

Plasma levels of vitamin D metabolites in the bovine species during the perinatal period

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Summary. Plasma vitamin D metabolites (25-OH D ; 24,25-(OH)₂D and 1,25-(OH)₂D) were measured simultaneously in the blood plasma samples of young cows and their calves. Four of the calves were chronically catheterized *in utero* at least 2 weeks before the expected time of parturition. No significant hypocalcaemia occurred in the dams at calving. Plasma vitamin D metabolites showed no significant variations for 2 weeks before and 4 days after calving. But rapid changes in plasma 1,25-(OH)₂D concentrations were observed in one calf during the first 48 postnatal hours. Before birth, the maternal and foetal concentrations of either 1,25-(OH)₂D or 24,25-(OH)₂D were positively correlated. 25-(OH) D (13 ng/ml), 24,25-(OH)₂D (1 ng/ml) and 1,25-(OH)₂D (76 pg/ml) were detected in one foetal plasma collected 35 days before term.

Introduction.

Vitamin D metabolism during gestation and lactation has been investigated in rats (Weisman *et al.*, 1976, 1978b ; Lester, Gray and Lorenc, 1978 ; Halloran, Barthell and De Luca, 1979 ; Pike *et al.*, 1979), sheep (Ross *et al.*, 1979) and humans (Kumar *et al.*, 1979 ; Seino *et al.*, 1980 ; Steichen *et al.*, 1980). However, to our knowledge, the interrelations between bovine maternal-foetal vitamin D metabolites are unexplored. The purpose of our study was to determine (i) whether vitamin D metabolites, 25-hydroxyvitamin D (25-OH D) ; 1,25-dihydroxyvitamin D (1,25-(OH)₂D) and 24,25-dihydroxyvitamin D (24,25-(OH)₂D), are detectable in calves during the last month of gestation, (ii) whether the foetal levels of these plasma metabolites are related to maternal concentrations, and (iii) if there are immediate pre-or postnatal changes in vitamin D metabolites in the term calf.

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Material and methods.

Animals. — Seven Jersey cows weighing 343 ± 9 kg (mean \pm SEM) at the first or second calving were used at the end of winter. Each cow was housed inside and received a daily ration of hay and grain concentrate containing 45 g of calcium and 55 g of inorganic phosphorus. The normal length of gestation in our Jersey herd is 289 ± 3 days. A catheter was chronically implanted in the artery of a cotyledon in 4 cows between days 250 and 275 of gestation, according to a previously described method (Dardillat, Lefaivre and Barlet, 1977). Blood was only collected 5 days after surgery to allow the animals time to recover. The 4 calves, chronically catheterized *in utero* and born alive on days 288 and 290 of gestation, weighed 24 ± 0.3 kg at birth. One was crushed by its mother just after delivery. The birthweight of the 3 control calves, born on days 289 and 290 of gestation, was 23 ± 0.7 kg. After birth, each calf was left with its dam and suckled colostrum *ad libitum* during the first neonatal week. Blood samples of dams and newborn calves were obtained by puncture of the external jugular vein.

The blood was centrifuged at 4 °C immediately after sampling, and the plasma was frozen at -20 °C until analysis.

Analysis. — Plasma calcium was determined by atomic absorption spectrophotometry (Perkin Elmer 400). Plasma inorganic phosphorus was measured by colorimetry (Technicon Autoanalyser).

Plasma vitamin D metabolites were measured with competitive protein assay after double-step purification of the 25-OH D, 24,25-(OH)₂D and 1,25-(OH)₂D fractions (i) on a Sephadex LH-20 column with 55 p. 100 chloroform-M hexane as solvent and then (ii) on a silicic acid column (Preece *et al.*, 1974) for 25-OH D or in a high pressure liquid system (Waters Associate, Milford, Massachusetts) for 24,25-(OH)₂D and 1,25-(OH)₂D, using a LC-18 column and a 50-100 p. 100 methanol-water elution gradient as solvent (Nguyen *et al.*, 1979). Rat serum protein was used for 25-OH D and 24,25-(OH)₂D binding assays (Preece *et al.*, 1974 ; Nguyen *et al.*, 1979) and chick intestinal cytosol for the 1,25-(OH)₂D binding assay (Shepard *et al.*, 1979).

Results.

Plasma vitamin D metabolite levels in one calf on day 255 of gestation (35 days before birth) were 2 ng/ml for 25-OH D, 0.7 ng/ml for 24,25-(OH)₂D and 76 pg/ml for 1,25-(OH)₂D (calcium : 13.2 mg/dl ; inorganic phosphorus : 7.2 mg/dl). At the same time in the dam they were 25-OH D : 13 ng/ml ; 24,25-(OH)₂D : 1.1 ng/ml ; 1,25-(OH)₂D : 76 pg/ml (calcium : 9.0 mg/dl ; inorganic phosphorus : 6.5 mg/dl).

Mean plasma calcium and inorganic phosphorus levels were significantly higher in foetuses and newborn calves than in dams, except on the first neonatal day. Neither hypocalcaemia nor hypophosphataemia was observed in the dams at parturition (table 1 ; fig. 1). Plasma 25-OH D levels were higher in dams than in foetuses or calves. There were no significant differences between dam and foetal (or dam and newborn) plasma concentrations of 24,25-(OH)₂D and 1,25-(OH)₂D (table 1 ; fig. 1). During

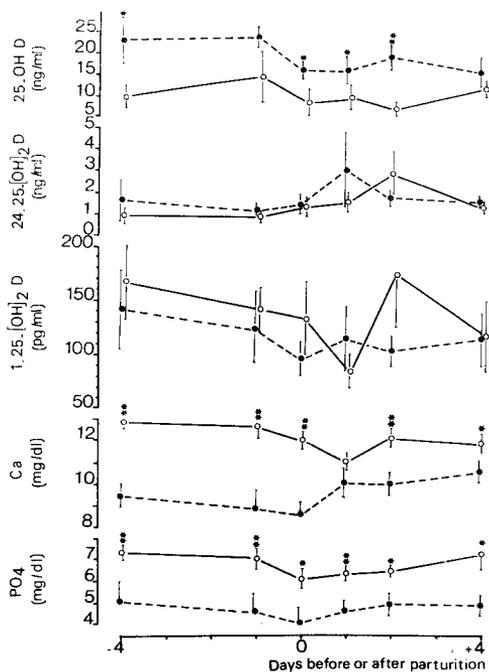
TABLE 1

Plasma calcium (Ca), inorganic phosphorus (PO_4), 25-hydroxyvitamin D (25-OH D), 24,25-dihydroxyvitamin D (24,25-(OH) $_2$ D) and 1,25-dihydroxyvitamin D (1,25-(OH) $_2$ D) levels in 7 cows and their calves for 2 weeks before calving, for 2 min after parturition and for 7 days after calving (mean \pm SEM)

	Before calving		At calving		After calving	
	Dams	Fœtuses	Dams	Calves	Dams	Calves
Ca (mg/dl)	9.7 \pm 0.3 (27)	12.8 \pm 0.2 ** (23)	8.9 \pm 0.6 (7)	11.9 \pm 0.5 ** (6)	9.9 \pm 0.2 (25)	11.7 \pm 0.2 ** (25)
PO_4 (mg/dl)	4.8 \pm 0.2 (23)	6.7 \pm 0.1 ** (23)	4.3 \pm 0.6 (7)	5.8 \pm 0.5 * (6)	5.3 \pm 0.3 (25)	6.4 \pm 0.3 ** (22)
25-OH D $_3$ (ng/ml)	19.5 \pm 1.8 (18)	11.4 \pm 3.4 ** (16)	16.7 \pm 1.8 (7)	7.4 \pm 2.4 ** (6)	16.6 \pm 1.7 (20)	8.8 \pm 0.8 ** (19)
24,25-(OH) $_2$ D $_3$ (ng/ml)	1.2 \pm 0.2 (17)	1.1 \pm 0.1 (17)	1.5 \pm 0.4 (7)	1.4 \pm 0.4 (6)	2.3 \pm 0.6 (20)	3.6 \pm 1.2 (19)
1,25-(OH) $_2$ D $_3$ (pg/ml)	124 \pm 14 (18)	126 \pm 18 (16)	97 \pm 16 (7)	134 \pm 35 (6)	113 \pm 12 (19)	118 \pm 12 (19)

Student's *t* tests was used to compare simultaneously calves and dams (* $P < 0.05$; ** $P < 0.01$). () number of assays.

FIG. 1. — Plasma levels of 25-hydroxyvitamin D (25-OH D), 24,25-dihydroxyvitamin D (24,25-(OH) $_2$ D), 1,25-dihydroxyvitamin D (1,25-(OH) $_2$ D), calcium (Ca), and inorganic phosphorus (PO_4) in Jersey cows (dotted lines) and their calves (solid lines) for 4 days before (4 cows and their 4 fœtuses) and 4 days after (7 cows and their 6 calves) parturition (mean \pm SEM). Student's *t* test was used to compare the plasma values measured simultaneously in cows and calves (* $P < 0.05$; ** $P < 0.01$).



4 days before and 4 days after calving, mean plasma 25-OH D and 24,25-(OH)₂D levels showed no significant variation in the calves (fig. 1). However, there was a decrease in plasma 1,25-(OH)₂D in the calves at birth. This decrease was significant ($P < 0.05$) when compared with the mean 1,25-(OH)₂D concentrations measured 1 day before (142 ± 21 pg/ml) and 1 day after (84 ± 15 pg/ml) birth (fig. 1). We observed plasma vitamin D metabolite changes in one calf during the first neonatal day; its plasma 25-OH D and 24,25-(OH)₂D levels did not vary during the first 24 postnatal hrs (fig. 2). However, the plasma 1,25-(OH)₂D level sharply decreased from 147 pg/ml at birth to 75 pg/ml 2 hrs later and then to 58 pg/ml at 6 postnatal hrs, followed by an increase to 150 pg/ml at 12 and 24 postnatal hrs (fig. 2).

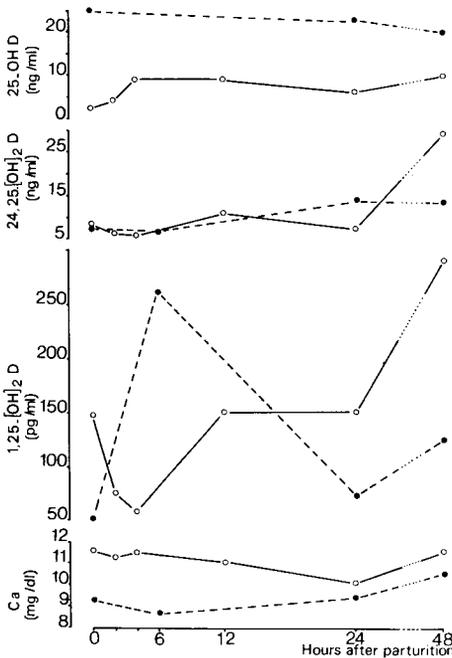


FIG. 2. — Plasma levels of 25-hydroxyvitamin D (25-OH D), 24,25-dihydroxyvitamin D (24,25-(OH)₂D), 1,25-dihydroxyvitamin D (1,25-(OH)₂D) and calcium (Ca) in a 3-year old Jersey cow (dotted line) and its calf (solid line) in the hours following parturition (second calving). This female calf (birth-weight 23.7 kg) was born on day 290 of gestation. Note the increase of maternal 1,25-(OH)₂D from 51 to 255 pg/ml between 0 and 6 hrs : maternal plasma calcium decreased simultaneously from 9.2 to 8.7 mg/dl.

No significant change in any of the studied vitamin D metabolites was observed in cow plasma concentration (fig. 1).

In foetuses and newborns there was no significant relation between plasma calcium and 25-OH D, 24,25-(OH)₂D or 1,25-(OH)₂D. In foetuses, a negative linear relationship was observed between plasma 25-OH D (y) and inorganic phosphorus (x), ($y = -15x + 112$; $r = -0.68$; $n = 16$; $P < 0.01$). In pregnant cows, plasma 1,25-(OH)₂D (y) and calcium (x) levels were negatively correlated ($y = -22x + 324$; $r = -0.58$; $n = 18$; $P < 0.01$), as were plasma 24,25-(OH)₂D (y) and calcium (x) ($y = -0.34x + 4.3$; $r = -0.80$; $n = 17$; $P < 0.01$), and plasma 24,25-(OH)₂D (y) and phosphorus (x) ($y = -0.23x + 2.3$; $r = -0.53$; $n = 16$; $P < 0.05$) levels. These relationships disappeared after parturition.

There was no relationship between maternal and foetal plasma levels of 25-OH D₃. However foetal (y) and maternal (x) 1,25-(OH)₂D plasma levels were positively correlated ($y = -0.76x + 38$; $r = 0.63$; $n = 17$; $P < 0.01$), as were 24,25-(OH)₂D plasma levels in foetal and newborn calves (y) and in cows (x) ($y = 0.9x + 0.4$; $r = 0.60$; $n = 47$; $P < 0.01$).

Discussion.

To our knowledge, this study is the first one to measure simultaneously the main vitamin D metabolites in dams and foetuses or newborns during the perinatal period.

In pregnant rats, vitamin D₃ and 25-OH D₃ are transferred across the placenta of the mother to the foetus (Haddad, Boisseau and Avioli, 1971). There appears to be little maternal-foetal transfer of 1,25-(OH)₂D (Noff and Edelstein, 1978). Moreover, 25-OH D₃ conversion into 1,25-(OH)₂D₃ occurs in the foeto-placental unit, and more precisely, in the human placenta (Weisman *et al.*, 1979), rat placenta (Weisman *et al.*, 1978b; Gray, Lester and Lorenc, 1979; Tanaka *et al.*, 1979), and the kidneys of the 19-day rat foetus (Weisman *et al.*, 1976), the 26-day rabbit foetus (Sunaga *et al.*, 1979) and the 61-day guinea-pig foetus (Fenton and Britton, 1980). In the present study of the bovine species, similar amounts (75 pg/ml) of 1,25-(OH)₂D were measured in the plasma of one foetus and its dam 35 days before parturition. During the last 2 weeks of gestation, the foetal blood 25-OH D level (11 400 pg/ml) was ten times greater than that of 24,25-(OH)₂D (1 100 pg/ml) and ninety times greater than that of 1,25-(OH)₂D (126 pg/ml). At the same time in pregnant cows, the 25-OH D/24,25-(OH)₂D ratio was about 16, while the 25-OH D/1,25-(OH)₂D ratio was 157 (table 1). Thus, plasma 24,25-(OH)₂D and 1,25-(OH)₂D levels were easily detectable for 2 weeks before term, and they did not differ from those measured simultaneously in the dams. Our results do not indicate whether foetal 1,25-(OH)₂D and 24,25-(OH)₂D were synthesized in the foeto-placental unit or in the mother's kidneys. In the sheep foetus, portions of kidney cortex can hydroxylate ³H-25-OH D₃ in the carbon 1 position 4 to 7 days before term. In addition, these kidneys produce more ³H-24,25-(OH)₂D₃ than ³H-1,25-(OH)₂D₃ (Ross *et al.*, 1979). There is a good correlation in sheep between maternal and foetal plasma 25-OH D₃ (Ross *et al.*, 1976; Barlet *et al.*, 1978a) which was not observed in the cows of the present experiment during the 4 days before birth. However, we have demonstrated a positive linear relationship between maternal and foetal 1,25-(OH)₂D and 24,25-(OH)₂D measured in the dams and their calves before and after birth. This would tend to indicate that the bovine placenta, like the ovine placenta (Ross *et al.*, 1979), is permeable to vitamin D₃ metabolites.

As already suggested in the ovine species (Barlet *et al.*, 1978b; Ross *et al.*, 1979), one physiological role for 1,25-(OH)₂D₃ during pregnancy might be to stimulate active calcium transfer from the cow placenta to the calf. The recent discovery of specific 1,25-(OH)₂D₃ receptors in placental tissue (Christakos and Norman, 1980; Pike *et al.*, 1980) supports this hypothesis. Thus, 1,25-(OH)₂D₃ would contribute to the high plasma calcium levels measured in foetal calves (table 1).

In women, a highly significant correlation has been observed between 25-OH D₃ concentrations in the mother's serum and those in the mixed arterial-venous cord

serum of her infant (Hillman and Haddad, 1974 ; Bouillon, Van Baelen and De Moor, 1977 ; Paunier *et al.*, 1978 ; Shimotsuji *et al.*, 1979). According to Hillman, Slatopolsky and Haddad (1978), there is no correlation between 24,25-(OH)₂D₃ levels in maternal and cord sera, and mean foetal 24,25-(OH)₂D₃ (2.25 ± 0.26 ng/ml) is similar to the mean maternal concentration. However, Weisman *et al.* (1978a) reported positive correlations between maternal and cord sera for both 25-OH D₃ and 24,25-(OH)₂D₃. In cows, there is no correlation between maternal and calf plasma 25-OH D concentrations after birth. Yet there is a positive correlation between plasma 1,25-(OH)₂D and 24,25-(OH)₂D concentrations. The plasma 1,25-(OH)₂D levels measured in the young parturient Jersey cow used in this experiment are similar to those reported by Horst *et al.* (1977) in young non-pareptic Holstein cows 2 days after calving (100 pg/ml). Plasma 1,25-(OH)₂D concentrations were significantly lower in calves on the first neonatal day than in foetuses on the day just before birth (fig. 1). Rapid changes in circulating 1,25-(OH)₂D after birth were also thoroughly studied in one calf during the first neonatal day (fig. 2). These rapid changes should be further investigated and taken into consideration when studying vitamin D metabolism in the early postnatal period.

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Résumé. Nous avons mesuré les taux plasmatiques des principaux métabolites de la vitamine D (25-OH D ; 24,25-(OH)₂D ; 1,25-(OH)₂D) chez 7 jeunes vaches Jersiaises et leurs veaux, dont 4 étaient porteuses d'un cathéter inséré de façon chronique sur une artère cotylédonnaire au moins 2 semaines avant la parturition. Aucune hypocalcémie significative n'est apparue chez les mères à la mise-bas. Durant les 4 jours précédant et suivant celle-ci, les taux plasmatiques moyens des métabolites ne variaient pas de façon significative ni chez les vaches, ni chez les veaux. Cependant des variations individuelles importantes et rapides des concentrations en 1,25-(OH)₂D ont pu être mises en évidence chez un veau pendant les 48 premières heures de vie postnatale.

Avant la naissance, les taux plasmatiques de 1,25-(OH)₂D, comme ceux de 24,25-(OH)₂D, étaient corrélés positivement chez les foetus et leurs mères. Ils étaient corrélés négativement à la calcémie chez les mères avant la parturition. Enfin, le 25-(OH) D (13 ng/ml), le 24,25-(OH)₂D (1 ng/ml) et le 1,25-(OH)₂D (76 pg/ml) ont pu être décelés dans le plasma d'un foetus prélevé 35 jours avant la naissance.

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