mares from Farm A and Farm B. Quadratic curves were fit to the data from 7 d pre-partum to 2 d post-partum and from 2 to 5 d post-partum.

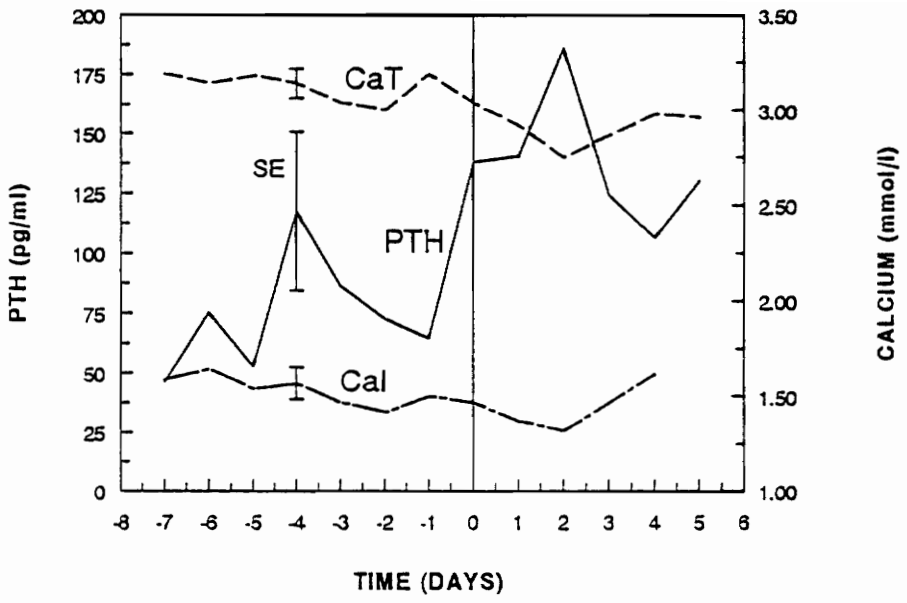


Figure 4. Serum [PTH], [TCa], and [ICa] in periparturient mares. Values are lsmeans from d 8 pre-partum to d 6 post-partum. The shown SE bars are representative.

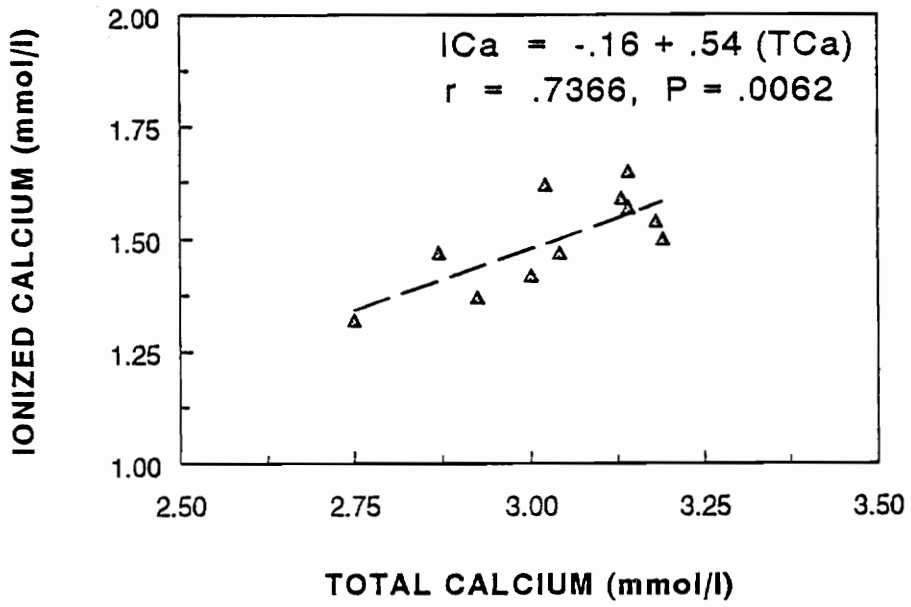


Figure 5. Mean serum [ICa] was linearly related to mean serum [TCa] pre-partum to d 5 post-partum in mares.

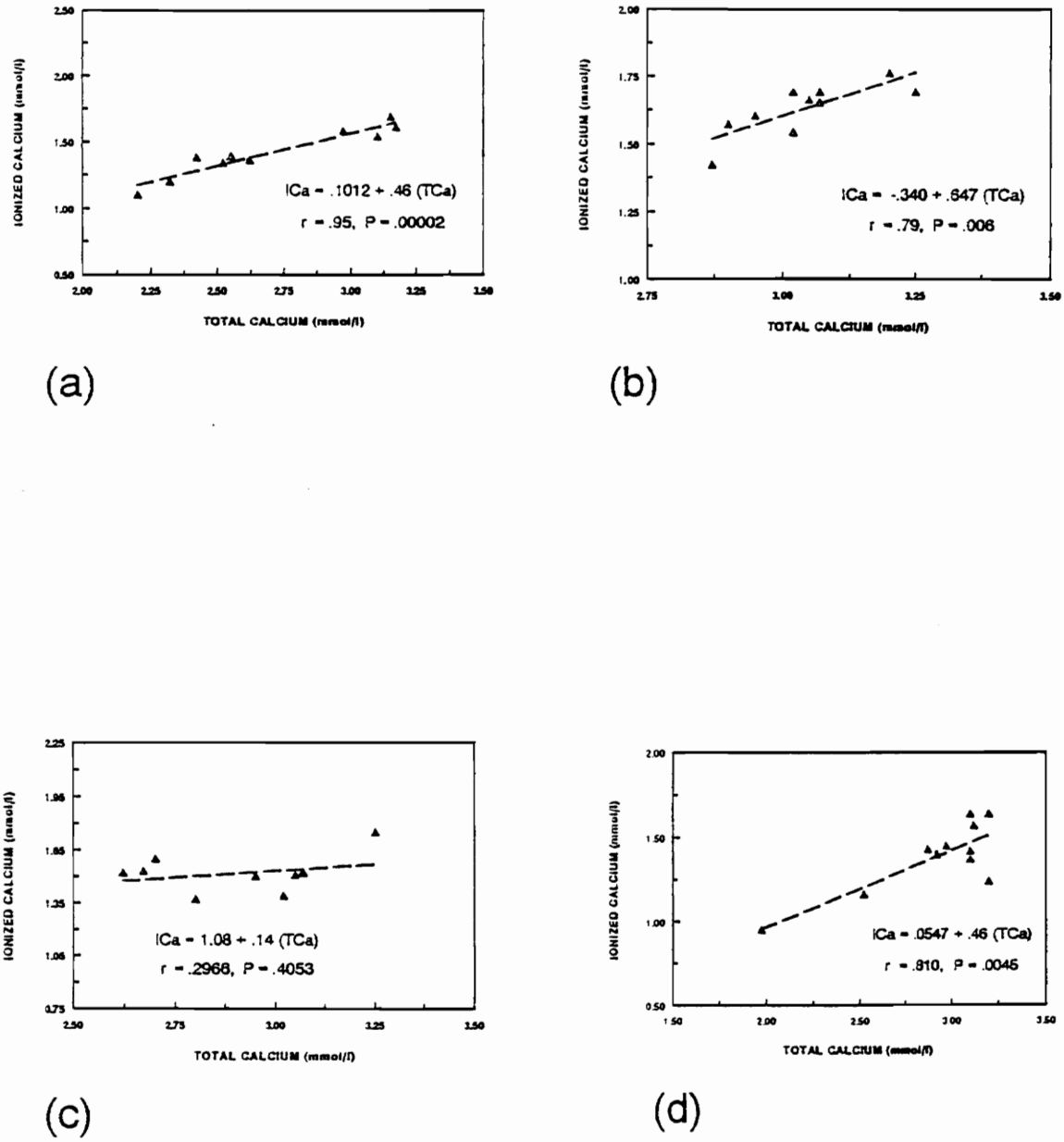


Figure 6. Linear relationships between serum [ICa] and serum [TCa] from d 6 pre-partum to d 5 post-partum were found in three out of four individual mares.

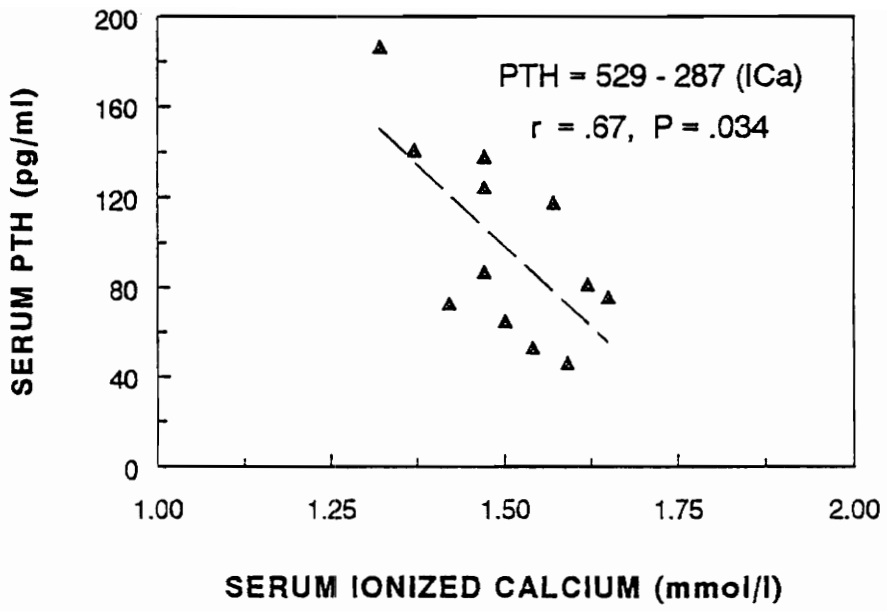


Figure 7. Mean serum [PTH] was related linearly to mean serum [ICa] from d 6 pre-partum to d 5 post-partum.

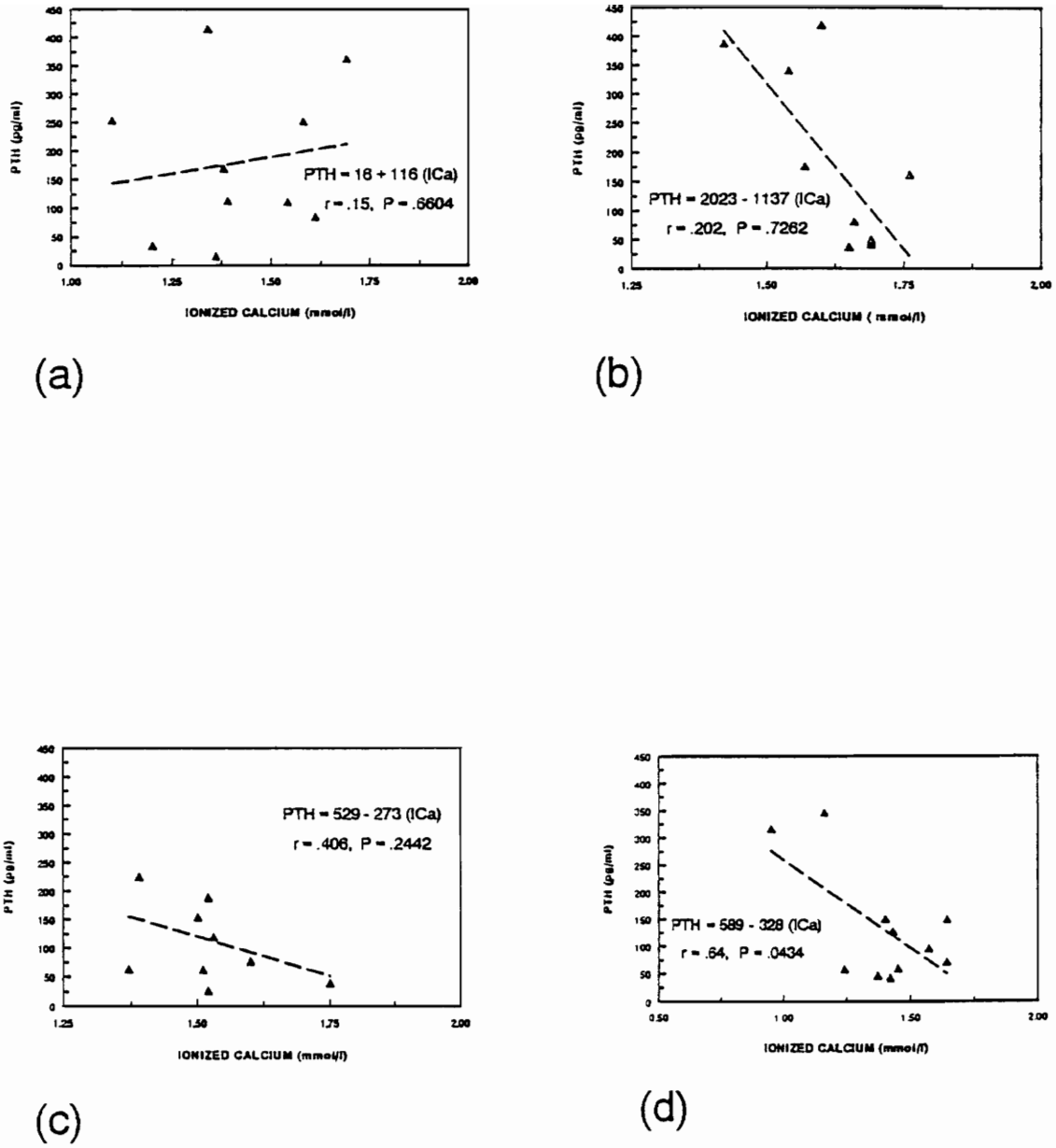


Figure 8. In only one of four individual mares, serum [PTH] was correlated with [ICa] from d 6 pre-partum to d 3 or 4 post-partum.

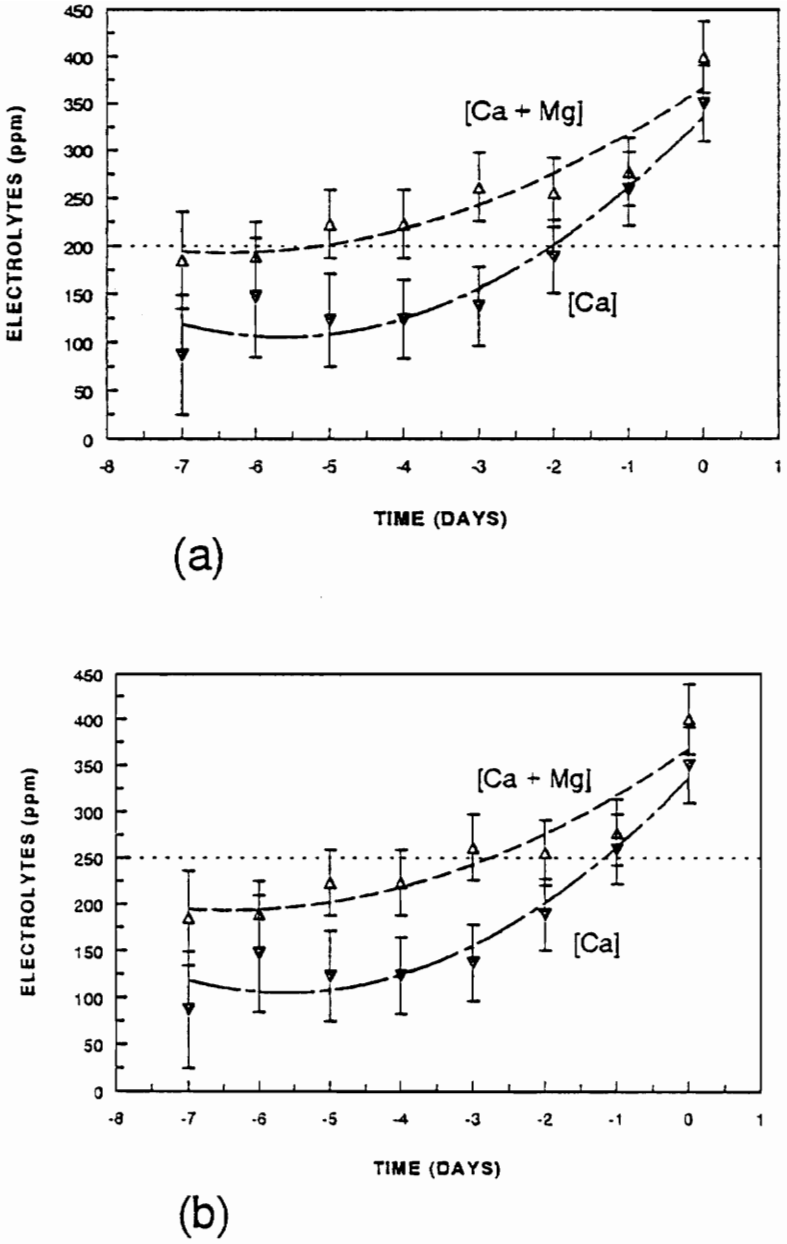
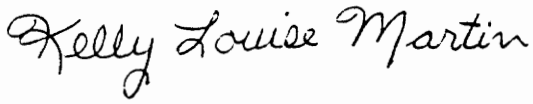


Figure 9. The [Ca + Mg] and [Ca] in mammary secretions increase with time approaching parturition. Values are lsmeans ± SE. The dotted line represents the critical level of milk [Ca] required to induce parturition or to predict foaling.

Vita

Kelly Louise Martin, daughter of James and Donna Martin of Pottersville New Jersey, was born on April 22, 1970. She attended high school at the Purnell School and graduated in 1988. She received her Bachelor of Science degree in Large Animal Science from Delaware Valley College in 1992, where she was President of her class for sophomore, junior, and senior years and a member of Who's Who Among Students in American Universities and Colleges in 1992.



Kelly Louise Martin