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EQUAL CALCITONIN RESPONSE IN PARETIC AND NON-PARETIC COWS AFTER INTRAVENOUS CALCIUM INFUSION*

By

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FORSLUND, K., K. LUNDSTRÖM and L.-E. EDQVIST: *Equal calcitonin response in paretic and non-paretic cows after intravenous calcium infusion.* Acta vet. scand. 1980, *21*, 171—184. — Seventeen cows divided into 3 groups were studied: Group 1 consisting of newly delivered cows (1—3 days post partum) suffering from parturient paresis; Group 2 comprising newly delivered (1—4 days post partum) healthy cows and Group 3 consisting of non-pregnant, non-lactating cattle. All animals were given an intravenous standard Ca infusion, and blood samples were obtained before treatment and at intervals during a 3-hour period thereafter. Plasma levels of calcitonin (CT), calcium (Ca) and inorg. phosphate (P) were determined. No significant difference for the mean CT response existed between animals in Groups 1 and 2. Blood levels of inorg. P increased significantly with time in animals of Groups 1 and 2 but not in animals of Group 3.

The finding here of a similar CT response after Ca stimulation in both paretic and non-paretic cows demonstrates that the C-cells in both groups of animals have the same capacity to secrete CT.

parturient paresis; cows; calcitonin; RIA; calcium; inorganic phosphate.

In the high-yielding dairy cow parturition is occasionally associated with parturient paresis. The etiology of this hypocalcemic condition is unknown. An endocrinological etiology has been suggested.

Ochs et al. (1964) described a hypocalcemic factor present in the blood of cows with milk fever. Furthermore, *Capen & Young* (1967) following histological and ultrastructural studies

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of the thyroid parafollicular cells reported evidence of a more active secretion of the calcium reducing hormone calcitonin (CT) in cows with parturient paresis as compared to cows without the disease.

When administered to dairy cows, CT has been shown to decrease the plasma Ca and to cause clinical signs similar to parturient paresis (*Barlet* 1968). *Mayer* (1970) on the other hand showed that CT gave a negligible response in the Ca level in old cows and in cows immediately after parturition.

Care et al. (1970) and *Garel & Barlet* (1975) measured blood plasma levels of CT in cows at intervals before and after parturition and reported elevated CT levels in cows with parturient paresis. In contrast to this, *Mayer et al.* (1975) after analysing the blood plasma concentration of CT and Ca during the time period 1 month before to 1 month after parturition in 23 cows suggested that the development of parturient paresis was not related to an increased CT secretion.

The role of calcitonin as an etiological factor in parturient hypocalcemia in cows is thus still unclear. In the present study we investigated the calcitonin secretory capacity in cows with and without parturient paresis as well as in non-pregnant non-lactating cattle. The calcitonin secretion was stimulated through the infusion of a fixed amount of Ca, and the resulting calcitonin discharge was measured by radioimmunoassay.

MATERIAL AND METHODS

Animal experiments

Seventeen cows divided into the following 3 groups were studied:

Group 1. Cows with parturient paresis. This group consisted of 7 cows (4 Swedish Red and White Breed, SRB; 1 Swedish Friesian Breed, SLB; 2 crosses, Swedish Jersey Breed × SRB and SLB × SRB) with an age varying between 5 and 10 years. All cows in this group showed typical signs of parturient paresis 1—3 days post partum. In conjunction with the clinical examination a blood sample was obtained through puncture of the jugular vein (Vacutainer, Heparin 10 i.u./ml, Becton Dickinson, New Jersey, USA). Thereafter, all cows were given an intravenous infusion of 9.3 g Ca^{++} in 500 ml of a Ca-borogluconate solution (boric acid 20 g, calcium gluconate 100 g, sterile water ad 500 ml, sodium bicarbonate ad pH 7.2). The infusion was given into the jugular vein, and blood samples were drawn at intervals (0—1, 5, 10, 20, 30, 45, 60, 90, 120 and 180 min) timed from the beginning of the infusion. The blood samples were immediately placed in ice-water and centrifuged as soon as possible. Plasma was removed and immediately frozen on dry ice and kept frozen until analysed. Centrifugation and freezing of the samples were completed within 15 min after collection.

Group 2. Post-parturient non-paretic cows. This group consisted of 5 cows of the SRB, and their age varied between 4 and 7 years. The animals belonged to the University's research farm. During the immediate post-partum period these animals were "treated" identically as the animals in Group 1. The time interval from parturition to Ca infusion for the cows in Group 2 varied between 1 and 4 days. These animals showed no signs of parturient paresis neither before nor after being treated. In this study animals in Group 2 served as a control group.

Group 3. Non-pregnant, non-lactating cattle. This group consisted of 5 crosses between SRB $\times$ SLB with an age varying between 1 and 4 years. The 3 youngest animals were of a smaller size, and the infused amount of Ca^{++} was reduced from 9.3 to about 8 g.

The treatment and blood sampling schedule and handling applied in Group 2 and Group 3 were identical to those previously described for the cows in Group 1.

CT levels were determined by radioimmunoassay (RIA), according to *Forslund & Stridsberg* (1980). The RIA system utilizes an antiserum to ovine calcitonin, and porcine CT was used as reference standard and for labelling. The practical detection limit of the assay system was 0.25 µg/l. All hormone data presented here represent the mean of triplicate determinations.

Plasma inorg. phosphate values were determined according to a method described by *Itaya & Ui* (1966). Plasma calcium was determined using an atomic absorption spectrophotometer.

Statistical methods

All statistical calculations were performed using the Statistical Analysis System (*Barr et al.* 1976). The following models were assumed to describe the data for Groups 1 and 2:

Model I (all observations per testing occasion were used in the calculations):

$$Y_{ijkl} = \mu + g_i + c_j + a_{ijk} + e_{ijkl}$$

where

Y_{ijkl} = the *ijkl*th observation

μ = general mean

g_i = effect of the *i*th group ($i = 1,2$)

c_j = effect of the *j*th age class ($j = 1,2$)

a_{ijk} = effect of the *k*th animal within the *i*th group and *j*th age class

e_{ijkl} = residual random term with variance σ^2_e

The effects of group and age class were regarded as fixed and the effect of animal as random. The restrictions $\Sigma_i g_i = \Sigma_j c_j = 0$ were imposed on the model. The interaction between group and age class was non-significant in all cases and was therefore ignored.

The 10 samples drawn after the Ca infusion were used to calculate the mean and maximum value and the area under the CT response curve for each cow. The obtained values were used in the following model (Model II):

$$Y_{ijk} = \mu + g_i + c_j + e_{ijk}$$

where the elements have the same meaning as before.

The effect of age could not be tested using all groups due to confounding between age and group. All paretic cows were between 5 and 10 years, while all non-pregnant non-lactating cows were between 1 and 4 years. The age effect was therefore only studied using paretic and non-paretic cows. The age was divided into 2 classes with Class 1 including cows between 4 and 6 years, and Class 2 including cows older than 6 years. A comparison between all three groups was made including only the effect of group in the statistical model and using 1 observation per testing occasion (Model III).

The effect of sampling time within the group was studied using the following model (Model IV):

$$Y_{ijk} = \mu + t_i + a_j + e_{ijk}$$

where the elements have the same meaning as before and in addition

t_i = effect of the *i*th sampling time ($i = 1, 2, \ldots 10$)

RESULTS

The infusion time for the administration of the Ca-borogluconate solution to the cows in Group 1 varied from 6 to 11 min, while the corresponding infusion times for animals in Groups 2 and 3 were almost exactly 10 min.

Table 1. Mean, standard deviation (s) and range for the pretreatment Ca and P levels.

Substance	Group 1	Group 2	Group 3
Ca mmol/l			
mean	1.31	2.24	2.66
s	0.30	0.37	0.10
range	0.88—1.66	1.75—2.57	2.53—2.78
P mmol/l			
mean	0.62	1.25	1.12
s	0.36	0.37	0.18
range	0.23—1.30	0.78—1.66	0.96—1.42

The cows in Group 1 showed clinical signs of recovery within 1—3 h after the intravenous administration of Ca. All animals except 1 recovered after treatment. Re-examination of the cow not recovering 24 h after the initial treatment revealed a normalized blood Ca level in combination with a highly elevated ASAT (primarily designated GOT) concentration. None of the treated animals showed any signs of adverse side effects in conjunction with the Ca infusion.

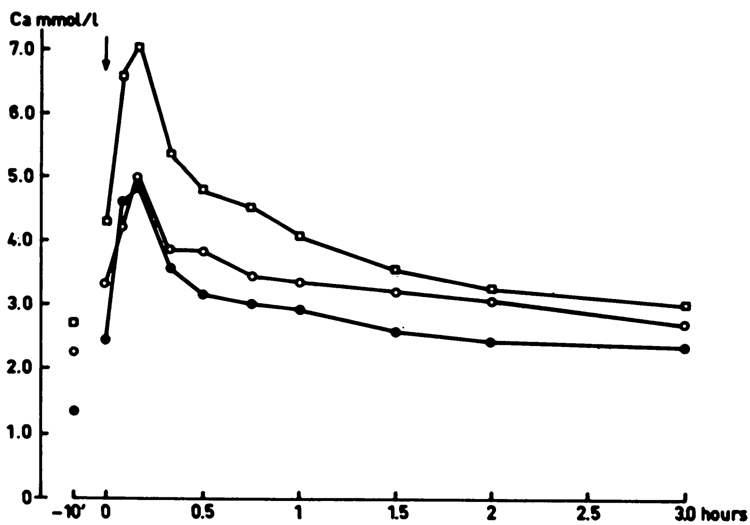
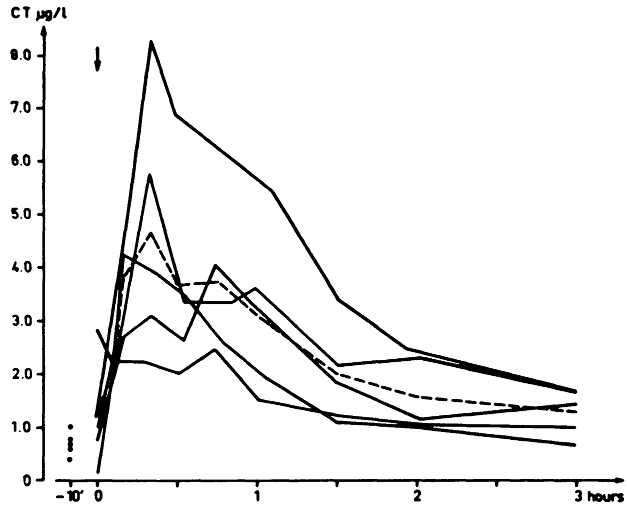


Figure 1. Mean blood Ca levels for cows in Group 1 (●——●), Group 2 (○——○) and Group 3 (□——□) 10 min before (—10'), during and at intervals after the intravenous infusion of Ca-borogluconate (0 : ↓).


CT µg/l
9.0
7.0
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4.0
3.0
2.0
1.0
0
−10'
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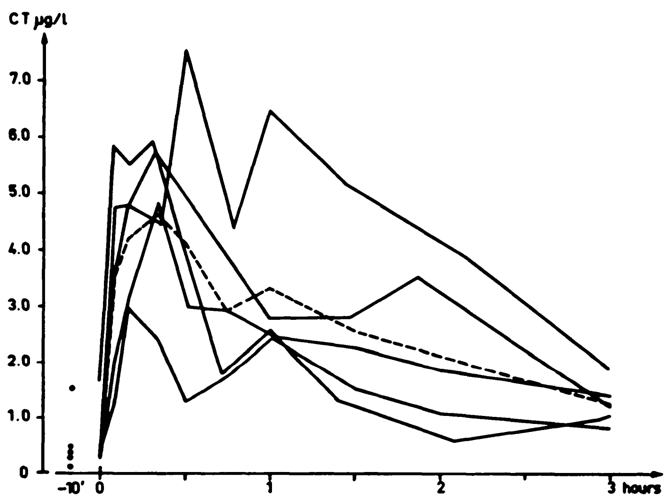
1
2
3 hours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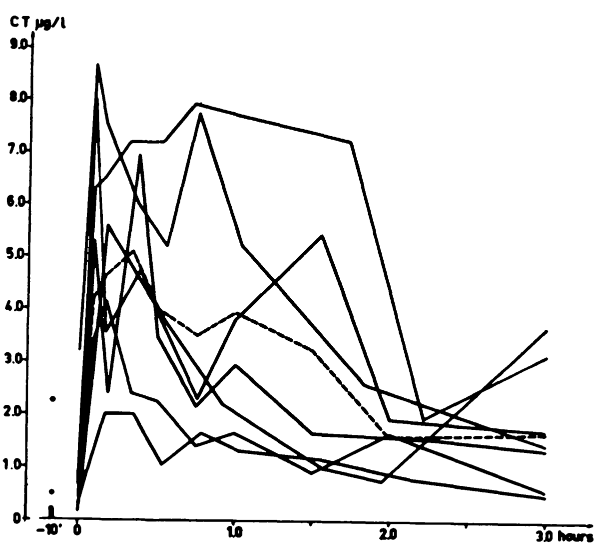
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3.0 hours

Table 2. Levels of significance for the effects studied (Model I).

Substance	Level of significance group	age class	animal
Ca	n.s.	n.s.	n.s.
CT	n.s.	n.s.	**
P	n.s.	n.s.	***

Levels of significance: n.s. = not significant ($P > 0.05$);
* = $P \leq 0.05$; ** = $P \leq 0.01$; *** = $P \leq 0.001$.

The mean, range and standard deviation for the pre-treatment Ca and P levels for the different groups of animals are presented in Table 1. The intravenous infusion of Ca-borogluconate resulted in an increase of the peripheral blood Ca levels in all animals (Fig. 1). The pre-treatment CT levels were low with most of the values being below the practical detection limit of the assay procedure (< 0.25 μg/l). In conjunction with the Ca infusion the peripheral blood plasma concentration of CT increased rapidly in all animals (Fig. 2).

As pointed out previously the animals in Group 3 were considerably younger than the animals of Groups 1 and 2. Since the main object of this study was to compare the CT response after the infusion of Ca to paretic and non-paretic cows, animals belonging to Group 3 have in certain cases been deleted from the statistical treatment of the data. In Table 2 are given the levels of significance for the substances measured in paretic and non-paretic cows using Model I for the calculations. The differences between individual animals were found to be significant for CT and P. Least-square means for the effect of age class and levels of significance for the effects studied using Model II are presented in Table 3. Paretic cows had significantly lower mean Ca levels after the Ca-borogluconate infusion as compared to the non-paretic cows. The mean CT response as well as the area under the CT response curve was significantly higher in the younger cows (age 4—6 years) as compared to the older cows

Figure 2. Blood plasma levels of CT in individual cows (———) before (—10'), during and after the intravenous infusion of Ca-borogluconate to paretic cows (below), newly delivered non-paretic cows (middle) and non-pregnant non-lactating cattle (top). Interrupted line indicates the mean value. (↓) Ca given at 0 hours.

Table 3. Least square means ± standard error of the mean (s.e.m.) for the effect of age class, and level of significance for the effects studied (Model II).

Substance	Least-square mean for age class ± s.e.m. 1 (4—6 years)	2 (> 6 years)	Level of significance group	age class
Ca mmol/l				
mean	3.33±0.10	3.15±0.12	*	n.s.
maximum	5.21±0.23	4.83±0.29	n.s.	n.s.
CT µg/l				
mean	3.88±0.37	2.22±0.47	n.s.	**
maximum	6.88±0.88	5.06±1.12	n.s.	n.s.
CT				
area	137.30±16.37	80.61±20.70	n.s.	*
P mmol/l				
mean	1.32±0.14	0.79±0.17	n.s.	*
maximum	1.80±0.17	1.22±0.22	n.s.	*

Levels of significance: n.s. = not significant ($P > 0.05$);
* = $P \leq 0.05$; ** = $P \leq 0.01$.

(age > 6 years). However, no significant differences were found for the mean CT response when comparing paretic to non-paretic cows.

Table 4 shows the overall means for the substances studied in the blood of all 3 groups of animals using Model III. Non-pregnant, non-lactating animals reached significantly higher Ca concentrations than paretic and non-paretic cows. Non-paretic cows had significantly higher maximum P levels than paretic cows. No significant differences between the groups of animals were found for the CT mean, CT maximum and for the area under the CT curve. Furthermore, no significant differences for the mean Ca/CT ratio were found between groups of animals. Since the pre-treatment Ca levels varied considerably between the groups the Ca/CT ratio was also calculated after subtraction of the pre-treatment Ca level. Also when using this Ca/CT ratio no significant differences were found for the mean ratios between the groups of animals.

Although only Ca but no phosphate was infused, inorg. P levels increased significantly with time in the paretic and non-paretic cows as compared to the animals in Group 3 (Model IV). For paretic cows all P levels between 20 min and 3 h after the injection of Ca-borogluconate were significantly higher than the

Table 4. Overall means ($\bar{x}$) and standard deviations (s) for the blood substances studied.

Substance	Group 1 (n=7) $\bar{x}$	Group 1 (n=7) s	Group 2 (n=5) $\bar{x}$	Group 2 (n=5) s	Group 3 (n=5) $\bar{x}$	Group 3 (n=5) s	Diff. sign by Duncan test (P < 0.05)
Ca mmol/l							
mean	3.01	0.26	3.48	0.13	4.40	0.32	1—2; 1—3; 2—3;
maximum	5.08	0.59	5.12	0.37	7.45	0.98	1—3; 2—3;
CT μg/l							
mean	3.00	1.50	2.69	0.92	2.54	0.87	
maximum	6.16	2.39	5.38	1.69	5.27	1.82	
area	105.91	54.17	97.69	41.04	91.12	44.06	
P mmol/l							
mean	0.87	0.40	1.27	0.35	1.19	0.10	
maximum	1.21	0.50	1.76	0.41	1.43	0.17	1—2;
Ca/CT ratio							
mean	1.32	0.68	1.40	0.19	1.21	0.29	

pre-treatment value ($P < 0.05$). Non-paretic cows had P levels between 45 and 90 min which were significantly higher than the pre-treatment values ($P < 0.05$). For the non-pregnant, non-lactating animals no significant change in the P level was observed (Fig. 3).

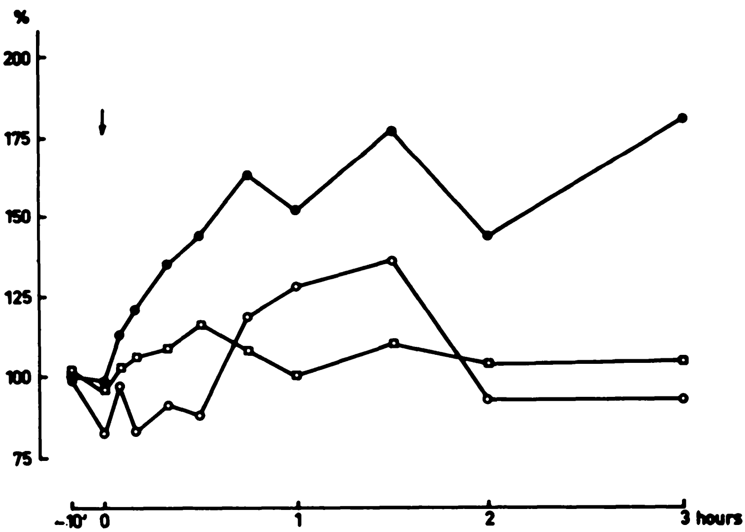

Figure 3. Blood P levels after intravenous infusion of Ca-borogluconate to cows in Groups 1 (●—●), 2 (○—○) and 3 (□—□). The pre-treatment mean values (—10') obtained 10 min before the Ca infusion (↓) have arbitrarily been transformed to 100 %.

DISCUSSION

The main object of the present study was to compare the Ca-stimulated CT response in paretic and non-paretic cows. The data for the animals in Group 3 were already available when planning the experiment and have subsequently been included in some of the statistical analyses.

The clinical signs in combination with the low blood Ca concentration verify a diagnosis of parturient hypocalcemia for the cows in Group 1. The cow in Group 1 still recumbent after treatment with Ca-borogluconate had normalized blood Ca levels but was found to have an elevated ASAT level upon re-examination 24 h after treatment. The increased ASAT concentration is an indication of a skeletal muscle disorder. It has previously been shown that one of the major causes for paretic cows to become downers is a trauma of the skeletal muscles (*Jönsson & Pehrson* 1969). One of the cows in Group 2 had lowered Ca levels which were not accompanied by clinical signs of hypocalcemia. This is usually referred to as "physiological puerperal hypocalcemia" and is accepted as normal (*Hallgren et al.* 1959, *Mayer et al.* 1966).

The higher Ca level after infusion found in animals of Group 3 when compared to the other 2 groups is probably due to differences in body weight. Although a reduced Ca amount was infused into some of the animals in Group 3, the infused amount in relation to body weight was on an average presumably higher in this group. The relatively lower serum Ca levels recorded after the infusion into the paretic cows is explained by these animals being hypocalcemic at treatment.

It is well established that the Ca infusion into the bovine species will cause a measurable discharge of CT (*Littledike et al.* 1972, *Deftos et al.* 1972). In this study all 3 groups of animals investigated responded with a marked and rapid elevation of the blood plasma CT levels as a result of the Ca infusion, which clearly demonstrates similar secretory ability of the calcitonin producing C-cells in all groups. This is in contrast to *Capen & Young* (1967) who observed a diminished CT content in the thyroid glands of cows with parturient paresis indicating an exhaustion of the C-cells.

CT does not only exert a hypocalcemic effect but also a hypophosphatemic effect (*Talmage et al.* 1972, *Simonnet et al.* 1978). The hypophosphatemic effect has been explained by CT increasing the renal P excretion and/or inhibiting the osteoclastic activity (*Singer et al.* 1976).

In parturient paresis there is a marked hypophosphatemia, which may be caused by the increased parathyroid hormone (PTH) levels (*Mayer et al.* 1969, *Blum et al.* 1974, *Jönsson et al.* 1980). Infusion of Ca decreases the PTH level in paretic cows (*Blum et al.*) leading to an increased renal P reabsorption. This may be the explanation of the observed increase in the plasma inorg. P after the Ca infusion, provided that this P increasing effect overcomes the hypophosphatemic effect of the CT increase (*Talmage et al., Simonnet et al.*). The small increase in plasma inorg. P in the non-paretic animals may have the same explanation.

The finding here of a similar CT response in both the paretic and non-paretic control cows after the intravenous infusion of Ca contradicts the results obtained by *Black & Capen* (1973). These authors therapeutically administered Ca to paretic cows but 1 h later were unable to record a significant increase in the blood plasma calcitonin-like activity. This difference in results is probably explained by the method used for the determination of the CT levels, *Black & Capen* using a relatively insensitive bioassay system. Furthermore, these authors determined plasma CT-like activity 1 h after the administration of the Ca. In the present study the majority of the animals reached maximum CT concentrations within 20 min and had declining CT levels 1 h after the Ca infusion.

Black & Capen also found the plasma CT-like activity/Ca ratio to be high in both paretic and control cows 24 h post partum when compared to samples obtained 1 month prepartum, at parturition and 1 month post partum. Since at this time the serum Ca level was relatively low the authors suggest that the increase in plasma CT-like activity was mediated by some other factor than the blood Ca concentration. In the present study no significant difference was found in the Ca/CT ratio regardless of whether the pre-treatment Ca levels were subtracted or not.

The significantly higher CT response recorded here in the younger cows might indicate a change in the CT secretion with advancing age. Such an age effect of serum CT has been described in man (*Samaan et al.* 1975).

It is noteworthy that individuality (effect of animal) significantly influenced the CT response to the Ca infusion. Consequently the CT data obtained from few animals are likely to be inconclusive. This might explain some of the controversies which exist concerning the possible role of CT as an etiological factor for the development of parturient paresis. Thus, *Care et al.* (1970) and *Garel & Barlet* (1975), after having determined CT levels in 1 paretic cow each, and 1 and 2 control cows, respectively, concluded that in parturient cows, the CT secretion escapes from the control of the Ca concentration, but the cause of the CT release remains obscure. *Mayer et al.* (1975), after investigating CT levels in 10 paretic and 13 control cows, concluded that parturient hypocalcemia was not due to an increased secretion of the hormone. On the contrary, their data suggested that milk fever may be preceded by a diminished secretion of the CT.

In conclusion, the results of the present study, yielding a similar CT response in both paretic and non-paretic cows, clearly demonstrate that the C-cells in both groups of animals have the capacity to secrete CT in response to a Ca infusion.

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SAMMANFATTNING

Samma calcitonin-svar efter kalkinfusion till kor med och utan kalvningsförlamning.

De calcitoninproducerande C-cellernas förmåga att frisätta calcitonin (CT) som svar på en intravenös Ca-boroglukonatinfusion har undersökts hos nykalvade kor med (7 djur) och utan (5 djur) puerperal pares samt hos ej lakterande, ej dräktiga djur (5 stycken). De nykalvade korna med puerperal pares behandlades 1—3 dagar efter förlossningen medan motsvarande tidsintervall för de nykalvade korna utan sjukdomen var 1—4 dagar.

Blodplasmakoncentrationen av CT, Ca och oorganisk P bestämdes före, under och efter infusionen. Ingen signifikant skillnad i CT-nivåer förelåg mellan nykalvade kor med eller utan puerperal pares. Blodplasmakoncentrationen av oorganisk P ökade signifikant 60 minuter efter Ca-infusionen till de nykalvade korna. Motsvarande ökning förelåg inte hos de ej lakterande, ej dräktiga djuren.

Resultatet av föreliggande undersökning visar bl a att C-cellerna hos nykalvade kor med eller utan puerperal pares har samma kapacitet att frisätta CT.

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