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Original article

Plasma osteocalcin concentrations in cows around parturition. The influence of a regular *versus* a very short dry period

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Summary — Plasma Ca, P, Mg and OC concentrations were compared between 2 groups of Holstein cows around 2nd calving. Thirteen cows were milked until their daily milk production was lower than 2 kg, which occurred 4 d before parturition. The control group (8 cows) was normally dried 8 weeks before the expected time of calving. Apart from the week following the initiation of the dry period where plasma OC concentrations were significantly lower in dried cows than in the control group (which would indicate lower bone remodeling in dried cows), no significant difference concerning Ca, P, Mg and OC concentrations were observed between the 2 groups of cows. This indicates that the only lack of 1 8-week-long dry period probably has no major immediate effect upon Ca and bone metabolism in young dairy cows given a convenient Ca and P daily intake. Nevertheless, the lack of the dried period might protect the cow against parturient hypocalcemia: the decrease in plasma Ca and P concentrations observed 12 h after calving was less intense in non-dried cows than in controls.

cow / lactation / dry period / hypocalcemia / osteocalcin

Résumé — Influence du non-tarissement sur le métabolisme calcique de la vache laitière aux alentours de la parturition. *Les concentrations plasmatiques en calcium (Ca), phosphore minéral (P), magnésium (Mg) et ostéocalcine (OC; un marqueur de l'activité ostéoblastique) ont été mesurées chez des vaches Holstein aux alentours du deuxième vêlage. Huit animaux ont été taris normalement 8 semaines avant la date prévue de vêlage. Les 13 autres ont continué à être traits jusqu'à ce que leur production devienne inférieure à 2 kg de lait par jour, c'est-à-dire jusqu'à 4 jours en moyenne avant vêlage. Chez les 21 vaches les concentrations plasmatiques en Ca et P les plus faibles ont été observées 12 h après mise-bas. À l'exception de la semaine suivant le tarissement des 8 vaches chez lesquelles l'ostéocalcinémie était plus faible que chez les treize non taries (ce qui traduit vraisemblablement un brusque ralentissement du remodelage osseux chez les premières), nous n'avons observé aucune différence significative entre les paramètres plasmatiques mesurés dans les deux lots. Ainsi, la seule absence d'une période de tarissement de 8 semaines ne semble pas avoir d'effets immédiats très importants sur le métabolisme calcique et osseux de jeunes vaches laitières recevant une alimentation phosphocalcique équilibrée. Néanmoins, la baisse de la calcémie*

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et de la phosphatémie observée après vêlage étant moins intense chez les vaches non taries que chez les témoins, la suppression du tarissement pourrait avoir un effet prophylactique à l'encontre de l'hypocalcémie vitulaire.

vache / lactation / tarissement / hypocalcémie / ostéocalcine

INTRODUCTION

Bovine parturient paresis (milk fever) is a metabolic disorder occurring at the onset of lactation, when demands for calcium (Ca) and inorganic phosphorus (P) are substantially increased (Braithwaite, 1976). These elements are secreted into milk at a faster rate than they are removed from bone (Yarrington *et al,* 1976) and absorbed from intestine (Hove and Hilde, 1984). Although a decrease in plasma Ca and P concentrations is a normal event in all parturient cows, the hypocalcemia and hypophosphatemia is far more important in paretic animals (Moodie *et al,* 1955; Larvor *et al,* 1961; Barlet, 1969). In pregnant dry cows, Ca metabolism is in balance: that is input from intestine and bone equals output into bone, feces, urine and fetus. At delivery, the exchange into the fetus stops, but the output to colostrum is greater than towards the fetus in the dry period and may induce hypocalcemia (Ramberg *et al,* 1970; Westerhuis, 1974). The parturient cow may, however, become resistant to hypocalcemia by enlarging the immediately available Ca pool, *ie* by decreasing Ca output from the blood, and/or by increasing Ca input into the blood. Decreasing Ca output from the blood may be effected by incomplete milking for some days following calving. In fact the efficiency of milking during the days after parturition does not seem to have any significant influence on the incidence of milk fever in Ayrshire, Guernsey and Jersey cows (Owen, 1954). Decreasing Ca input into the immediately available Ca pool just before calving would make Ca metabolism more adaptable. This can be done by removing Ca by milking pre-partum. In fact, results obtained by Smith and Blosser (1947) in a group of 46 cows and by Kendall *et al* (1968) using 22 animals do not support or reject this theory.

Working on the influence of the dry period length on subsequent milk production, Coppock *et al* (1974) also studied the incidence of parturient paresis in cows used in their experimental design. Sixty-five Holstein herds, with a minimum herd size of 50 cows, were assigned to dry periods of 20, 30, 40, 50 and 60 d. These durations had no significant influence on parturient paresis which occurred in 13, 12.5, 11.8, 12.4 and 13.3% of the animals of each group, respectively. Nobody has, however, observed the influence of the suppression of the dry period on plasma Ca, P and osteocalcin (OC: a marker for bone remodeling (Delmas *et al,* 1986)) concentrations in dairy cows at parturition. This was the aim of the present study.

MATERIALS AND METHODS

Animals

Twenty-one Holstein cows were divided into 2 groups at the end of their first lactation. The predicted times for calving, milk production and milk composition during the first 4 months of the first lactation, the age of the animals at the first calving and their body weight were the criteria used for grouping of cows. Eight cows were normally dried 8 wk before the expected time of calving (in our herd the length of gestation is 281 ± 9 d (mean ± SEM). The 13 other cows were milked until their daily milk production was lower than 2 kg/d. Thus the length of the dry period was 58 ± 15 days and 4 ± 6 d for the dried (D) and non dried (ND) cows, respectively. During the experimental period each cow was fed grass silage, hay and grain concentrate. Forty-eight h after calving, the body weight of the D and ND cows was 662 ± 56 kg and 661 ± 57 kg, respectively. During the first 2 wk after calving the daily dry matter (DM) intake increased in parallel in both groups and was not different in ND (17.6 kg) and D (16.1 kg) cows. Thus, the mean daily intake of each cow was 120 g, 80 g and 25 g for Ca, P and Mg, respectively. This corresponds to INRA dietary recommendations (7 g Ca and 4 g P per kg dry matter intake for a daily milk production of 20 kg) (Guéguen *et al*, 1988).

After calving each cow was milked twice daily, at 6 am and 5 pm. Daily milk production increased in parallel in both groups. It was lower in ND than in D cows and maximal (27 kg and 33 kg for D and ND cows, respectively) during the 7th week of lactation.

Sampling and assays

Heparinized blood samples were collected from each cow by puncture of the caudal vein before and during the dry period, 12 h after calving, and 10 and 70 d later. After centrifugation, plasma was frozen until analysis. Plasma Ca and Mg concentrations were measured by atomic absorption spectrophotometry. Plasma P was determined by colorimetry (Biotrol kit, Paris, France).

Plasma osteocalcin (OC) concentration was measured by homologous radioimmunoassay using the osteocalcin RIA kit from Incstar Corporation (Stillwater, MN, USA). In our experimental conditions, the sensitivity of the method was 0.15 ng/ml. The intra- and interassay precision was 6 and 9% respectively.

Results are expressed as means ± SEM. Student's *t*-test was used to compare values between the 2 groups of animals. One-way analysis of variance was used for comparisons within 1 group of cows.

RESULTS

In both groups of cows parturition was associated with a significant decrease in plasma Ca and P concentrations (fig 1). In D cows calcemia and phosphatemia decreased from 10.6 ± 0.1 mg/dl and 8.3 ± 0.4 mg/dl at first sampling to 10.1 ± 0.1 mg/dl ($P < 0.05$) and to 6.7± 0.2 mg/dl ($P < 0.05$) at parturition, respectively. At the same time, in ND cows, plasma Ca and P concentrations decreased from 10.5 ± 0.1 mg/dl and 6.7 ± 0.3 mg/dl to 8.5 ± 0.3 mg/dl ($P < 0.01$) and to 5.2 ± 0.4 mg/dl ($P < 0.05$), respectively. Twelve h after calving, plasma Ca and P concentrations were lower in D than in ND cows. On the other hand, plasma Mg concentrations (which were never significantly different in both groups) were then higher ($P < 0.05$) in D (2.6 ± 0.1 mg/dl) than in ND cows (2.1 ± 0.1 mg/dl).

In both groups of cows, plasma OC concentrations gradually decreased from the first sampling (ND: 28.8 ± 3.2 ng/ml; D: 26.3 ± 3.1 ng/ml) to calving (ND: 13.1 ± 2.5 ng/ml ($P < 0.01$); D: 12.2 ± 1.6 ng/ml ($P < 0.01$). A negative linear regression was observed between plasma OC concentrations *(y)* and days before calving *(x)* in both D ($y = 0.09\ x + 17.1$; $r = -0.402$; $P < 0.05$) and ND cows ($y = 0.14\ x + 17.2$; $r = -0.506$; $P < 0.01$). Values measured 70 d after calving (ND: 25.5 ± 2.6 ng/ml; D: 30.1 ± 3.5 ng/ml) were not different from those measured in the first sampling. Plasma OC concentrations were never different in both groups of cows except those measured 50 days before calving, which were lower in D (17.6 ± 1.2 ng/ml) than in ND cows (24.7 ± 1.8 ng/ml; $P < 0.05$).

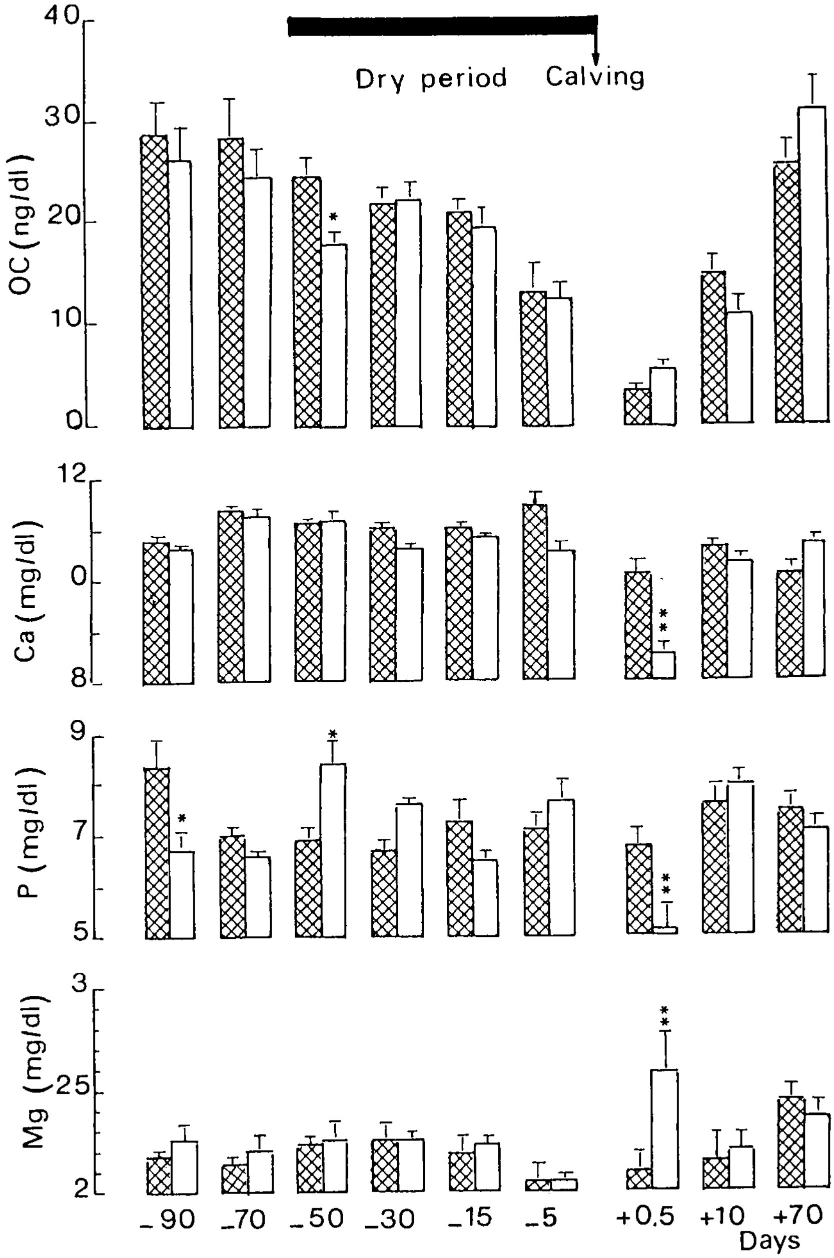

Fig 1. Plasma osteocalcin (OC), calcium (Ca), inorganic phosphorus (P) and magnesium (Mg) concentrations measured in 8 dried (white bars) and 13 continuously milked (hatched bars) Holstein cows at the second calving (means ± SEM; * $P < 0.05$, ** $P < 0.01$; comparison between the 2 groups of cows).

DISCUSSION

In the bovine (Van Mosel and Corlett, 1990) as in the ovine (Farrugia *et al*, 1989; Pastoureau *et al*, 1991) and human species (Delmas *et al*, 1986) plasma OC concentrations are strongly correlated to osteoblastic activity and bone growth. Our results confirm previous studies demonstrating a decrease in plasma OC concentration in cattle near parturition (Davicco *et al*, 1990; Naito *et al*, 1991) Thus, this would indicate a simultaneous reduction in osteoblastic activity (Van Mosel and Corlett, 1990). This might be due to a decrease in bone remodeling (and bone resorption) following the initiation of the dry period: plasma OC concentrations were lower in D than in ND cows immediately after milking stopped (fig 1). Such a difference was not observed between the 2 groups during the last 40 d of pregnancy. However, it must be borne in mind that during the last days of milking in ND cows, Ca intestinal absorption in these young (3-yr-old) cows was probably high enough to supply Ca for a very low milk production (less than 5 kg/d) and thus to prevent active bone resorption.

No clinical symptom of parturient paresis was observed in these young animals (Braithwaite, 1976). The lowest values for plasma Ca and P concentrations were measured in both groups 12 h after calving, associated with the highest values for magnesemia (fig 1). A significant and still unexplained negative relationship has already been observed between plasma Ca and Mg concentrations in postparturient paretic (Marr et al, 1955; Blum *et al*, 1972) and healthy cows (Moodie *et al*, 1955).

Twelve h after calving plasma Ca and P concentrations were significantly higher in ND than in D cows (fig 1). This indicates that continuous milking might protect the cow against parturient hypocalcemia. However, the way in which this protection might occur remains obscure. Parathyroid hormone-related peptide (PTHrP) is a newly characterized calciotropic factor which was originally isolated from tumors associated with the paraneoplastic syndrome of humoral hypercalcemia of malignancy (Broadus *et al*, 1988; Martin and Suva, 1989). PTHrP might be the putative calciotropic factor functioning during lactation: it has biological effects very similar to PTH (Horiuchi *et al*, 1987; Orloff *et al*, 1989); it is synthetized in lactating mammary tissue (Thiede and Rodan, 1988; Ratcliffe *et al*, 1992) and increases calcium and phosphorus secretion into milk (Barlet *et al*, 1992); in rats, serum levels of PTHrP are increased during lactation and this coincides with stimulation of bone resorption (Miller *et al*, 1991).

Thus, higher plasma PTHrP levels in ND than in D cows might be responsible for higher plasma Ca concentrations measured in these animals after calving (fig 1). This would not explain higher plasma P concentrations (fig 1) since PTHrP is a hyperphosphaturic and hypophosphatemic hormone (Horiuchi *et al*, 1987). However, PTHrP can increase plasma 1,25-dihydroxyvitamin D_3 concentrations (Horiuchi *et al*, 1987; Barlet *et al*, 1990). These would stimulate Ca and P intestinal absorption, and thus prevent hypocalcemia and hypophosphatemia (fig 1). It has recently been demonstrated that suckling or milking increases plasma PTHrP concentrations in lactating rats (Yamamoto *et al*, 1991) and goats (Ratcliffe *et al*, 1992), respectively. However, the effect of milking on plasma PTHrP concentrations in dairy cows remains unknown.

In conclusion, our results indicate that the only suppression of a 60-d dry period does not seem to have a long-lasting effect on bone and Ca metabolism (as evaluated by plasma Ca, P and OC concentrations) in young, adequately fed dairy cows. Nevertheless, higher plasma Ca and P concentrations measured in non-dried cows just after calving indicate that continuous milking might have a protective effect against parturient hypocalcemia.

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