1   **Original Article**

2

3   Addition of magnesium sulphate to ropivacaine for spinal analgesia in dogs undergoing tibial

4   plateau levelling osteotomy

5

6   C.Adami,[a] D.Casoni,[b] F.Noussitou,[c] U.Rytz,[c] C.Spadavecchia[b]

7   a. Department of Clinical Sciences and Services, Royal Veterinary College, University

8   of London, Hawkshead Campus, North Mymms, AL97TA Hatfield, Herts, UK

9   b. Department of Veterinary Clinical Science, Anaesthesiology and Pain Therapy

10   Division, Vetsuisse Faculty, University of Berne, Länggassstrasse 124, CH-3012

11   Berne, Switzerland

12   c. Department of Veterinary Clinical Science, Surgery Division, Vetsuisse Faculty,

13   University of Berne, Länggassstrasse 124, CH-3012 Berne, Switzerland

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15   **Corresponding author:**

16   Chiara Adami DMV, MRCVS, DACVAA, DECVAA, RCVS Specialist in Anaesthesia,

17   EBVS® European Specialist in Veterinary Anaesthesia and Analgesia, PhD

18   Department of Clinical Sciences and Services, Royal Veterinary College, Hawkshead Lane,

19   AL97TA, Hatfield, UK

20   Email: cadami@rvc.ac.uk

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25

26 **Abstract**

27 The aim of this blinded, randomised, prospective clinical trial was to determine whether the

28 addition of magnesium sulphate to spinally-administered ropivacaine would improve peri-

29 operative analgesia without impairing motor function in dogs undergoing orthopaedic surgery.

30 Twenty client-owned dogs undergoing tibial plateau levelling osteotomy were randomly

31 assigned to one of two treatment groups: group C (control, receiving hyperbaric ropivacaine

32 by the spinal route) or group M (magnesium, receiving a hyperbaric combination of magnesium

33 sulphate and ropivacaine by the spinal route). During surgery, changes in physiological

34 variables above baseline were used to evaluate nociception. Arterial blood was collected before

35 and after spinal injection, at four time points, to monitor plasma magnesium concentrations.

36 Post-operatively, pain was assessed with a modified Sammarco pain score, a Glasgow pain

37 scale and a visual analogue scale, while motor function was evaluated with a modified Tarlov

38 scale. Assessments were performed at recovery and 1, 2 and 3 h thereafter. Fentanyl and

39 buprenorphine were administered as rescue analgesics in the intra- and post-operative periods,

40 respectively.

41

42 Plasma magnesium concentrations did not increase after spinal injection compared to baseline.

43 Group M required less intra-operative fentanyl, had lower Glasgow pain scores and

44 experienced analgesia of longer duration than group C (527.0 ± 341.0 min vs.

45 176.0 ± 109.0 min). However, in group M the motor block was significantly longer, which

46 limits the usefulness of magnesium for spinal analgesia at the investigated dose. Further

47 research is needed to determine a clinically effective dose with shorter duration of motor block

48 for magnesium used as an additive to spinal analgesic agents.

49

50 **Introduction**

51 Prevention and control of pain is one of the most important ethical obligations of veterinarians.

52 As a result, various aspects of this fascinating branch of anaesthesia have been explored, and a

53 number of novel techniques have been developed over the past decades to improve peri-

54 operative pain management. It is likely that a multimodal approach has increased efficacy and,

55 consequently, there has been particular interest in agents that, although not classified as

56 analgesics, do exert antinociceptive effects (KuKanich, 2013, Madden et al, 2014, Crociolli et

57 al, 2015; Norkus et al., 2015).

58

59 The use of magnesium has generated widespread interest as it could prevent central

60 sensitisation by acting as a non-competitive antagonist at N-methyl-D-aspartate receptors in

61 the dorsal horn, in a voltage-dependent fashion. Magnesium sulphate is commercially available

62 in Europe, and the formulation developed for parenteral use is inexpensive, stable at room

63 temperature and approved for use in dogs. Several studies in both human patients and dogs

64 suggest that magnesium sulphate exerts antinociceptive effects (Bahrenberg et al., 2015), and

65 consistently prolongs the duration of analgesia of various local anaesthetics and opioid

66 combinations when administered via either the epidural or spinal route (Buvanendran et al,

67 2002, Oezalevli et al, 2005, Arcioni et al, 2007). Additionally, a study in dogs investigating the

68 neurotoxicity of intrathecal magnesium sulphate found that a dose rate of 3 mg/kg did not cause

69 neurological deficits or histopathological changes in the spinal cord (Simpson et al., 1994).

70

71 Overall, these findings supported our hypothesis that a clinical trial investigating the effects of

72 spinally administered magnesium in client-owned dogs would be feasible and ethically

73 acceptable. The aim of this study was to compare the intensity and duration of peri-operative

74 analgesia and motor block in client-owned dogs undergoing elective orthopaedic surgery, after

75 spinal administration of either ropivacaine, or a combination of ropivacaine–magnesium

76 sulphate. Our hypothesis was that the inclusion of magnesium sulphate would provide longer

77 lasting, better quality analgesia than ropivacaine alone, without impairing neurological

78 function of the pelvic limbs and/or prolonging the duration of the motor block.

79

80 **Materials and methods**

81

82 *Animals and determination of sample size*

83 Twenty client-owned dogs undergoing elective tibial plateau levelling osteotomy (TPLO)

84 between May 2014 and March 2015 were enrolled in the study. On arrival, a pre-anaesthetic

85 physical examination was performed, as well as venous blood sampling for haematology and

86 chemistry. Exclusion criteria were an American Society of Anaesthesiologists risk category

87 higher than 2, infectious skin diseases affecting the lumbosacral area, and bleeding disorders.

88 The clinical trial was approved by the Committee for Animal Experimentation, Canton of

89 Berne, Switzerland (approval no. BE11/14, 28 April 2014), and performed with informed

90 owner consent.

91

92 *Study design*

93 The study was designed as an investigator-blinded, block-randomised, prospective clinical

94 trial. Dogs were randomly allocated to one of two treatment groups using a block

95 randomisation method, based on shuffle and drawing of treatment assignments inside an

96 opaque, sealed envelope. One operator not involved in the study was in charge of the

97 allocations list, which was disclosed only at the end of the trial.

98

99 A sample size calculation determined that 10 dogs were needed in each treatment group, to

100 achieve a power of 0.9 with an α of 0.05, to detect a minimum difference of 60 min in the mean

101 duration of analgesia (defined as the time elapsed from the spinal injection to the first

102 administration of rescue analgesics, either intra-operative fentanyl or post-operative

103 buprenorphine), between groups.

104

105 *Anaesthetic protocol and procedures*

106 After IM premedication with acepromazine (0.03 mg/kg, Prequillan, Aprovet), an

107 appropriately sized IV catheter was placed in a cephalic vein. General anaesthesia was

108 induced with IV propofol (Propofol, Fresenius Kabi) titrated to effect to enable orotracheal

109 intubation, and maintained with isoflurane (IsoFlo, Abbott) vaporised in an oxygen–air

110 mixture and delivered via a circle system. All dogs received IV lactated Ringer's solution

111 (Ringer-Lactate, Fresenius Kabi) at a rate of 10 mL/kg/h during anaesthesia. The dorsal

112 pedal artery of the non-surgical pelvic limb was catheterised to allow blood sampling and

113 continuous measurement of the systolic (SAP), mean (MAP) and diastolic (DAP) arterial

114 blood pressures. A multiparametric monitor was used to assess cardiovascular (SAP, MAP,

115 DAP, heart rate [HR]) and respiratory (end-tidal carbon dioxide, $P_{E'}CO_2$; peak inspiratory

116 pressure, PIP; respiratory rate, RR; tidal volume, TV; inspired fraction of oxygen, FiO_2; end-

117 tidal isoflurane tension, $P_{E'Iso}$) variables, as well as oesophageal temperature (T, °C). Data

118 were manually recorded every 5 min until the end of anaesthesia. The dogs were allowed to

119 breathe spontaneously unless $P_{E'}CO_2$ was >45 mmHg, in which case pressure-controlled

120 ventilation with PIP set at 10 cm H_2O was used to maintain $P_{E'}CO_2$ within the normal range.

121 A constant $P_{E'Iso}$ of 1.3%, equivalent to the minimum alveolar concentration (MAC) for the

122 species (Steffey and Mama, 2007), was targeted during anaesthesia.

123

124 Hypotension, defined as MAP lower than 60 mmHg, was treated with a crystalloid bolus

125 (10 mL/kg lactated Ringer's delivered IV over 10 min). Non-responsive hypotension was

126 treated initially with a colloid bolus (2 mL/kg tetrastarch delivered IV over 10 min), and then

127 with dopamine infusion, starting at a rate of 5 µg/kg/min. The dose was increased by

128 2.5 µg/kg/min every 10 min until MAP was above 60 mmHg.

129

130 Bradycardia, defined as HR lower than 45 beats per min (bpm), was treated with IV

131 glycopyrronium, 10 µg/kg, administered as a bolus. Any clinical signs compatible

132 with hypermagnesaemia, including cardiac bradyarrhythmias and persistent hypotension,

133 were recorded.

134

135 After tracheal extubation, carprofen (4 mg/kg) was administered IV to all dogs. Dogs were

136 discharged from the hospital 24 h after surgery.

137

138 *Spinal injection*

139 Once the plane of anaesthesia was judged as adequate on the basis of clinical assessments (jaw

140 relaxation, absence of active blinking, slight or absent palpebral reflex, immobility and

141 physiological variables within normal ranges for the species), spinal injection was performed

142 by one of two anaesthetists (C.A. or D.C.), who were blinded to the treatment. The dogs were

143 positioned in lateral recumbency with the limb to be operated on in a dependent position, with

144 both pelvic limbs pulled symmetrically cranially to maximise the length of the dorsal lumbar

145 intervertebral spaces. The iliac wings and the dorsal spinous processes of L5, L6 and L7 were

146 used as anatomical landmarks. After surgical preparation of the area, a 75 mm × 19 G spinal

147 needle was inserted towards the epidural space, with the bevel facing cranially, through the

148 interspinous ligament between L6 and L5. The stylet was then withdrawn and the needle slowly

149 advanced until cerebrospinal fluid was observed at the hub of the needle. A 'dry tap' after the

150 third attempt of needle insertion was considered an exclusion criterion.

Treatment groups

Group C (control) received ropivacaine (Naropin 1%, AstraZeneca), at a dose of 1 mg/kg

(0.1 mL/kg). Group M (magnesium) received a mixture of magnesium sulphate (2 g/10 mL,

Magnesio Solfato, Galenica Senese), at a dose of 2 mg/kg (equivalent to a volume of

0.01 mL/kg), and ropivacaine at a dose of 1 mg/kg. All treatments were administered spinally.

For both treatments, the solution for injection was made hypertonic immediately before the

injection by adding 50% glucose (Glucose 50% BBraun, 0.002 mL/kg) to the solution. The

specific gravity of the solutions, measured with a refractometer, was 1.032 and 1.035 at 25 °C

for groups C and M, respectively. The solution was injected over 1 min. Doses and volumes

were based on previous reports in both human and veterinary medicine (Oezalevli et al, 2005,

Arcioni et al, 2007, Bilir et al, 2007, Sarotti et al, 2011).

Assessment of nociception

Intra-operatively, any increase in HR, MAP and/or RR of 20% above baseline values

(defined as the values recorded before skin incision, after $P_{E'Iso}$ values of 1.3% had been

recorded consecutively for 15 min) was considered indicative of nociception. When such

increases were seen for at least two of these parameters, fentanyl (3 µg/kg, IV) was

administered as rescue analgesia.

Post-operatively, pain was assessed with a modified multifactorial pain score (Sammarco et

al, 1996, Adami et al, 2012) and the Glasgow pain scale (Holton et al., 2001). Additionally, a

10 cm visual analogue scale (VAS) with end points labelled 'worst pain imaginable' (0) and

'no pain' (10) was used. Cut-off values to administer rescue buprenorphine (Temgesic,

10 µg/kg IV) were one or more pain scores exceeding 40% of the maximum value possible

176 (>4 for the VAS, >6 for the Sammarco pain score, and >10 for the Glasgow pain scale).

177 Neurological function of the pelvic limbs and the degree of motor block were assessed with a

178 modified Tarlov scale (Table 1) (Buvanendran et al., 2002).

179

180 *Blood sampling*

181 The assessments were performed as soon as the dogs were conscious enough to respond to

182 stimulation (vocal call and incitement to sit or stand up) and then at 60, 120, 180, 240 and

183 300 min thereafter. The evaluations were performed by one of two observers (C.A. and D.C.),

184 who were blinded to the treatment. During preliminary tests, comparable pain and motor block

185 scores were determined by the two observers when independently evaluating the same dogs.

186

187 *Statistical analysis*

188 Normality of data was tested with the Kolmogorov–Smirnov test and the Shapiro–Wilk test.

189 Repeated measures ANOVA, followed by Tukey–Kramer's multiple comparison test, was used

190 for the plasma magnesium concentrations, for physiological variables and for post-operative

191 pain and Tarlov scores, with treatment (group) and time of data collection as factors.

192 Physiological variables used for statistical analysis were recorded at three predefined time

193 points: (1) before the beginning of surgical stimulation (used as baseline); (2) immediately after

194 skin incision; and (3) immediately after the beginning of tibial osteotomy.

195

196 The duration of anaesthesia and analgesia, as well as the number of intra-operative fentanyl

197 and post-operative buprenorphine boluses received by each group, was tested using either one

198 way ANOVA followed by a Bonferroni multiple comparison test, or Kruskal–Wallis ANOVA

199 on ranks followed by Dunn's test. The proportion of dogs within each group showing

200 hypotension and/or bradyarrhythmias was analysed with Fisher's exact test.

201

202 Commercially available software (NCSS-2007, SigmaStat; SigmaPlot 12, Systat Software)

203 was used. P values < 0.05 were considered statistically significant.

204

205 **Results**

206 Eleven female dogs (seven of which were neutered) and nine males (six of which were

207 neutered) were enrolled in the study. The dogs weighed 35.5 ± 22.0 kg, with a mean age of

208 12.5 ± 5.8 years. Data for age, bodyweight, intra-operative fentanyl requirement, duration of

209 anaesthesia and analgesia and plasma magnesium concentrations were normally distributed.

210 The number of cases per treatment group was equally distributed between the two observers.

211

212 Duration of anaesthesia was 267.3 ± 36.0 min (mean $\pm$ standard deviation) in group M and

213 282 ± 36.0 min in group C; this difference was not statistically significant ($P = 0.37$). Group M

214 experienced significantly longer analgesia (527.0 ± 341.0 min; Fig. 1) and required fewer intra-

215 operative fentanyl boluses (median 0, range 0–1; Fig. 2) than group C (176.0 ± 109.0 min,

216 $P = 0.015$; median 1.5, range 0–4 boluses, $P = 0.0018$; respectively).

217

218 Intra-operative physiological variables remained within normal ranges for the species and no

219 differences were detected between treatments or time points (Fig. 3). However, one dog in

220 group M and one dog in group C showed moderate sinus bradycardia (40 beats per min) and

221 arterial hypotension (MAP, 50 and 55 mmHg, respectively) shortly after the spinal injection,

222 which responded to glycopyrronium and colloid administration, respectively. None of the dogs

223 required rescue buprenorphine before the final pain assessment.

224

225   Post-operatively, there were no significant differences between groups or time points in VAS

226   (P = 0.36 and P = 0.57) and Sammarco (P = 0.17 and P = 0.16) pain scales (Fig. 4). However,

227   group M had significantly lower scores for the Glasgow pain scale (P = 0.012) and the Tarlov

228   scale (P = 0.049) compared to group C (Fig. 4). The Glasgow (P = 0.08) and the Tarlov

229   (P <0.001) scores significantly increased over time in both groups. Two dogs in group M

230   showed a persistent motor block, accompanied by loss of deep pain sensation, which lasted 24

231   and 18 h; neurological function of the pelvic limbs normalised progressively and no long-term

232   complications were observed, although these two dogs required longer hospitalisation and were

233   discharged 72 h after surgery.

234

235   Total plasma magnesium concentrations remained within physiological ranges for the species

236   (Fig. 5) and no significant differences were observed between subjects (P = 0.015) or between

237   time points (P = 0.61).

238

239   **Discussion**

240   The main finding of this study is that spinal administration of magnesium potentiates the

241   analgesia provided by ropivacaine in dogs undergoing elective orthopaedic surgery. However,

242   magnesium also prolongs the duration of motor block, which makes it a less attractive

243   adjunctive analgesic for peri-operative pain in client-owned dogs, especially in those

244   undergoing TPLO, as they are frequently large breed dogs. Persistent motor block is likely to

245   cause discomfort and to increase the costs of hospitalisation. This is in contrast with the

246   findings of most reports focusing on the neuroaxial use of magnesium in both humans and

247   canine patients, which indicated a lack of effect of magnesium on motor function when

248   administered by either epidural or spinal routes (Buvanendran et al, 2002, Yousef, Amr, 2010,

249   Shahi et al, 2014, Bahrenberg et al, 2015). Nonetheless, magnesium was found to prolong and

250   enhance brachial plexus motor block when used with lidocaine in human patients (Haghighi et

251   al., 2015).

252

253   The mechanisms by which magnesium causes motor block are unknown. Magnesium sulphate

254   is an inorganic salt which readily dissolves in water and becomes almost completely

255   dissociated across a wide pH range, from the low pH of the stomach to the neutral pH of

256   extracellular and cerebrospinal fluids1. An in vitro study on isolated mammalian dorsal root

257   ganglion neurons showed that bivalent and trivalent metal cations transiently block voltage-

258   activated calcium channel currents (Busselberg et al., 1994). The ionised magnesium released

259   by its salt could have acted so, blocking the calcium currents by altering the resting potential

260   of the neuronal membrane within the spinal cord.

261

262   Another possible explanation is that, because the magnesium sulphate solution used was

263   hyperosmolar, it might have altered the osmotic homeostasis of cerebrospinal fluid and spinal

264   cord, leading to axonal shrinking and transient neurological dysfunction. In vitro studies have

265   shown that osmotically perturbed neurons are capable of regulating their membrane

266   capacitance, structural organisation and topology, and that these changes are reversible (Wan

267   et al, 1995, Mills, Morris, 1998). Furthermore, dynamic changes in neuronal volume and

268   surface area caused by osmotic manipulation of isolated ganglia resulted in blockade of

269   transmembrane sodium channels (Mills and Morris, 1998), which is also a well-recognised

270   mechanism of action by which local anaesthetics interrupt sensory and motor transmission. For

271   most solutions, however, osmolarity and specific gravity usually change in parallel, and

272   therefore this explanation is less likely because the solution for injection in both groups had

273   very similar specific gravity.

274

275   Spinal administration of local anaesthetics has been shown to provide adequate analgesia to

276   dogs undergoing orthopaedic procedures (Sarotti et al., 2011), and is a commonly used

277   technique in clinical practice. For this reason, ropivacaine was selected for use in the positive

278   control group in this trial.

279

280   Spinal administration of magnesium did not increase total plasma magnesium concentrations

281   in the dogs enrolled in this trial. However, one limitation of our methods is that plasma

282   magnesium concentrations do not correlate with tissue concentrations, with the exception of

283   interstitial fluid and bone, nor does it reflect total body magnesium (Elin, 2010). Moreover,

284   only total magnesium, rather then the ionised, biologically active form of the ion, could be

285   measured. Another limitation is that blood was collected over a relatively short period of time;

286   more frequent sampling over a longer period, though not feasible in client-owned animals,

287   would have provided a more complete picture of magnesium uptake and distribution. However,

288   because clinical signs compatible with hypermagnesaemia were not observed, it is reasonable

289   to assume that ionised magnesium stayed within acceptable ranges for the species.

290

291   Although cardiovascular variables remained within physiologically acceptable limits, spinal

292   injection in both groups resulted in a transient decrease in heart rate and arterial blood pressure.

293   Additionally, one dog in each group experienced persistent hypotension and bradycardia,

294   which required treatment with colloids and anticholinergics. Administration of ropivacaine by

295   the spinal route might result in decreased sympathetic outflow to the cardiovascular system

296   (Levin et al., 1998). However, because the incidence of cardiovascular side effects did not

297   differ between treatments, it is unlikely that they were caused by magnesium.

298

299   **Conclusions**

300   The addition of magnesium sulphate to spinal ropivacaine increased the intensity and the

301   duration of peri-operative analgesia in dogs undergoing orthopaedic surgery, but the potential

302   for prolonged motor block could limit its utility in clinical practice. Further research might help

303   identify a dose with similar analgesic effects but with less potential for prolonged motor block.

304

305   **Acknowledgements**

308

309   **Conflict of interest**

310   None of the authors have financial or personal relationships with individuals or organisations

311   that could inappropriately influence or bias the content of the paper.

312

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387

388   Figure legends:

389   Fig. 1. Duration of analgesia (min) in dogs receiving a spinal injection of either ropivacaine
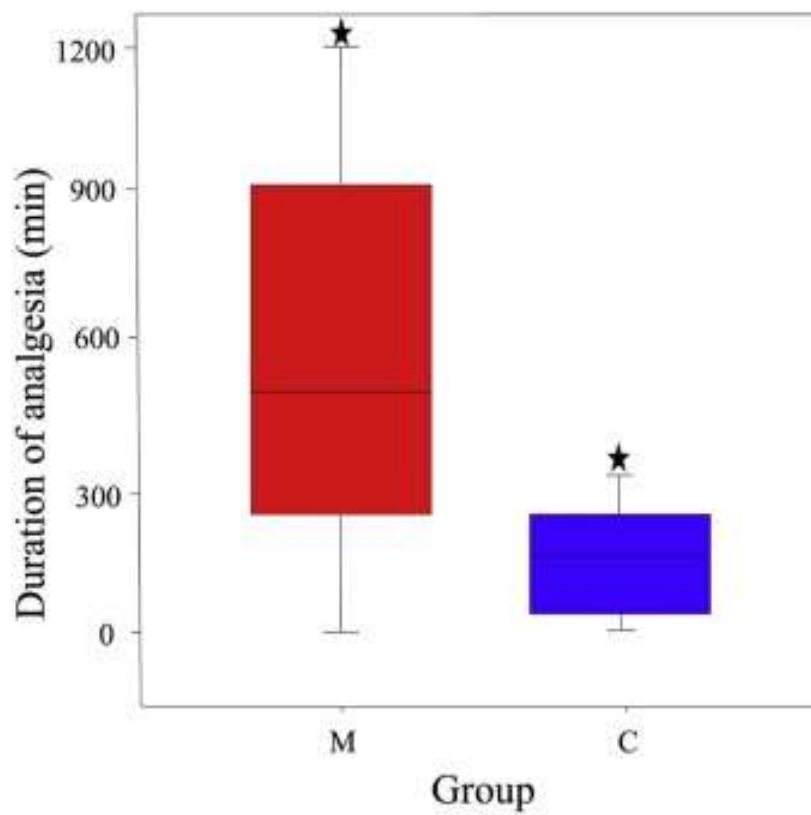
390   alone (group C, $n = 10$) or a combination of ropivacaine and magnesium (group M, $n = 10$).

391   The asterisk indicates a statistically significant difference between treatments ($P < 0.05$). The

392   lines indicate median values. The upper and lower boxes indicate the 75% and 25% of the

393   values which fall below the upper and lower quartiles, respectively. The upper and lower

394   whiskers indicate the maximum and minimum values.


395

396

397   Fig. 2. Number of intra-operative fentanyl boluses (3 µg/kg each bolus) administered to 20

398   dogs receiving a spinal injection of either ropivacaine alone (group C, $n = 10$) or a combination

399   of ropivacaine and magnesium (group M, $n = 10$). The lines indicate median values. The upper

400   and lower boxes indicate the 75% and 25% of the values which fall below the upper and lower

401   quartiles, respectively. The upper and lower whiskers indicate the maximum and minimum

402   values. The dots indicate the outliers. The asterisks indicate a statistically significant difference

403   between treatments ($P < 0.05$).
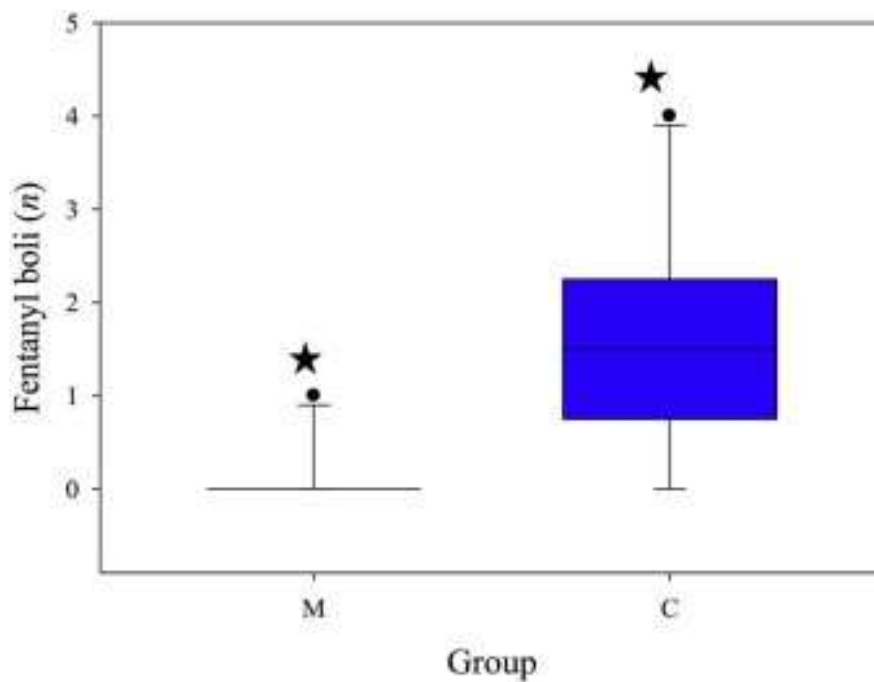
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405

406   Fig. 3. Values for heart rate (HR, beats per min [bpm]) and mean arterial pressure (MAP,

407   expressed in mmHg) for 20 dogs receiving a spinal injection of either ropivacaine alone (group

408   C, n = 10) or a combination of ropivacaine and magnesium (group M, n = 10). Data were

409   recorded at three different time points: (1) before the beginning of surgical stimulation (used

410   as baseline); (2) immediately after skin incision; and (3) immediately after the beginning of

411   tibial osteotomy. The lines indicate median values. The upper and lower boxes indicate the

412   75% and 25% of the values which fall below the upper and lower quartiles, respectively. The

413   upper and lower whiskers indicate the maximum and minimum values.


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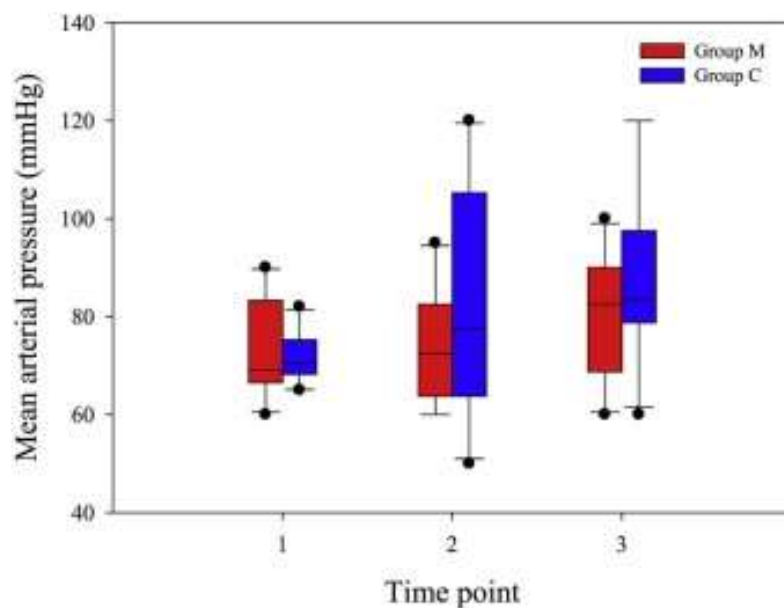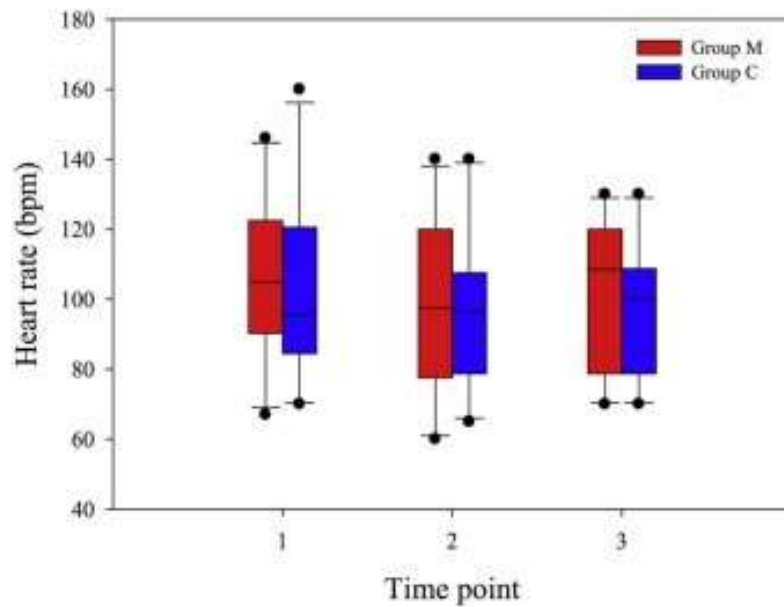
415   Fig. 4. Values for Sammarco pain score, Glasgow pain scale, Visual Analogue Scale (VAS)

416   and Tarlov scale for 20 dogs receiving a spinal injection of either ropivacaine alone (group C,

417   n = 10) or a combination of ropivacaine and magnesium (group M, n = 10). Data were recorded

418   at four different time points: at recovery, as soon as the dogs were conscious enough to be

419   examined (time point 1), and then 1, 2 and 3 h after that (time points 2, 3 and 4, respectively).

420   The lines indicate median values. The upper and lower boxes indicate the 75% and 25% of the

421   values which fall below the upper and lower quartiles, respectively. The upper and lower

422   whiskers indicate the maximum and minimum values. The asterisks and the daggers indicate

423   statistically significant differences between treatments and between time points, respectively

424   (P <0.05).
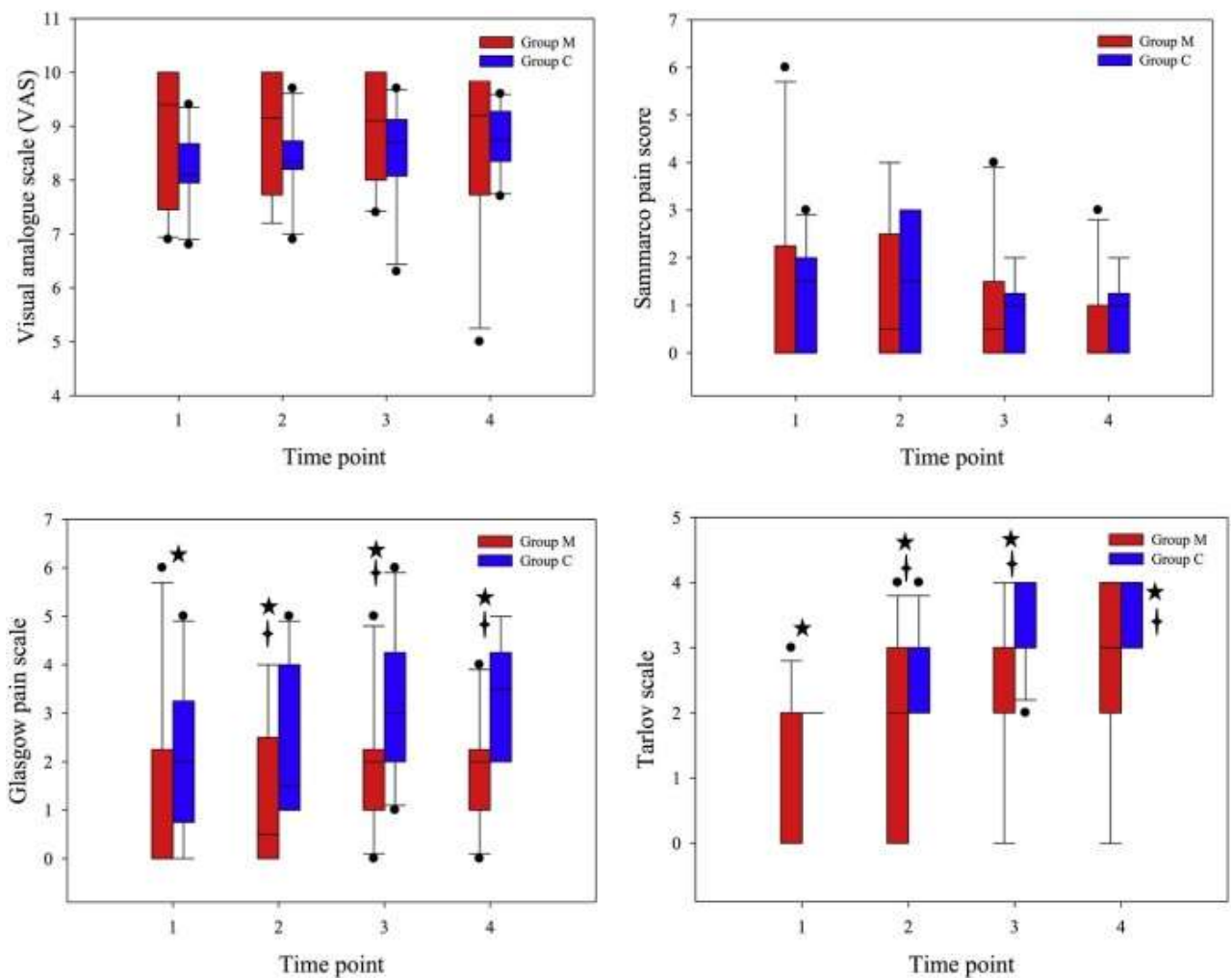
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426

427   Fig. 5. Mean values (±standard deviations) of total plasma magnesium concentrations

428   (mmol/L) for 20 dogs receiving a spinal injection of either ropivacaine alone (group C, n = 10)

429   or a combination of ropivacaine and magnesium (group M, n = 10). Blood was sampled at the

430   following time points: before spinal injection (0, baseline), and then at 15, 60, 120 and 240 min

431   thereafter.
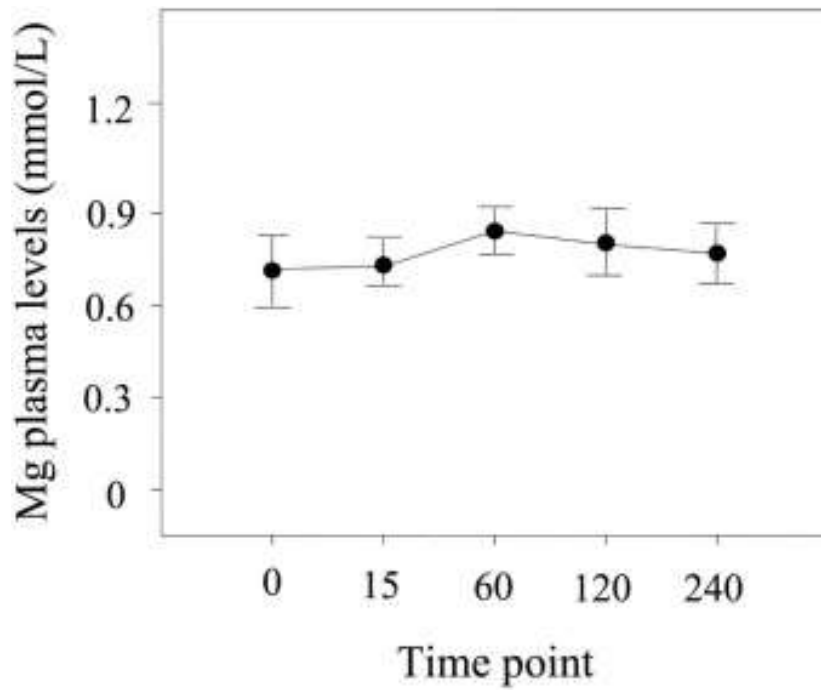
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434   Table 1. Tarlov's scale (modified from Buvanendran et al., 2002) used for neurological

435   assessment.

Grade	Description
Grade 0	Flaccid paraplegia, no movements of the pelvic limbs, possible loss of bowel/urinary bladder control
Grade 1	Spastic paraplegia with moderate or vigorous purposeless movements of the pelvic limbs. No sitting, unable to walk
Grade 2	Good movements of the pelvic limbs but unable to stand
Grade 3	Able to stand but unable to walk normally, hips and pelvic limbs obviously unstable, moderate to severe ataxia
Grade 4	Able to stand and walk normally, some muscle weakness of the pelvic limbs may be seen

436