

Foetal plasma magnesium levels during maternal hypo- or hypermagnesaemia in ewes

BY J.-P. BARLET, MARIE-JEANNE DAVICCO, MICHÈLE MONCAUP
AND J. LEFAIVRE

INRA Theix, 63110 Beaumont, France

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1. The influence of maternal hypo- or hypermagnesaemia on foetal plasma magnesium levels was studied in pregnant ewes between the 120th and the 145th day of gestation using a permanent catheter inserted *in utero* into the left aorta of the foetus on the 115th day of gestation.

2. In seven ewes, carrying eleven foetuses, the injection of 1α -hydroxycholecalciferol induced a hypomagnesaemia which lasted 72 h. This had no significant effect on foetal plasma Mg levels.

3. In five ewes, carrying six foetuses, hypermagnesaemia induced by a 2 h magnesium chloride infusion had no significant effect on foetal plasma Mg levels.

4. These results provide evidence that in sheep during the last 20 d of gestation the foetal regulation of plasma Mg is independent of the maternal plasma Mg levels.

Very little is known about the regulation of plasma magnesium levels during the perinatal period and the foetal life. Capillary blood samples from newborn babies showed an increase in Mg concentration on the 1st day post partum and a decrease towards the 3rd day (Jukarainen, 1971). At birth, the Mg level in cord blood (0.72 mM) was similar to that measured in maternal blood plasma (0.73 mM) (Bogden *et al.* 1978). In newborn kids, calves and foals plasma Mg levels were slightly higher or similar to those measured in dams (Barlet *et al.* 1971; Garel & Barlet, 1976). Plasma Mg levels measured in rats from 16.5 to 21.5 d of gestation during the 1st week after birth proved to be invariably higher in the foetus than in the mother. The highest level observed was in the 16.5-d-old foetus. A small decrease occurred between 16.5 and 17.5 d of gestation; thereafter the plasma Mg levels remained constant up to 19.5 d and then subsequently decreased between 19.5 and 21.5 d. After birth an increase in plasma Mg occurred with suckling; the levels then remained constant through the 1st week (Garel & Barlet, 1974). In nineteen pregnant Scottish Blackface ewes, carrying twenty-eight foetuses, the mean maternal and foetal plasma Mg levels between 90 and 145 d of gestation were respectively 0.78 and 0.92 mM (Mellor & Matheson, 1977). In nineteen Limousine ewes, carrying thirty foetuses, the foetal plasma Mg levels (0.94 mM) between the 40th and the 26th day before parturition were also slightly higher than the maternal plasma Mg levels (0.83 mM), this difference then disappeared until birth (Barlet, Davicco *et al.* 1978). As far as we are aware, the influence of variations in maternal magnesaemia on foetal plasma Mg levels has not been studied.

Hypermagnesaemia can be easily induced by Mg infusion. Hypomagnesaemia was induced by injection of 1α -hydroxycholecalciferol (1α -OH D₃), a synthetic analogue of cholecalciferol. This is rapidly hydroxylated to $1\alpha,25$ -dihydroxycholecalciferol ($1,25$ -(OH)₂D₃), a biologically-active metabolite of cholecalciferol, when injected into rats (Holick *et al.* 1976). It has already been demonstrated that 1α -OH D₃ injected into cows (Barlet, 1975, 1977; Sansom *et al.* 1976) induced hypercalcaemia and hyperphosphataemia, associated with a slight but significant decrease in plasma Mg level.

Thus, the present study was undertaken to study the influence on foetal plasma Mg of experimentally-induced variations of maternal Mg.

EXPERIMENTAL

Animals. Twenty-four 15-month-old primiparous Limousine ewes, weighing 55–60 kg, were selected after radiography 100 d after mating: fourteen ewes carried a single foetus, ten were bearing twins. The length of gestation of the Limousine breed is 145 d. After radiography each ewe was placed in an individual cage and fed on hay and grain concentrate. Thus the daily intake (g) for each animal was: calcium 4.1, inorganic phosphorus 3.5, Mg 1.5, meeting the nutritional requirements of pregnancy for 60 kg ewes (Thériez & Guéguen, 1978).

Surgical procedure. Catheters were chronically implanted in the left aorta of 115-d-old foetuses, according to a method described by Mellor & Matheson (1975). In ewes bearing twins, both foetuses were catheterized (Barlet, Davicco *et al.* 1978). In this way each permanent catheter allowed the collection of foetal blood from the time of surgery until at least 24 h before birth. However, a period of 10 d was allowed to enable each animal to recover fully from surgery before the experimental period. Three lambs died from haemorrhage at the time of delivery, after displacement of the catheter. The thirty-one other lambs were born alive in good condition: their mean (\pm SE) body-weight at birth (3.1 ± 0.3 kg) did not differ from that of unoperated control lambs from primiparous ewes of the same breed.

Maternal blood samples were obtained via a catheter implanted in the left carotid artery of the ewe during the anaesthesia for foetal surgery. Maternal infusions were given through a catheter implanted in the right jugular vein of the ewe before each experiment.

Mg infusions and 1α -OH D_3 injections in ewes. A group of five ewes bearing six foetuses and a second group of six ewes bearing eight foetuses (two bearing twins) were used on the 125th and 140th day of gestation respectively. Magnesium chloride (2 mg Mg/kg body-weight in 20 ml sodium chloride (9 g/l)) was infused with a peristaltic pump into each ewe at a rate of 10 ml/h.

Seven ewes bearing eleven foetuses (four bearing twins) were injected intramuscularly with 1α -OH D_3 (Leo Laboratories, Vernouillet, France; $0.2 \mu\text{g/kg}$ body-weight in 2 ml propylene glycol, given in two equal doses) at 09.00 hours on days 135 and 136 of gestation. Six control ewes bearing nine foetuses (three bearing twins) were injected with propylene glycol alone.

Blood analysis. After centrifugation, the blood plasma was collected and frozen until analysed. Plasma Mg and Ca were measured by atomic absorption spectrophotometry (Perkin Elmer 400). Plasma inorganic phosphorus was determined by colorimetry using a Technicon Autoanalyzer.

RESULTS

No significant variation was observed either in foetal (0.87 ± 0.07 mM; 76 observations) or in maternal (0.78 ± 0.4 mM; 35 observations) plasma Mg levels from the 125th to the 145th day of gestation. Foetal plasma Mg was never significantly different from maternal levels. However foetal Ca (2.89 ± 0.09 mM; 76 observations) and phosphate (2.14 ± 0.18 mM; 74 observations) were always higher ($P < 0.01$) than maternal plasma Ca (2.31 ± 0.10 mM; 33 observations) and phosphate (1.55 ± 0.15 mM; 37 observations) levels.

A significant increase occurred in plasma Ca and inorganic P, both in dams and foetuses (Table 1) in ewes injected with 1α -OH D_3 ($0.1 \mu\text{g/kg}$ body-weight on days 135 and 136 of gestation). Maternal hypercalcaemia and hyperphosphataemia were associated with a significant decrease in plasma Mg, occurring 24 h after the second injection, and lasted 72 h (Fig. 1). Injection of 1α -OH D_3 in pregnant ewes had no significant effect on foetal plasma Mg (Fig. 1). The injection of propylene glycol alone had no significant effect on foetal or maternal plasma electrolytes levels (Fig. 1, Table 1).

On the 125th and 140th days of gestation, the intravenous infusion of MgCl_2 induced a

Table 1. The effect of intramuscular injections of 1 α -hydroxycholecalciferol in pregnant ewes (0.2 μ g/kg body-weight, in 0.2 ml propylene glycol, given in two equal doses at 0 and 24 h) on maternal and foetal plasma calcium and phosphate levels (mM) and control ewes injected with solvent alone

Period after injection (h)	Control animals						Treated animals									
	Ewes (6)			Foetuses (9)			Ewes (7)			Foetuses (11)						
	Ca	P		Ca	P		Ca	P		Ca	P					
Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE					
-24	2.47	0.04	1.32	0.10	2.75	0.05	2.25	0.05	2.44	0.04	1.32	0.07	2.75	0.05	2.27	0.03
0	2.48	0.05	1.34	0.03	2.76	0.05	2.29	0.05	2.45	0.03	1.31	0.07	2.76	0.07	2.27	0.03
12	2.48	0.05	1.31	0.06	2.76	0.02	2.26	0.07	2.59	0.05	1.51	0.09	2.84	0.05	2.34	0.05
24	2.46	0.03	1.30	0.06	2.75	0.02	2.26	0.06	3.48	0.05*	1.78	0.05*	2.89	0.06*	2.46	0.03*
36	2.47	0.06	1.31	0.04	2.78	0.02	2.30	0.03	3.58	0.05**	1.88	0.06**	3.07	0.06**	2.63	0.03*
48	2.43	0.07	1.32	0.04	2.76	0.05	2.28	0.05	3.75	0.07**	2.03	0.09**	3.22	0.07**	2.65	0.03*
60	2.41	0.07	1.35	0.05	2.76	0.04	2.31	0.06	2.81	0.04*	2.00	0.10*	3.19	0.08**	2.60	0.10*
72	2.41	0.08	1.35	0.06	2.72	0.03	2.27	0.05	2.73	0.05*	1.96	0.12*	3.16	0.07*	2.58	0.03**
96	2.43	0.08	1.32	0.03	2.73	0.06	2.26	0.07	2.58	0.04*	1.61	0.04*	3.06	0.06*	2.46	0.06*
120	2.46	0.08	1.34	0.03	2.75	0.06	2.24	0.09	2.51	0.08	1.51	0.07	2.87	0.09	2.36	0.10
144	2.45	0.09	1.29	0.03	2.71	0.04	2.25	0.07	2.51	0.08	1.38	0.07	2.84	0.10	2.29	0.06
168	2.43	0.10	1.32	0.08	2.74	0.09	2.26	0.06	2.46	0.03	1.33	0.05	2.78	0.07	2.26	0.06

Statistical significance of differences between treated and control ewes and between foetuses from control ewes and foetuses from treated ewes: * $P < 0.05$, ** $P < 0.01$.

Table 2. *The effect of intravenous infusion of magnesium chloride in pregnant ewes on days 125 and 140 of gestation (2 mg magnesium/kg body-weight, given in 20 ml sodium chloride (9 g/l) during 2 h) on maternal and foetal plasma calcium and phosphate levels (mM)*

(Mean values with their standard errors; no. of animals in parentheses)

Period of gestation(d) . . .	125						140									
	Ewes (5)			Foetuses (6)			Ewes (6)			Foetuses (8)						
	Ca	P		Ca	P		Ca	P		Ca	P					
Period after infusion (min)	Mean	SE		Mean	SE		Mean	SE		Mean	SE					
-30	2.44	0.06	1.59	0.12	2.72	0.17	2.13	0.19	2.34	0.08	1.71	0.13	2.82	0.16	2.14	0.15
0	2.42	0.04	1.55	0.10	2.79	0.14	2.09	0.20	2.36	0.03	1.69	0.09	2.80	0.16	2.10	0.17
30	2.39	0.06	1.57	0.07	2.70	0.13	2.10	0.18	2.39	0.07	1.67	0.07	2.80	0.14	2.10	0.09
60	2.38	0.05	1.61	0.12	2.75	0.10	2.13	0.20	2.30	0.05	1.67	0.12	2.77	0.07	2.15	0.18
90	2.30	0.03	1.53	0.14	2.77	0.18	2.15	0.20	2.30	0.05	1.59	0.14	2.77	0.05	2.17	0.07
120	2.28	0.05*	1.49	0.07*	2.73	0.07	2.10	0.16	2.20	0.01*	1.48	0.08*	2.81	0.13	2.19	0.11
150	2.38	0.07	1.55	0.22	2.68	0.11	2.03	0.20	2.37	0.05	1.55	0.12	2.83	0.17	2.21	0.17
240	2.33	0.04	1.47	0.13	2.78	0.09	1.97	0.15	2.38	0.10	1.49	0.13	2.77	0.16	2.15	0.10

Statistical significance of difference from values measured at zero time in the same group of animals: * $P < 0.05$.

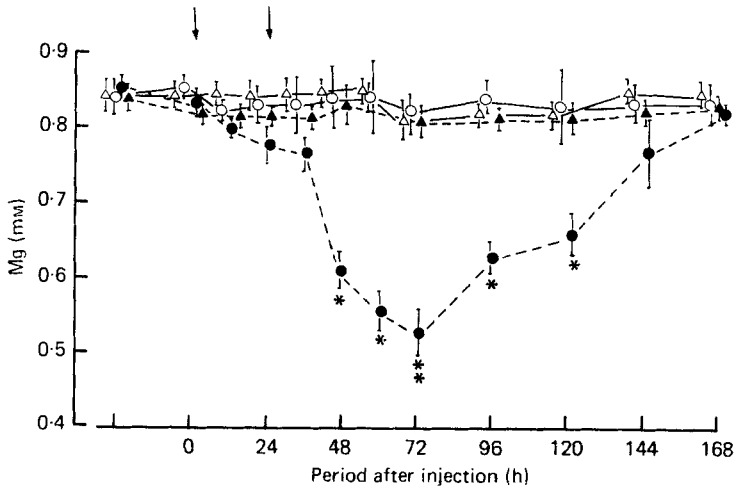


Fig. 1. The effect of intramuscular injections (↓) of 1α -hydroxycholecalciferol ($0.2 \mu\text{g}/\text{kg}$ body-weight, given in two equal doses on 135th and 136th days of gestation, in 1 ml propylene glycol) on plasma magnesium levels (mM) in seven pregnant ewes (● --- ●) and their eleven foetuses (○—○). Six control ewes (▲ --- ▲) carrying nine foetuses (△—△) were injected with solvent alone. Mean values with their standard errors represented by vertical bars. * $P < 0.05$, ** $P < 0.01$; treated and control ewes or foetuses of treated ewes and foetuses of control ewes were compared at the same time interval by Student's t test.

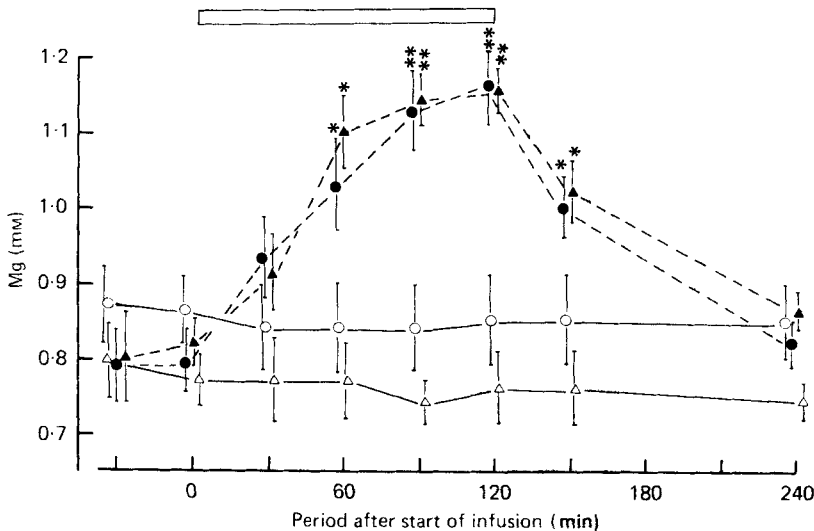


Fig. 2. The effect of intravenous infusion of magnesium chloride (□) magnesium/kg body-weight, given in 20 ml sodium chloride (9 g/l for 2 h) on plasma Mg levels in five ewes (● --- ●) carrying six foetuses (○—○) on 125th day of gestation and in six ewes (▲ --- ▲) carrying eight foetuses (△—△) on 140th day of gestation. Mean values with their standard errors represented by vertical bars. * $P < 0.05$, ** $P < 0.01$; comparison with values measured at zero time in the same group of animals.

significant rise in maternal plasma Mg levels, starting 60 min after the initiation of the infusion and lasting a further 90 min (Fig. 2). The Mg infusion also induced a slight but significant decrease in maternal plasma Ca and phosphate levels (Table 2). Mg infused

intravenously in ewes did not affect foetal plasma Mg, Ca and phosphate levels significantly (Fig. 2, Table 2).

DISCUSSION

Over the last 20 d of gestation, plasma Ca and phosphate levels were higher in the foetus than in the mother (Tables 1 and 2), confirming the results of previous experiments in primiparous pregnant ewes of the Limousine breed (Barlet, Davicco *et al.* 1978). Despite this however no significant difference between maternal and foetal plasma Mg levels during the last 20 d of gestation was observed (Figs. 1 and 2). Thus, the higher mean plasma Mg level in foetuses compared to ewes reported by Mellor & Matheson (1977) probably resulted from high foetal levels before the 119th day of gestation (Barlet, Davicco *et al.* 1978).

In five lactating Masham ewes, weighing 55–60 kg, receiving an intramuscular injection of 1α -OH D_3 daily for 10 d (5 μ g/animal per d) the hypercalcaemia and hyperphosphataemia induced resulted mainly from an increased intestinal absorption of Ca and phosphate (Braithwaite, 1978). A significant hypercalcaemia and hyperphosphataemia occurred both in dams and foetuses from the 24th until the 96th day post-injection in four pregnant Limousine ewes, carrying six foetuses, intravenously injected with 1α -OH D_3 (0.1 μ g/kg body-weight). However foetal plasma Mg remained unchanged, while maternal plasma Mg levels were only slightly and not significantly decreased (Barlet, Davicco *et al.* 1978). The lack of significant fall in maternal plasma Mg levels in this experiment was probably due to the fact that only four ewes were used, and these were injected with a lower dose of 1α -OH D_3 (0.1 μ g/kg body-weight, in a single injection) than that used in the present study (0.1 μ g/kg body-weight on 135th and 136th days of gestation). It has also been reported that 1α -OH D_3 injection in dairy cows induced hypercalcaemia and hyperphosphataemia with a decrease in plasma Mg (Barlet, 1975, 1977; Sansom *et al.* 1976). Such a decrease might result from an increased urinary excretion of Mg: a single dose of ergocalciferol (125 μ g/animal) greatly increased the renal clearance of Mg in vitamin D-deficient rats (Lifshitz *et al.* 1967). It might also be due to an increased Mg uptake by different tissues, mainly bone: the administration of 2.5–5 mg calciferol to adult dogs daily for 5 weeks caused hypercalcaemia and hypomagnesaemia, associated with an increase of 20% in the Mg content of pancreas, liver and bone (Wallach *et al.* 1966).

Maternal hypercalcaemia induced by Ca infusion does not modify foetal calcaemia either in the ovine (Bawden & Wolkoff, 1967; Garel *et al.* 1974) or in the bovine (Barlet *et al.* 1979) species. Ca placental transfer in the pregnant ewes is a one way process, from the dam to the foetus (Braithwaite *et al.*, 1969; Ramberg *et al.* 1973). Thus the foetal hypercalcaemia observed after the administration of $1,25$ -(OH) $_2D_3$ analogues to pregnant ruminant females results either from an increased placental transfer of Ca from the dam to the foetus, or from placental transfer of cholecalciferol metabolites to the foetus, which would increase foetal calcaemia by a yet unknown process (Barlet, Davicco *et al.* 1978). Several recent results appear to be in favour of placental transfer of 25 -OH D_3 (Ross *et al.* 1976; Barlet, Argémi *et al.* 1978) and $1,25$ -(OH) $_2D_3$ (Ross *et al.* 1979). However, in this instance, $1,25$ -(OH) $_2D_3$ would not induce hypomagnesaemia in the foetus. Our results merely allow the conclusion that maternal hypomagnesaemia, induced by 1α -OH D_3 injection in the ewe, does not modify foetal magnesaemia which remains remarkably constant (Fig. 1).

The slight hypocalcaemia and hypophosphataemia observed in ewes injected with $MgCl_2$ might have resulted from increased calcitonin (CT) release. It has been demonstrated in rats (Radde *et al.* 1968), cats (Nielsen, 1970), pigs (Care *et al.* 1971) and steers (Barlet, 1971) that an increase in plasma Mg level is able to stimulate CT secretion. Furthermore, it has

been shown *in vivo* (Buckle *et al.* 1968), and *in vitro* (Targovnik *et al.* 1971) that Mg ions were able to reduce parathyroid hormone release: such an inhibition could increase the fall in plasma Ca induced by CT.

Maternal hypercalcaemia induced by Ca infusion does not modify foetal plasma Ca levels in sheep (Bawden & Wolkoff, 1967; Garel *et al.* 1974). Similarly, maternal hypermagnesaemia induced by Mg infusion does not modify foetal plasma Mg levels either on the 125th or 140th day of gestation (Fig. 2). However, in pregnant women treated with magnesium sulphate for toxæmia of pregnancy, an increase in plasma Mg level was observed in cord blood at birth (Lipsitz & English, 1967; Tsang, 1972). These species differences may reflect differences of placentation between primates and ruminants. The syndesmochorial ovine placenta is probably very important in preventing excessive mineral transfer from the dam to the foetus. Thus, during the last 20 d of gestation, the ovine foetus is able to regulate its own magnesaemia independently from maternal plasma Mg level. This seems to be the situation under our experimental conditions, where fluctuations in maternal magnesaemia do not induce severe disturbances in the mother.

In conclusion, during the last 3 weeks of gestation in ewes, neither hypomagnesaemia induced by 1α -OH D₃ injection, nor hypermagnesaemia after MgCl₂ infusion, have any significant effect on foetal plasma Mg levels.

Pregnant ewes used in this study were supplied by M. Thériez (Laboratoire de Production Ovine). 1α -OH D₃ was a gift from Dr J. L. Le Bossé (Laboratoires Léo, France).

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