

Short Communication

Calcium metabolism in cows receiving an intramuscular injection of 1,25-dihydroxyvitamin D₃ combined with prostaglandin F_{2α} closely before parturition

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To determine the effect of exogenous 1,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃] combined with induced parturition on calcium (Ca) metabolism, cows received a single intramuscular injection of 1,25(OH)₂D₃ and prostaglandin F_{2α} (PGF_{2α}) closely before calving. Ten late-pregnant, multiparous Holstein cows were assigned to 1,25(OH)₂D₃ group (five treated with both 1,25(OH)₂D₃ and PGF_{2α}) and control group (five treated with PGF_{2α}). 1,25(OH)₂D₃ group showed an increase in plasma Ca concentration around parturition, whereas control group revealed a decrease in plasma Ca level. Plasma Ca concentration in 1,25(OH)₂D₃ group were significantly higher than that in control group during -0.5 to 3 days after parturition.

Key words: cow, calcium, 1,25-dihydroxyvitamin D₃, hypocalcemia, parturition

1,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃], a physiologically active form of vitamin D₃ metabolites, has been used to elevate plasma calcium (Ca) concentration during 1 to 4 days post-injection and thereby prevent parturient paresis (parturient hypocalcemia) in dairy cows [2,4,5]. This metabolite has the advantage of a shorter biological life than vitamin D₃; therefore, toxicity problems are reduced [9]. The shorter biological life also requires a more accurate prediction of the time of parturition for full effectiveness [4]. Because a decrease in the plasma Ca level is most likely to occur within 1 or 2 days postpartum [11], we suggest that exogenous 1,25(OH)₂D₃ should be administered to cows during 1 to 3 days prepartum. Parturition can be induced to dairy cows using prostaglandin F_{2α} (PGF_{2α}) within 2 or 3

days after injection [6]. In this short communication, cows received a single intramuscular injection of 1,25(OH)₂D₃ and PGF_{2α} closely before calving. The objective of this study was to determine the effects of exogenous 1,25(OH)₂D₃ combined with PGF_{2α} on Ca metabolism around parturition and to discuss the ability of this prophylactic regimen to prevent parturient paresis in cows.

The protocol and experimental design were approved by the Obihiro University of Agriculture and Veterinary Medicine, Laboratory Animal Care and Use Committee. Ten late-pregnant, multiparous Holstein cows (aged 3 to 4 years) were assigned to 1,25(OH)₂D₃ group (five treated with both 1,25(OH)₂D₃ and PGF_{2α}) and control group (five cows treated with PGF_{2α}). The cows stayed in an outside paddock during dry period until 275 days gestation, and were housed in an individual pen until 5 days postpartum. The cows were fed a ration of good quality hay, grass and corn silages, and commercial concentrate; providing daily 0.3% Ca and 0.2% phosphorus (P) of dry matter (DM) prepartum and 0.8% Ca and 0.4% P of DM postpartum.

Intramuscular injection of 1,25(OH)₂D₃ and/or PGF_{2α} was performed as close to 1 or 2 days before the predicted date of parturition. Date of parturition was predicted twice a day by rectal temperature, by observation of udder filling and oedema, and by swelling and relaxation of the vulva and pelvic ligaments [1]. The cows of 1,25(OH)₂D₃ group treated with a 1 µg/kg body weight 1,25(OH)₂D₃ dissolved in ethanol and 25 mg PGF_{2α} (Dinoprost; Pharmacia & Upjohn, Japan). The 1,25(OH)₂D₃ use in this study was the gift of Mercian Corporation, Japan. The cows of control group treated with 25 mg PGF_{2α} and ethanol.

Heparinized blood samples were obtained from the jugular vein from 277 days gestation to 5 days postpartum; the samples were immediately chilled in ice water and then centrifuged at 4°C. The obtained plasma was frozen at -30°C. The plasma 1,25(OH)₂D₃ concentrations were determined

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Table 1. Plasma 1,25(OH)₂D₃, Ca, inorganic phosphorus (iP) and Mg concentrations around parturition in 1,25(OH)₂D₃ and control groups (mean ± SD)

Items	Days after parturition									
	Prepartum	-1	-0.5	0	0.5	1	2	3	5	
1,25(OH) ₂ D ₃ (pg/ml)										
Control	36.8±9.8	53.6±22.1	52.9±14.3	73.3±35.3	75.4±23.8	96.6±25.9	95.8±31.7	95.4±23.0	57.4±16.2	
1,25(OH) ₂ D ₃	28.7±13.4	1185.0±384.1*	694.5±526.7**	596.6±338.4**	391.4±188.9*	51.9±59.1	65.2±17.5	45.9±11.6	20.2±12.5*	
Ca (mg/dl)										
Control	9.7±0.3	9.4±1.0	9.4±0.6	8.3±1.2	8.6±0.9	8.3±1.0	8.5±0.8	9.1±0.6	10.0±1.1	
1,25(OH) ₂ D ₃	9.8±0.6	10.7±0.8	11.0±0.9*	10.8±1.0*	11.5±1.0*	11.1±1.1**	11.1±0.9*	10.3±0.7*	9.6±0.5	
iP (mg/dl)										
Control	5.0±0.5	3.3±2.0	3.5±1.4	2.5±1.5	4.1±0.8	4.9±1.0	4.3±1.3	4.7±1.6	3.6±1.0	
1,25(OH) ₂ D ₃	4.5±0.9	5.8±1.2	6.3±1.5*	6.0±1.9*	7.9±2.1*	7.8±2.6	8.1±2.3	6.8±2.3	4.3±0.3	
Mg (mg/dl)										
Control	2.3±0.2	1.9±0.9	2.4±0.3	2.6±0.5	2.7±0.2	2.7±0.3	2.5±0.3	2.3±0.4	2.3±0.7	
1,25(OH) ₂ D ₃	2.4±0.2	2.4±0.6	2.3±0.4	2.3±0.3	2.3±0.2	2.3±0.3	2.5±0.2	2.3±0.3	2.4±0.5	

Prepartum: 277 days gestation. Significant difference at the timed value between the groups: * $p < 0.05$; ** $p < 0.01$.

using a 1,25(OH)₂D RIA kit (Immunodiagnostic Systems, UK). The levels of Ca, inorganic phosphorus (iP), and magnesium (Mg) were analysed using a TBA-30R automatic analyser (Toshiba Medical Systems, Japan).

The actual time (mean ± SD) of parturition after the intramuscular injection was 29.4 ± 8.9 hours in 1,25(OH)₂D₃ group and 27.6 ± 11.7 hours in control group. There were not any specific clinical signs seen in cows of 1,25(OH)₂D₃ group by using exogenous 1,25(OH)₂D₃ compared to those of control group. Only one cow of control group developed retained fetal membrane. Parturient paresis occurred in one cow of control group within 10 hours of parturition and in another of 1,25(OH)₂D₃ group at 4 days postpartum. These two cows received Ca treatment (an intravenous infusion of 500 ml of 25% Ca borogluconate solution) [10,12] by the referring veterinarian, and recovered immediately. The criteria to start this treatment were that the cow was in recumbency and was unable to stand up itself.

Wilcoxon rank-sum test was used to observe the difference of the values between two groups on the each day after parturition. The data after Ca treatment were excluded in two cows with parturient paresis. The procedures for statistical analyses were done using JMP 5.0.1J software (SAS, USA). The significance was set at $p < 0.05$.

Table 1 shows plasma 1,25(OH)₂D₃, Ca, iP and Mg concentrations around parturition in cows of 1,25(OH)₂D₃ and control groups. Plasma 1,25(OH)₂D₃ concentrations in 1,25(OH)₂D₃ group (391.4 ± 188.9 to 1185.0 ± 384.1 pg/ml) were significantly higher during -1 to 0.5 days after parturition than those in control group (52.9 ± 14.3 to 75.4 ± 23.8 pg/ml; $p < 0.05$ or $p < 0.01$). The levels of 1,25(OH)₂D₃ at 5 days postpartum in 1,25(OH)₂D₃ group (20.2 ± 12.5 pg/ml) were significantly lower compared with the level in control group (57.4 ± 16.2 pg/ml; $p < 0.05$). 1,25(OH)₂D₃ group showed a marked increase in plasma Ca and iP

concentrations around parturition, whereas control group seemed to reveal a mild decrease in plasma Ca and iP and a small rise in Mg level around calving. Plasma concentrations of Ca (10.3 ± 0.7 to 11.5 ± 1.0 mg/dl) and iP (6.0 ± 1.9 to 7.9 ± 2.1 mg/dl) in 1,25(OH)₂D₃ group were significantly higher than those in control during -0.5 to 3 days (8.3 ± 1.0 to 9.4 ± 0.6 mg/dl; $p < 0.05$ or $p < 0.01$) and during -0.5 to 0.5 days (2.5 ± 1.5 to 4.1 ± 0.8 mg/dl; $p < 0.05$) after parturition, respectively. There was no significant difference in plasma Mg concentration between two groups.

The increase in plasma Ca and iP concentrations following the 1,25(OH)₂D₃ injection described here was similar to that observed by some previous investigations [2,4,5]. It has been shown that exogenous supplied 1,25(OH)₂D₃ does not stimulate increased bone resorption and that hypercalcemia and hyperphosphatemia result from an increased rate of intestinal absorption [4,8,9].

Goff and Horst [3] indicated that the main problem to impede the widespread use of exogenous 1,25(OH)₂D₃ or its analogues for the prevention of parturient paresis was the difficulty in timing the treatment. In the present study, we suggested that the prophylactic regimen using the intramuscular injection of 1,25(OH)₂D₃ combined with the induced parturition was successful to prevent hypocalcemia closely near calving. However, some disadvantages in 1,25(OH)₂D₃ group, i.e., the significant low plasma 1,25(OH)₂D₃ concentration at 5 days postpartum and parturient paresis in one cow at 4 days postpartum, seemed to result from an inhibition of renal 1- α -hydroxylase and endogenous 1,25(OH)₂D₃ synthesis by exogenous 1,25(OH)₂D₃ administration [7,8]. Repeated administration of low dose of 1,25(OH)₂D₃ as described by Hoffsis *et al.* [5] will be necessary to make the present prophylactic regimen complete.

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