Elsevier Editorial System(tm) for Journal of Equine Veterinary Science
Manuscript Draft

Manuscript Number:

Title: Feeding magnesium supplement to foals reduces osteochondrosis prevalence.

Article Type: Original Research

Keywords: osteochondrosis; magnesium; osteocalcin; CTX-1; horse

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Abstract: The influence of supplements containing magnesium on the aetiology of osteochondrosis is unknown. We did two studies to measure the effect of additional minerals (especially magnesium) on osteochondrosis. In study 1 (5 studs, in total 64 mares and foals aged 0 to 5 months, equally divided into two groups) supplementation with minerals and placebo was used. Blood samples were taken from foals at age of 2, 8 and 16 weeks. At the same time, milk samples were taken from the mare. Bone-biomarkers (osteocalcin and CTX-1) and minerals (calcium, phosphorus and magnesium) were measured in blood and the same minerals in milk of the mare. At the end of the study, the femoropatellar (FP = knee), tarsocrural (TC = hock) and metacarpophalangeal/metatarsophalangeal (MCP/MTP = fetlock) were radiographed and scored for the presence and grade of osteochondrotic lesions. In study 2 (6 studs, 54 foals, aged 5 to 12 months, equally divided into two groups) the same was repeated. At the start and end of the study, again blood samples were taken and analysed on the same parameters as in study 1. Also the same radiography was done.
In study 1 in the mineral supplemented group, 21.9 % were diagnosed with ostechondrosis compared to 41.9 % in the placebo group. In study 2, there was no change in osteochondrosis between 5 and 12 month in the placebo group while there was a drop of 14.3 % in incidence in the supplement group. We concluded that magnesium supplementation reduced osteochondrosis prevalence.

Suggested Reviewers:

Opposed Reviewers:

INTRODUCTION

Osteochondrosis (OC) is a disorder frequently diagnosed in horses. OC prevalence is very high and a prevalence of 25 to 40% is no exception in warmblood breeds (1), although cold blood horses also suffer from this disorder (2) (3). OC is a disturbance in the process of ossification that occurs in young animals. It is a dynamic disorder and lesions may repair or get worse during the first months until 12 months of age (4). It starts at birth or possiblyeven before. At an age offive months, prevalence is at its highest. Regression of lesions isjoint dependent, but no further substantial reduction in osteochondrosis is observed after an age of 12/twelve months (5).

Several factors do influence bone formation, and irregular ossification leads to the formation of loose fragments. Irrespective of a good genetic background, bone development depends on minerals such as calcium, phosphorus and magnesium, trace elements such as copper, zinc and manganese, and vitamins such as vitamin D and K. Previous studies mainly focused on copper, zinc and other trace elements, but the role of magnesium has not been studies so far (6-21). This study focuses on the effect of supplementing magnesium and phosphorus during the first 12 months of age of a foal on the development of OC. The second aim of this study was/is to evaluate the use of previous described biomarkers osteocalcin and CTx (C-terminal telopeptide of type I collagen) (3, 22-42) as an alternative for radiography to diagnose OC.

MATERIALS AND METHOD

Study 1

Sixty-four mares living at five different stud farms were selected for this study. Blood was taken from mares two weeks before calculated day of parturition. Three mares had already given birth to their foal at the start of the experiment. From the 64 foals, one foal died due to a bacterial infection before the end of the experiment, at week 16 after parturition. Until they reached the age of 16 weeks, foals were given 42 gram per day of an oral paste containing 4.05 gram magnesium, 2.50 gram phosphorus. The placebo group received the same oral paste without magnesium and phosphorus. The pastes were coded red and blue and no further difference in taste or labels existed. The mineral content of the feed was recorded in order to correct for differences between stud famrs. Blood samples were taken from the mares at the beginning of the experiment and levels of minerals and bone biomarkers were measured. At two, eight, and sixteen weeks after parturition, milk samples from mares and blood samples from foals were collected.

Study 2

Fifty-four foals living at six stud farms were selected for this study. Per stud farm, foals were divided randomly into two groups. One group received 200 gram pellets with 4 gram magnesium, 2,5 gram phosphorus and 1.7 gram calcium. The second group received no pellets. Whether or not foals received extra pellets was only known by the owner of the foals and the supervisor of the stud farms, but not to the researchers. The mineral content of the feed was recorded in order to correct for differences between stud farms. From the 54 foals, two died due to infections not related to this study.

In both studies, blood was collected from the jugular vein into plain tubes and sent to the laboratory as soon as possible/directly afterwards. After centrifugation (15 min at 2000 g) serum was stored at –20°C until analyses were performed.

Milk samples taken in study 1 were stored in plain tubes and sent to the laboratory together with the blood samples.

Milk samples were analyzed for calcium, magnesium and phosphorus after tenfold dilution with water, using ICP-OES. In a previous experiment we validated this method with two other methods: after acid digestion in a micro-wave and detection with ICP-OES and after precipitation of protein and detection with ICP-OES. All three methods gave the same results in our laboratory.

Blood samples were analyzed for calcium, magnesium and phosphorus, using ICP-OES.

Bone biomarkers were analyzed as follows: C-telopeptide type 1 (CTx) was analyzed with an ELISA-test kit (Immunodiagnostic Systems Inc. Scottsdale, USA). Osteocalcin was analyzed using an ELISA-test kit (Quidel, Metra Quidel MicroVue 8002, San Diego, USA). Both ELISA's were validated for use in horses. Linearity, stability, repeatability and within-lab-reproducibility, accuracy and selectivity were tested (29) and (27).

The following parameters were noted for each stud farm:

1. Management: use of chemicals, metals used for construction of stables, drinking system, feeding system, air ventilation, amount of light in stables.
2. Feeding of mares: hours spent in pasture, feeding additional concentrates, control of feeding amount and quality of grass.
3. Feeding of foals, additional to milk.
4. Water: origin of water source, control of water supply.
5. Physical exercise: from hours of movement of foals, inside or outside, and space opportunities during night, relative movement was calculated on a scale from 0 to 3.
6. From each foal, the growth was recorded.

Osteochondrosis was diagnosed using X-ray at an age of 5 and 12 months (1).

Statistical analyses of individual results were done with Stata 11 (StataCorp LP, College Station, Texas 77845 USA). Graphical analyses were done with SigmaPlot (SigmaPlot for Windows, version 11, Systat Software USA).

RESULTS

Blood analysis results of calcium, magnesium, phosphorus, C-telopeptide type 1, and osteocalcin at two weeks before parturition are shown in Figure 1. These values are within the reference ranges of the laboratory of Animal Health Services.

Average concentrations of calcium, phosphorus and magnesium in milk are shown in Figure 2.

Figure 3 shows different serum magnesium concentrations in blood from supplemented and non-supplemented foals during the first sixteen weeks of age. Supplementing magnesium did not have an effect on the concentrations of biomarkers osteocalcin or CTx (results not shown).

Figure 4 shows statistically significant higher magnesium levels ($P < 0.05$) at week 16 in blood from foals that were scored without OC at 5 months of age.

The average prevalence of OC in each stus farm is shown in Table 1.

Using the ratio osteocalcin / CTX-1 as a marker for active bone-metabolism, Table 2 shows a statistically significant difference ($p < 0.05$) in OC prevalence in foals at 8 weeks of age, based on their bone-metabolism. This difference did not exist, however, when foals were 5 months of age (results not shown).

Animals with OC at an age of 5 months had on average both a lower osteocalcin and a lower CTX-1 at an age of 2 to 8 weeks. However, this difference was not statistically significant at an age of 16 weeks.

Foals with no OC detected at an age of 5 month, had a statistically significant ($P<0.01$) higher average magnesium serum level at an age of 16 weeks. There was also a tendency that animals without OC had on average a higher phosphorus serum level, however, this was not statistically significant.

Most management parameters like water holders, drinking system, and feeding system did not differ between stud farms, and therefore, these parameters were not used in further calculations.

In Figure 5a, average relative movement is plotted against average OC prevalence per stud farm, showing an almost linear line. One stud farm, feeding foals extra minerals and extra magnesium above the level in the supplement, was clearly deviating. Combining extra feed and movement of the foals, Figure 5b shows a linear line.

Figure 6 shows a relation between growth and CTx at 12 month of age ($P < 0.001$). No such relation was found between growty and osteocalcin activity (results not shown).

Figure 7 shows the results of the blood samples for calcium, magnesium, phosphorus, osteocalcin, CTx and the ratio osteocalcin / CTx. In Figure 7f, bone activity of a full grown mare is pictured with a line (about 80 units).

In the second study, OC prevalence was measured at 5 and 12 months of age. The change in OC prevalence for supplemented and non-supplemented foals is listed in Table 3.

After logistic regression, the change in OC prevalence was only related with feeding supplement or not (odds ratio 4.6; $P < 0.05$).

Figure 8 shows that supplementation with magnesium resulted in a reduced prevalence of FP (knee joint) osteochondrosis at 12 months of age.

Figure 9 shows OC prevalences of both studies. After supplementing young foals with magnesium, at an age of 5 months the OC prevalence was significantly lower. In the second study, the addition of magnesium lowers the OC prevalence at 12 months compared to the situation at 5 months.

DISCUSSION

The two objectives in this study were: to measure the effect of supplementing magnesium in combination with phosphorus during the first 12 months of a foal on the prevalence of osteochondrosis (OC) and to evaluate the use of bone specific biomarkers osteocalcin and CTx (C-terminal telopeptide of type I collagen) to diagnose OC as an alternative for radiography. The three factors that have been related to OC are imbalanced feeding, fast growth and genetic factors (27). But also housing system can influence OC in foals (35, 42-45) and in fattening pigs (46). Therefore also in our study we noted for each stable parameters such as physical exercising of the foal. Although only 5 stud farms with 63 animals participated in this study, the relation is very suggestive.

The blood minerals and biomarkers of the mares two weeks before parturition didn't have any influence on the milk composition after parturition. The mineral content found in this study is comparable with previous studies (47, 48).

The concentrations of osteocalcin and CTx are comparable to other publications (3, 25, 27, 34). The activity of the bone metabolism (expressed as the ration between osteocalcin and CTx) was statistically significant higher at 8 weeks of age in foals diagnosed with OC after 5 months compared to animals without OC (23,8 % OC in the group with lower bone metabolism compared to 47,7 % OC in the group with higher bone metabolism).

In the second study, there was a negative correlation between CTx and the growth of the foal. But also in this second study, there was not a clear relation between the bone metabolism as measured by osteocalcin and CTx. Therefore, we concluded that measuring bone metabolism can't replace radiography in diagnosis of OC.

Feeding additional magnesium to foals resulted as expected in a higher magnesium in blood. In the first study, foals without OC had a significant lower magnesium in their blood at 16 weeks of age. The average prevalence of OC in this first study was statistically different in the placebo group (OC prevalence = 41.9%) compared to the group receiving the magnesium supplement (OC prevalence = 21.9%). The second study started with foals at an average age of 5,5 months. The average OC prevalence was 48,7 %. After the foal was randomly assigned in the supplement or placebo group, the OC score was measured without knowledge about the group the foal was placed to the authors doing the interpretation of the images. At the end of the second study, again the foals were radiographed but again blind. Only when all results were combined, it revealed that the placebo group had an average OC prevalence of 41.4 % and the supplement group 56.0 %. At the end of the study, the OC prevalence of the placebo was 42.9 % (statistically no change) but the group receiving the supplement had an OC prevalence of 41.7%, which was statistically significant lower. When looking more in detail, it was the OC of the knee joint that improved most. This is in concordance with the bone development of horses. As can be seen from figure 7F, some foals have reached the bone activity of a full grown horse at an age of 12 months.

The conclusion of our both studies is that magnesium-supplementation and more movement of the foal could lower the osteochondrosis prevalence significantly. Mainly the osteochondrosis prevalence of the knee joint was very low after supplementation of magnesium.

Acknowledgements

Thanks to M. Pijnappel and C. Helder who visited the stables and animals every week to check and monitor the animals. Also thanks to A. Koppejan who did all the biomarker analysis in serum.

A.J. van den Belt (University Utrecht): for second opinion of the x-ray pictures and the diagnosis of osteochondrosis.

V. Hinnen (Pavo Horsefeeds): for preparing the supplements of magnesium and phosphorus.

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AUTHOR CONTRIBUTIONS

G.H.M. Counotte (Animal Health Services): study setup, supervision of laboratory testing, statistical analysis and preparing the manuscript.

Gerrit Kampman (Den Ham): sampling the foals, taking the x-rays and interpretation of the x-rays.

Table 1. Prevalence of osteochondrosis in Mg supplemented and no

Stud farm	Mg-supplementation			
	No		Yes	
	Foals (n)	OC prevalence (%)	Foals (n)	OC prevalence (%)
1	5	20.0	5	40.0
2	12	33.3	13	15.4
3	6	50.0	6	33.3
4	4	75.0	5	20.0
5	4	50.0	3	0.0
Total	31	41.9	32	21.9

Table 2. Bone metabolism (ratio of osteocalcin and CTx) of foals

Stud farm	Bone metabolism at 8 weeks			
	Below average		Above average	
	Foals (n)	OC prevalence (%)	Foals (n)	OC prevalence (%)
1	8	12.5	2	100.0
2	15	20.0	10	30.0
3	8	37.5	4	50.0
4	7	28.5	2	100
5	4	25.0	3	33.3
Total	42	23.8	21	47.6

Table 3 Change in osteochondrosis prevalence between 5 and 12 mo

	Placebo		Supplement	
Stud farm	Foals (n)	Change in OCD (%)	Foals (n)	Change in OCD (%)
1	6	+16.7	6	0
2	2	0	2	-50
3	7	-8.9	9	-25.6
4	7	-28.5	1	0
5	3	+33	3	-33
6	3	+33	3	+33
Total	28	+1.5 %	24	-14.3

Figure 1 Boxplot of blood parameters from mares

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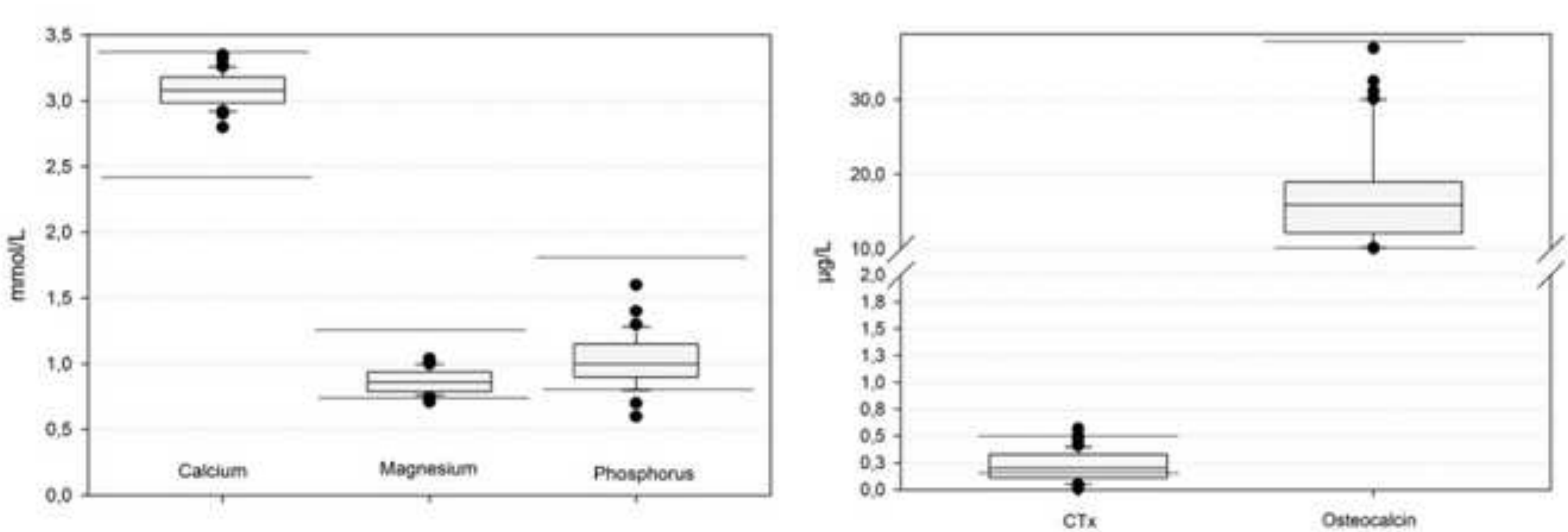

Figure 2 Calcium, magnesium and phosphorus in milk
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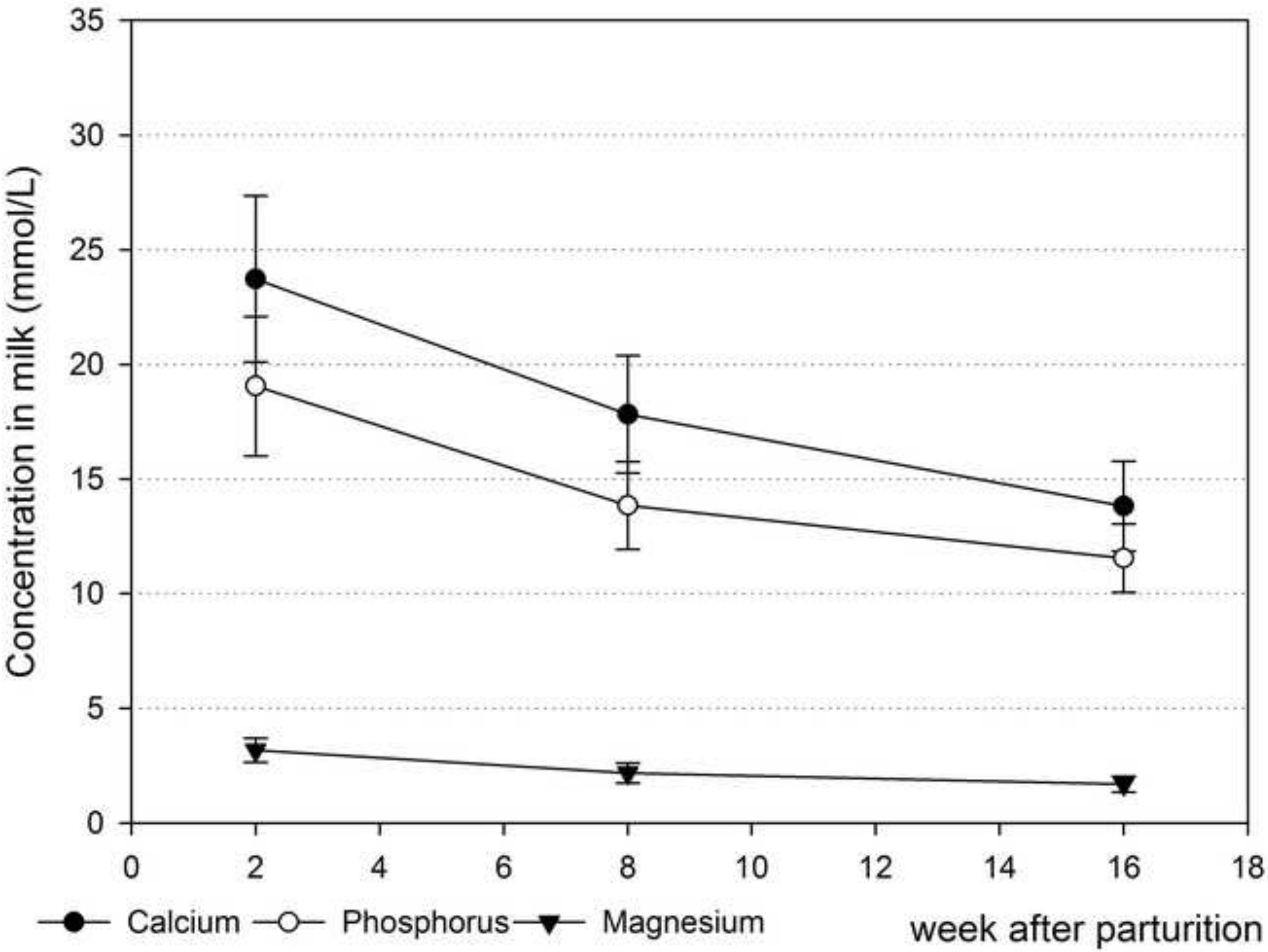

Figure 3 Magnesium in blood (foals) placebo or supplement
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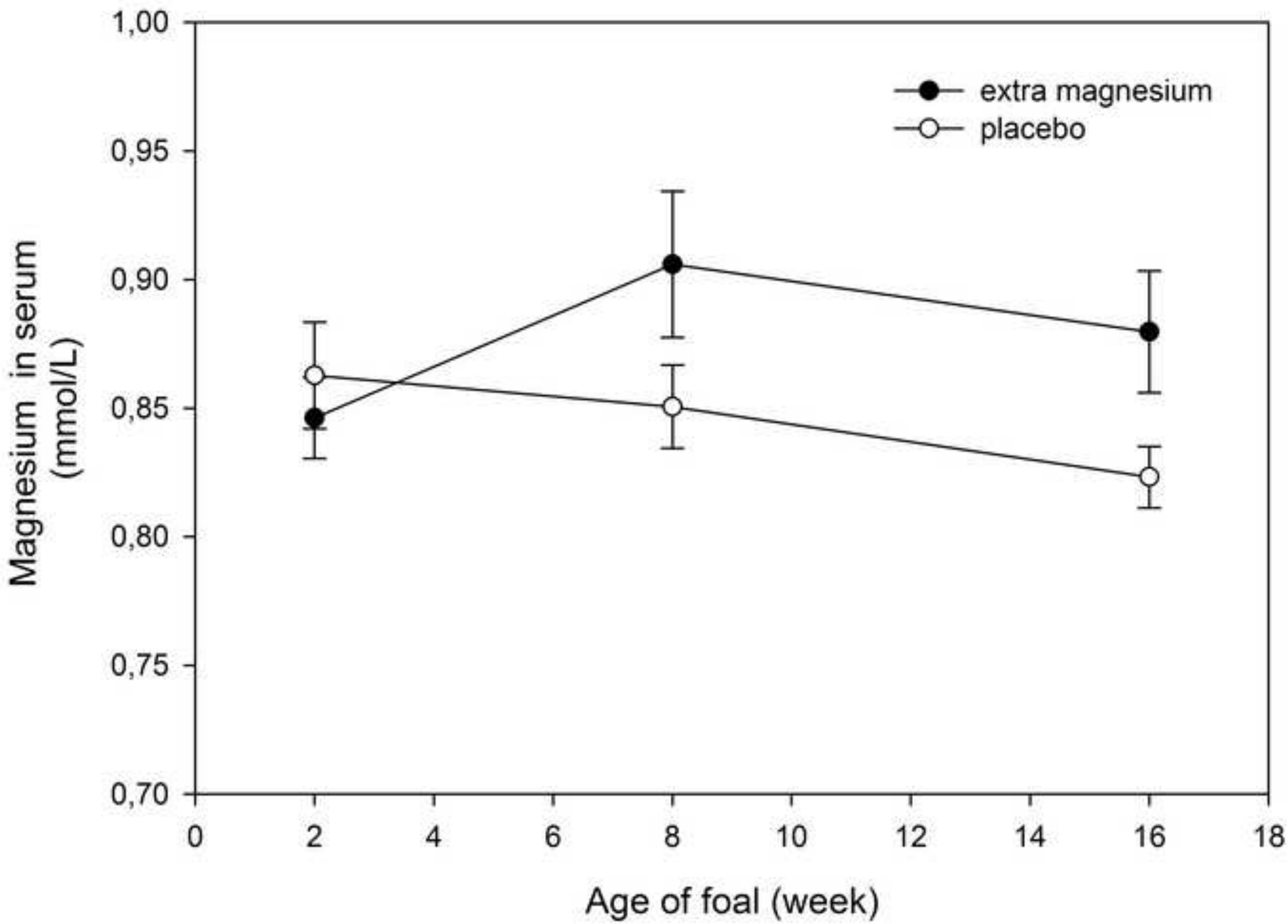

Figure 4 Magnesium in blood (foals) with or without OC
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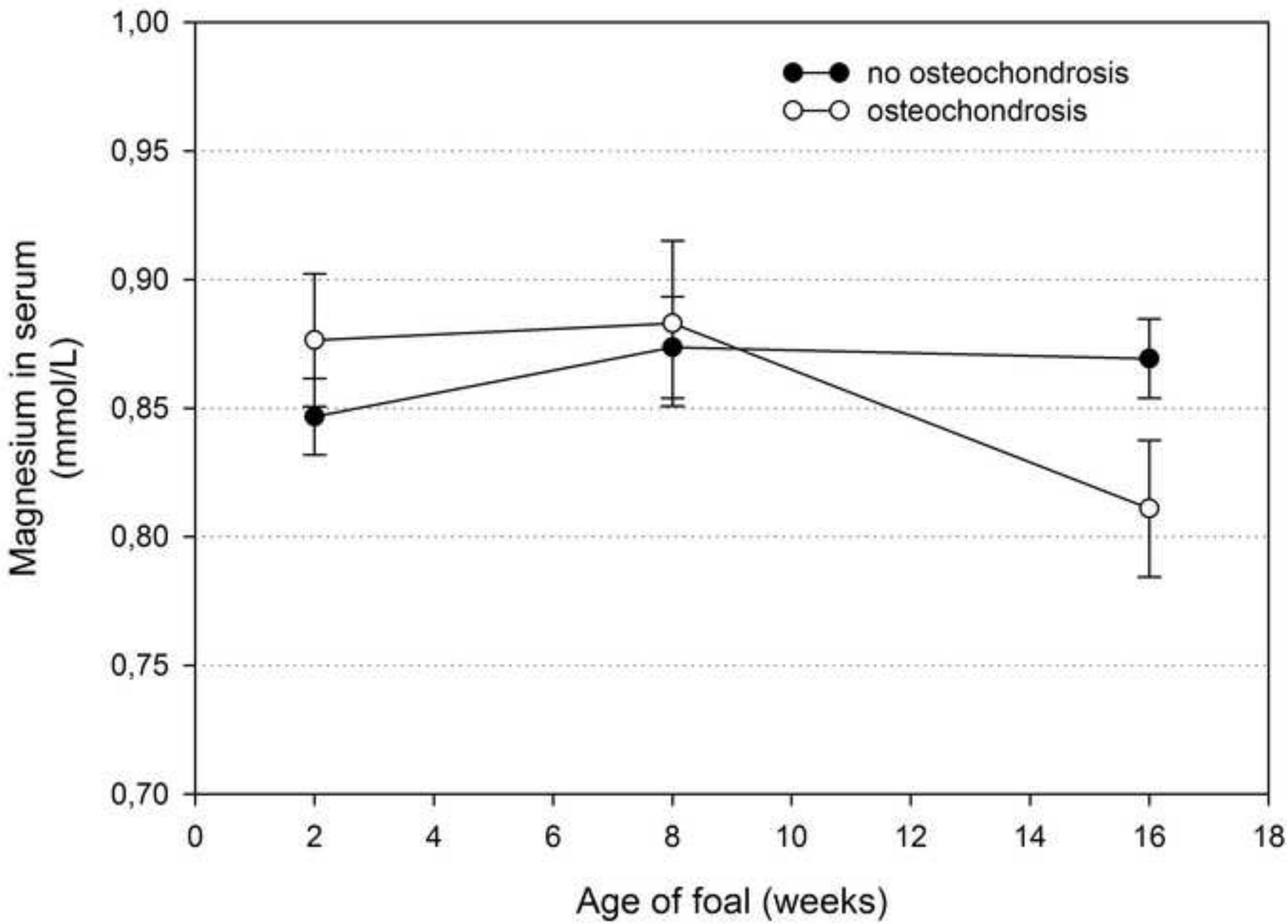

Figure 5 OC prevalence on 5 stud farms

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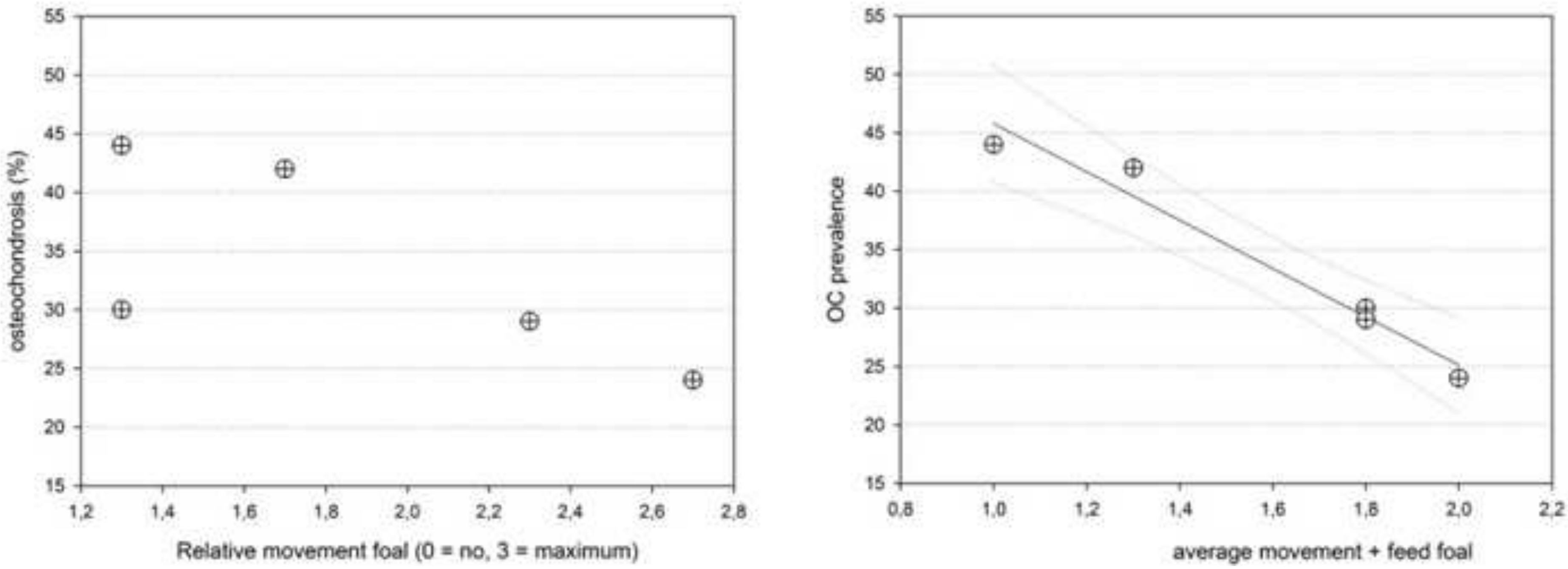

Figure 6 CTx as function of growth of foals
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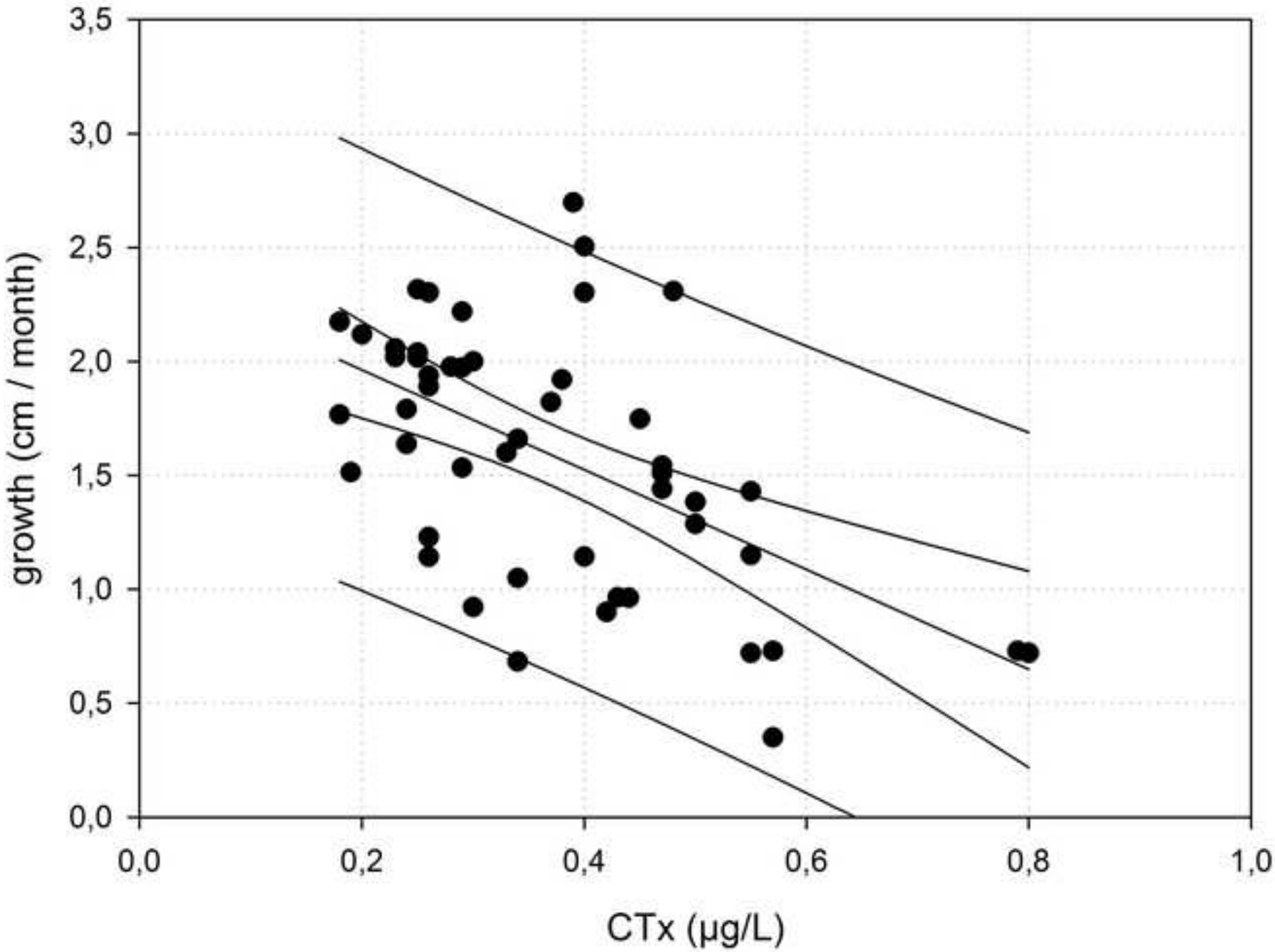

Figure 7 Blood parameters of foals (2 to 60 weeks)
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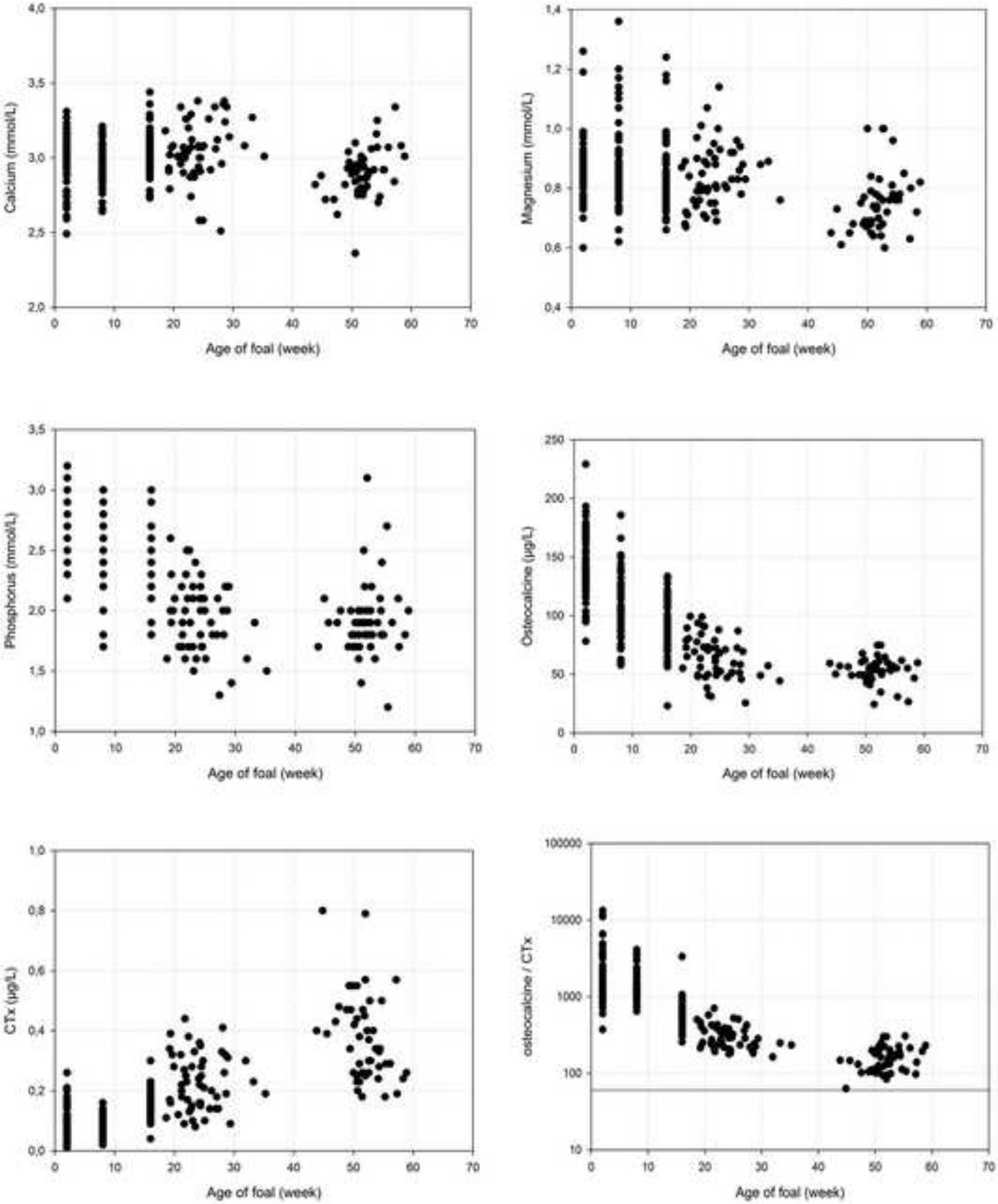

Figure 8 OC prevalence of three joints
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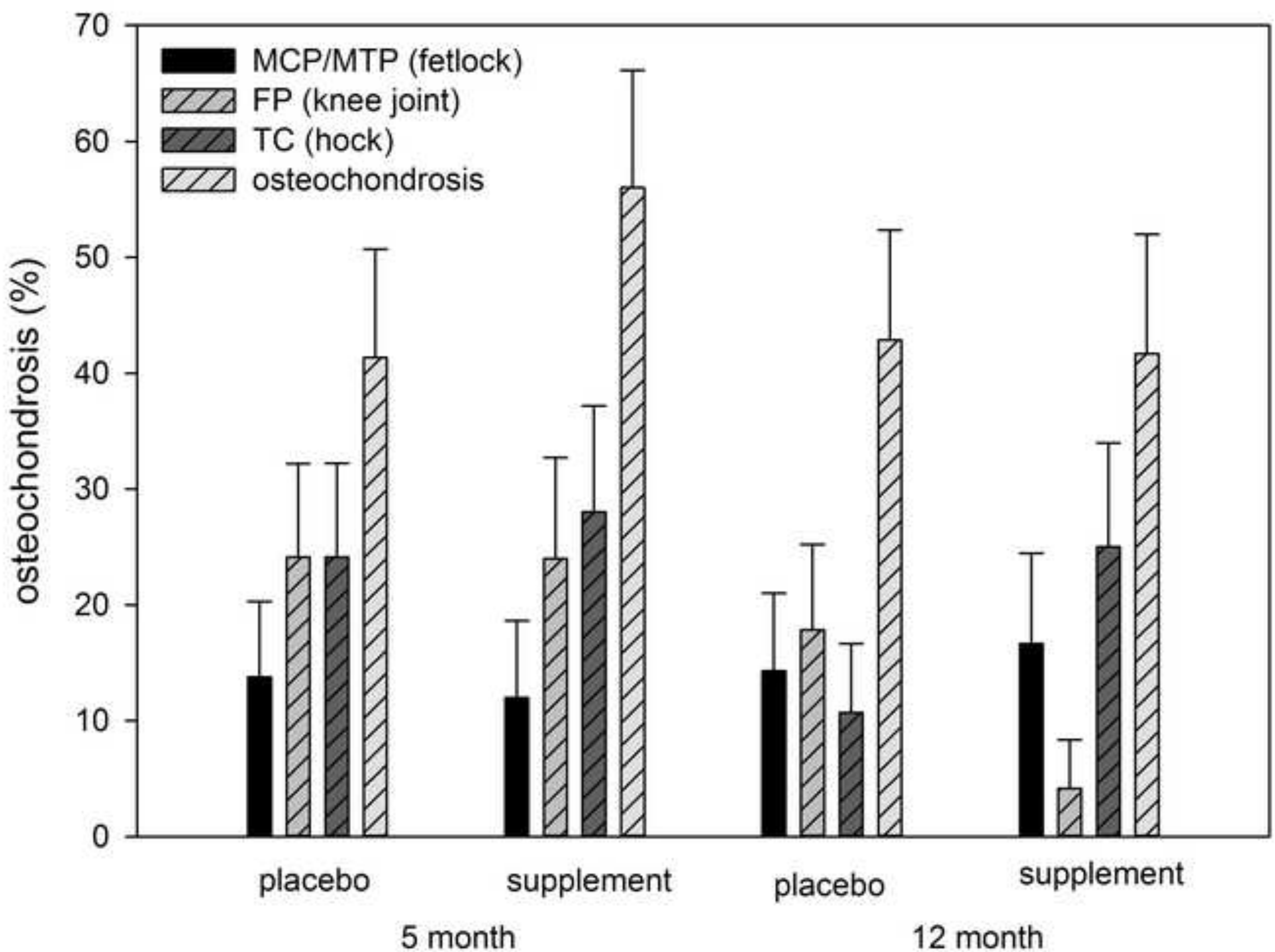

Figure 9 OC prevalence in combined studies
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