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RESEARCH PAPER

Combination of magnesium sulphate and ropivacaine epidural analgesia for hip arthroplasty in dogs.

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Running head: Epidural magnesium in dogs

Acknowledgements

Authors' contributions

1 **Abstract**

2 **Objective** The aim of this study was to determine whether lumbosacral epidural
3 administration of magnesium sulphate added to ropivacaine prolongs and improves
4 perioperative analgesia, without adverse effects on motor block duration or hind limb
5 neurological function, in dogs undergoing hip arthroplasty.

6 **Study design** Investigator-blind, controlled, randomized, prospective clinical trial.

7 **Animals** Twenty client-owned dogs undergoing hip arthroplasty were allocated
8 randomly to either group C (control, 1 mg kg⁻¹ epidural ropivacaine) or group M
9 (magnesium, epidural injection of 1 mg kg⁻¹ ropivacaine and 2 mg kg⁻¹ magnesium
10 sulphate).

11 **Methods** All dogs were premedicated with intramuscular acepromazine. General
12 anaesthesia was induced with propofol, and maintained with isoflurane in oxygen.
13 Intraoperatively, nociception was assessed based on changes in heart rate, respiratory
14 rate, and mean arterial pressure above baseline values. Postoperatively, pain was
15 evaluated with a Sammarco pain score, a Glasgow pain scale and a visual analogue
16 scale. The Tarlov's scale was used to quantify motor block. All dogs were evaluated at
17 recovery and then 1, 2, 3, 4, 5 and 24 hours after that. Rescue analgesia was provided
18 during surgery with fentanyl and, postoperatively, with buprenorphine. Groups were
19 compared using one way repeated measures analysis of variance followed by Holm-
20 Sidak method for multiple comparison, or non-parametric tests when appropriate.

21 **Results** The two treatment groups did not differ ($p > 0.05$) with respect to intraoperative
22 physiological variables, rescue analgesia, postoperative pain scores (Sammarco $q =$

23 1.00; Glasgow q = 3.10; VAS q = 0.50) and duration of the motor block (Tarlov's q =
24 2.40).

25 **Conclusions and clinical relevance** The addition of epidural magnesium to ropivacaine
26 did not improve or prolong the analgesia provided by ropivacaine alone. Further studies
27 are needed to determine whether an epidural magnesium dose higher than 2 mg kg⁻¹
28 would exert better analgesia, without causing adverse effects, in dogs undergoing
29 orthopaedic surgery.

30 **Keywords** dog, magnesium sulphate; neuroaxial anaesthesia, perioperative analgesia,
31 ropivacaine

32

33 **Introduction**

34 Total hip replacement is an innovative and invasive surgery used in dogs to treat hip
35 dysplasia and other pathological conditions affecting the coxofemoral joint.

36 Providing adequate perioperative analgesia during invasive orthopaedic procedures not
37 only is an ethical obligation for the veterinarian, but also plays a crucial role in the
38 outcome of the surgery itself (Conzemius et al. 2005). Indeed, effective prevention and
39 treatment of pain has been shown to significantly improve dogs' attitude, as well as
40 limb's use and function in dogs undergoing major orthopaedic surgery (Conzemius et
41 al. 2005).

42

43 As an alternative to systemic analgesia, loco-regional anaesthetic techniques offer the
44 advantage of a selective and targeted block of the anatomical area of interest. Among
45 neuroaxial techniques, epidural administration of analgesics is traditionally regarded as
46 safer and easier to perform than the spinal route. Owing to its popularity, practicality
47 and ease of performance, single epidural injection is usually preferred to constant rate
48 infusion of analgesics via this route, which can only be accomplished after insertion of
49 an epidural catheter. Placing an epidural catheter is a time-consuming procedure, which
50 requires a certain degree of expertise and carries the risk of complications (Ladha et al.
51 2013; Pumberger et al. 2013). Nevertheless, single epidural injections may provide
52 analgesia of insufficient duration when invasive and potentially long surgeries are
53 performed.

54

55 Within the last twenty years, there has been an increasing interest in the multimodal
56 approach to pain management in veterinary patients, especially with respect to the use
57 of agents which, despite not being listed among classical analgesics, exert
58 antinociceptive effects (Kukanich 2013; Madden et al. 2014; Crociolli et al. 2015;
59 Norkus et al. 2015). Among these, magnesium plays a central role in the prevention of
60 central sensitization by blocking the dorsal horn N-methyl-D-aspartate (NMDA)
61 receptors in a non-competitive, voltage dependent fashion. Magnesium sulphate is
62 inexpensive, and available in Europe as a formulation that is stable at room temperature
63 and approved for parenteral administration in dogs. The potential for neurotoxicity
64 when magnesium is administered intrathecally was investigated in dogs, and
65 neurological impairment and histopathological lesions of the spinal cord were not found
66 after a dose of 3 mg kg⁻¹ (Simpson et al. 1994). The studies investigating the clinical
67 role of magnesium as adjuvant in pain therapy show conflicting results. Intravenous
68 magnesium failed to improve perioperative pain in both humans and dogs (Rioja et al.
69 2012; Murphy et al. 2013). Conversely, several clinical trials showed that magnesium
70 effectively improves analgesia in human patients receiving combinations of local
71 anaesthetics and opioids, by either epidural or spinal route (Buvanendran et al. 2002;
72 Oezalevli et al. 2005; Arcioni et al. 2007). The antinociceptive effects of epidural
73 magnesium were demonstrated experimentally in dogs (Bahrenberg et al. 2015),
74 however there is a paucity of data regarding the clinical use of magnesium in this
75 species. A clinical trial suggests that adding spinal magnesium to ropivacaine increases
76 the duration and the intensity of analgesia, but also of the motor block, provided by
77 ropivacaine alone in dogs undergoing orthopaedic surgery (Adami et al. 2016).

78

79 The aim of this study was to determine whether the addition of magnesium sulphate to
80 epidural ropivacaine would result in better perioperative analgesia, defined as longer
81 duration and decreased rescue analgesia requirement, than ropivacaine alone, in client-
82 owned dogs anaesthetised for elective hip arthroplasty.

83 Our hypothesis was that the addition of magnesium to ropivacaine would improve
84 perioperative analgesia, without prolonging the motor block or causing neurological
85 dysfunction of the hind limbs.

86

87 **Materials and methods**

88 This clinical study was designed as an investigator-blind, controlled, randomized,
89 prospective trial.

90 Twenty client-owned dogs scheduled for hip arthroplasty between March 2014 and
91 February 2016 were recruited for this study. The number of dogs was determined based
92 on a sample size calculation. Each group was to be composed of a minimum of 10 dogs
93 to detect, with one-way analysis of variance (with power equal to 0.95 level of
94 confidence and α value and standard deviation set at 0.05 and 40 minutes, respectively),
95 a difference between groups in the mean duration of analgesia (defined as the time from
96 the epidural injection to the administration of the first dose of rescue analgesic agent)
97 equal to at least 60 minutes.

98 Inclusion criteria were American Society of Anaesthesiologists (ASA) risk category
99 lower than III and absence of skin infections at the level of the lumbosacral area. All
100 dogs underwent a preanaesthetic physical examination and a complete blood test,
101 including haematology and biochemistry, to rule out abnormalities. The permission of

102 the Ethical Committee of the Veterinary Teaching Hospital of the University of Turin
103 (Italy), as well as a written consent signed by the dogs' owners, was obtained prior to
104 enrolment.

105 All dogs were premedicated with intramuscular (IM) acepromazine (0.03 mg kg^{-1} ,
106 Prequillan; Fatro, Italy). Thereafter, intravenous (IV) propofol (Vetofol; Esteve, Spain)
107 was titrated to effect to induce general anaesthesia. After orotracheal intubation,
108 isoflurane (Isoflo; Esteve, Spain) was delivered in oxygen via a circle system and
109 lactated Ringer's solution was perfused IV ($10 \text{ mL kg}^{-1} \text{ hr}^{-1}$, Ringer Lattato; Fresenius
110 Kabi, Italy). Arterial blood pressure [systolic (SAP), mean (MAP) and diastolic (DAP)]
111 was measured continuously through an indwelling catheter placed in the dorsal pedal
112 artery. Monitoring during anaesthesia included both cardiovascular [SAP, MAP, DAP,
113 heart rate (HR) and rhythm) and respiratory [end tidal carbon dioxide ($P_{E'}\text{CO}_2$), peak
114 inspiratory pressure (PIP), respiratory rate (f_R), tidal volume (V_T), minute volume (V_E),
115 inspired fraction of oxygen (FIO_2), end tidal isoflurane tension ($P_{E'}\text{ISO}$)] parameters, as
116 well as oesophageal temperature (T° , C). Manual data recording was performed every 5
117 minutes for the entire duration of anaesthesia. Spontaneous breathing was preferred
118 unless $P_{E'}\text{CO}_2$ reached more than 45 mmHg (5.9 kPa) when mechanical ventilation was
119 used to maintain normocapnia. The target $P_{E'}\text{ISO}$ was 1.3%, which is equal to the
120 Minimum Alveolar Concentration (MAC) as determined in dogs (Valverde et al. 2003).
121 As soon as the anaesthesia plane was deemed surgical based on classical clinical
122 parameters (relaxation of the jaw, absence of blinking and movements, light palpebral
123 reflex and normal canine physiological parameters) the anaesthetist (EL), who was
124 unaware of the epidural treatment, performed all the epidural injections.

125 The dogs were positioned in sternal recumbency with the hind limbs cranial to
126 maximize the dorsal lumbosacral space. The ilium wings, together with the sacrum and
127 the dorsal spinous processes of L6 and L7, were used as anatomical landmarks. After
128 surgical preparation of the area, a 75 mm, 19 gauge spinal needle (BD Needles; Becton
129 Dickinson, Spain) was inserted percutaneously between L7 and S1, with the bevel
130 facing cranial, and then advanced through the intervertebral ligament into the epidural
131 space. Both the “popping” sensation, perceived while penetrating the interarcuate
132 ligament, and the hanging drop technique with saline were used as a first assessment of
133 proper needle placement. Radiographic exam followed to confirm correct positioning of
134 the needle between L7 and S1. A horizontal beam was used to maintain positioning in
135 sternal recumbency during injection.

136 A block randomization method was used to allocate the dogs into one of two epidural
137 treatment groups. Briefly, an operator not participating to the assessments was in charge
138 of keeping an opaque, sealed envelope from which treatment assignments were shuffled
139 and drawn. This same operator was also responsible for the list of allocations until the
140 end of data collection.

141 Epidural ropivacaine (Naropina 0.5%; AstraZeneca, Italy), 1 mg kg^{-1} (volume: 0.2 mL
142 kg^{-1}), was administered epidurally to group C (Control), while group M (Magnesium)
143 was treated with ropivacaine (1 mg kg^{-1} ; volume: 0.2 mL kg^{-1}) and magnesium sulfate
144 (Magnesio Solfato $2 \text{ g } 10 \text{ mL}^{-1}$; Galenica Senese, Italy) at the dose of 2 mg kg^{-1}
145 (volume: 0.01 mL kg^{-1}). The drugs were mixed in the same syringe and administered as a
146 single bolus over 1 minute. Doses were chosen based on the authors’ past clinical
147 experience, and human and veterinary medical literature (Arcioni et al. 2007; Bilir et al.

148 2007; Oezalevli et al. 2005). After the epidural injection was performed, the dogs
149 remained in sternal recumbency for 5 minutes.

150 A bolus of IV atropine (0.01 mg kg^{-1} , Atropina Solfato; ATI, Italy) was injected in the
151 event of bradycardia ($<45 \text{ beats minute}^{-1}$). Treatment of hypotension (MAP <60
152 mmHg) consisted of an IV bolus of lactated Ringer's solution (10 mL kg^{-1} over 10
153 minutes), followed by an IV colloid bolus (Voluven; Fresenius Kabi, Italy; 2 mL kg^{-1}
154 over 10 minutes), and then by an IV infusion of dopamine (Revivan; AstraZeneca, Italy;
155 starting at $10 \mu\text{g kg}^{-1} \text{ minute}^{-1}$, increased in increments of $2.5 \mu\text{g kg}^{-1} \text{ minute}^{-1}$ every 10
156 minutes until MAP increased above 60 mmHg) in the event of unresponsive
157 hypotension. Bradyarrhythmias and hypotension occurring shortly after the epidural
158 injection were regarded as clinical symptoms compatible with either sympathetic nerve
159 blockade or hypermagnesaemia, and their occurrence was recorded.

160 Intraoperative nociception was defined as an increase in HR, MAP and/or f_R of at least
161 20% compared to the baseline (recorded before skin incision, after PE₂ ISO had been
162 maintained constant at 1.3% for at least three consecutive measurements, over 15
163 minutes). When two of these three parameters increased above the defined values,
164 rescue fentanyl (Fentanest; Pfizer, Italy) was administered IV (0.003 mg kg^{-1}).

165 The duration of surgery and of anaesthesia (minutes) were recorded. The time elapsed
166 from termination of inhalational anaesthesia to recovery in intensive care unit (minutes)
167 was defined as "time to recovery", and recorded. The trachea was extubated after return
168 of swallowing and palpebral reflexes, accompanied by increased jaw tone. At this point,
169 all dogs were administered with IV carprofen (4 mg kg^{-1} , Rimadyl; Pfizer, Italy).

170 Postoperatively, a multifactorial pain score modified from Sammarco ranging from 0)
171 no pain to 13) extreme pain (Appendix 1; Sammarco et al. 1996; Adami et al. 2012) and
172 the short form of the Glasgow pain scale ranging from 0) no pain to 20) extreme pain
173 (Holton et al. 2001) were used to evaluate pain. Additionally, a 10 cm visual analogue
174 scale (VAS) with end points labelled 0) worst possible pain to 10) absence of pain was
175 utilized. Rescue analgesia consisted of 0.01 mg kg⁻¹ buprenorphine IV (Temgesic;
176 Schering Plough, UK), administered when at least one pain score was 40% or more of
177 the maximum value of the scale (<6 for the VAS, >5 for the multifactorial pain score
178 scale, >8 for the Glasgow pain scale). A modified Tarlov's scale (Appendix 2) ranging
179 from 0) neurological impairment to 4) no signs of motor block (Buvanendran et al.
180 2002; Adami et al. 2016) was used for neurological assessment of the hind limbs and
181 quantification of motor blockade. The same observer (EL), who was unaware of the
182 treatment, performed all the evaluations. All dogs were evaluated when deemed awake
183 enough to respond to vocal call and incitement to sit or stand up, and then 60, 120, 180,
184 240, 300 minutes and 24 hours after the end of surgery and before being discharged
185 from the hospital.

186 Statistical analysis was accomplished with commercially available software (SigmaStat
187 and SigmaPlot 12, Systat Software Inc.). Normality of data distribution was assessed
188 with the Kolmogorov-Smirnov test and with the Shapiro-Wilk test. Continuous
189 variables were analysed with either one way repeated measures analysis of variance
190 followed by Holm-Sidak method for multiple comparison, or Friedman repeated
191 measures analysis of variance on ranks followed by Tukey test, where it applied. For the
192 analysis of intraoperative cardiovascular and respiratory variables, only the values

193 recorded during three significant events were used: 0) before surgery (baseline as above
194 described), 1) 30 seconds after skin incision and 2) during femoral head osteotomy.

195 For non-continuous variables, either a T-test or Mann Whitney Rank Sum test were
196 used. Within each treatment group, the proportions of dogs which experienced
197 hypotension and bradyarrhythmias following epidural injection of magnesium were
198 analysed with the Fisher exact test. P values < 0.05 and q values < 2 were considered
199 statistically significant.

200

201

202 **Results**

203 Data are presented as either mean \pm standard deviation or median (range). Twenty dogs
204 (12 female and 8 male) of various breeds, aged 12 (9-144) months completed this study.

205 Heart rate, MAP, time to recovery and duration of anaesthesia were normally
206 distributed. Anaesthesia was uneventful in all dogs enrolled in the study and lasted
207 \pm 62 minutes in group M and 220 ± 32 minutes in group C, respectively; this difference
208 was not statistically significant. The treatment groups were not statistically different to
209 each other with respect to intraoperative physiological variables. However, HR

210 decreased over time in the control group while MAP increased in both groups (Fig. 1).

211 Respiratory rate increased over time in group M while it decreased in group C (Fig. 1).

212 Cardiovascular events compatible with hypermagnesaemia, namely bradyarrhythmias
213 and hypotension, were not observed during the anaesthetics. Three dogs of group M [0
214 (0-1)] and 4 of group C [0 (0-2)] required boluses of rescue fentanyl during surgery.

215 This difference was not statistically significant. There was no difference in the duration

216 of surgery, which lasted 120 (90-150) and 125 (100-150) minutes in groups M and C,
217 respectively, was detected between groups. Only one dog, assigned to group C, required
218 rescue buprenorphine before completion of pain assessments according to both the
219 Sammarco and VAS scores (7 and 6.8, respectively).

220 There was no significant difference between groups C and M in the VAS, Sammarco,
221 Glasgow and Tarlov's scores. In both groups, the Sammarco, the Glasgow and the
222 Tarlov's scores significantly increased over time, while VAS decreased (Fig. 2).

223 Recovery was smooth and normal motor function of the hind limbs was observed within
224 6 hours of the epidural injection in all dogs. Perianaesthetic complications were not
225 observed.

226

227 **Discussion**

228 This study failed to demonstrate that the addition of magnesium to epidural ropivacaine
229 provides superior perioperative analgesia, in terms of both duration and quality, than
230 ropivacaine alone in dogs undergoing total hip replacement. The duration of the motor
231 block was also comparable between the two groups, and the administration of
232 magnesium was not associated with neurological dysfunction of the hind limbs.

233

234 These findings were unexpected and not consistent with those of a previous study,
235 which found that the addition of spinal magnesium to ropivacaine potentiated the
236 intensity and the duration of analgesia in dogs after tibial plateau levelling osteotomy
237 (Adami et al. 2016), but also prolonged the duration of the motor block.

238 Possible explanations for this discrepancy are less effective analgesia when magnesium
239 is administered epidurally compared to the spinal route or, alternatively, a failure in the
240 methods used in the current study to detect a difference between treatments.

241 Besides the possibility of a direct analgesic effect of magnesium on the dorsal horn
242 NMDA receptors, Adami and colleagues (2016) hypothesized that the ionized
243 magnesium released by its salt may exert antinociception also by blocking the calcium
244 channels, which in turn could alter the resting potential of the neuronal membranes.
245 Alternatively, as a hyperosmolar salt, magnesium sulfate might cause osmotic
246 interference with the cerebrospinal fluid and spinal cord, leading to neuronal shrinking
247 and transient neurologic dysfunction (Busselberg et al. 1994). However, this hypothesis
248 could not be tested because the actual osmolality of the solution to be injected could not
249 be measured. Moreover, both mechanisms are more likely to occur when magnesium is
250 injected spinally rather than epidurally because we suspect that a higher concentration is
251 achieved in the cerebrospinal fluid when the dose is injected spinally.

252 Another reasonable explanation is that the epidural route of administration requires a
253 higher magnesium dose than the spinal one in order to detect appreciable analgesia.
254 Owing to ethical obligations, and not to cause any harm to client-owned dogs, it was
255 decided to use 2 mg kg^{-1} magnesium. This dose was proven to be safe in terms of risks
256 of direct neurotoxicity (Simpson et al. 1994) and hypermagnesaemia (Adami et al.
257 2016). Nonetheless, it cannot be excluded that a higher magnesium sulfate dose might
258 have resulted in more pronounced clinical effects.

259

260 Pain assessment in non-verbal patients can be extraordinarily challenging even for
261 experienced observers, especially when subjective indicators, namely behavioural signs
262 of pain, are evaluated (Conzemius et al. 1997; Reid et al. 2007). The choice of having
263 one single investigator in charge of all the assessments, as well as of using several pain
264 scales instead of one, should have helped overcome some potential intrinsic limitations,
265 namely the interobserver variability and the poor sensitivity and specificity of the scales
266 used to evaluate pain.

267 Lower pain score intervention levels might have resulted in detectable differences in
268 postoperative analgesia between treatments. For consistency, it was decided to use the
269 same cut-off value for all pain scales, which was set at 40% of the maximum possible
270 score. Similar cut-off values of the VAS have been previously used in dogs, as well as
271 in other animal species, to guide the administration of rescue analgesics during the
272 postoperative period (Adami et al. 2011; Adami et al. 2012). Moreover, it has been
273 suggested that, in human patients, 40% of the VAS scale may represent the limit
274 between mild and moderate pain (Serlin et al. 1995; Bodian et al. 2001;).

275 Another potential limitation of this study is the absence of irrefutable proof that the
276 needle had been correctly placed within the epidural space in all dogs. Although the
277 hanging drop technique was used to guide the needle's insertion, and radiography to
278 verify the needle's position within the targeted intervertebral space, only epidurography,
279 accomplished with the injection of a contrast medium, would have inarguably
280 confirmed that the tip of the needle had reached the adequate depth. Due to ethical
281 considerations for the client-owned dogs, the use of invasive or potentially harmful
282 techniques for this purpose was not considered. Failure to identify the exact injection
283 site could have distorted the results; however, the little or no postoperative rescue

284 analgesia requirement, together with the detection of motor blockade in all dogs at
285 recovery, suggests that the epidural injections were correctly performed.

286 Assuming that all the injections had been performed within the epidural space, an
287 alternative possible explanation for the lack of differences between the two treatments is
288 that ropivacaine alone, at the dose and concentration used in the current study, might
289 already be adequate as analgesic treatment for hip replacement. Moreover, carprofen
290 was administered to all dogs in recovery, which could have contributed to postoperative
291 analgesia and made the detection of differences between groups even more challenging.
292 In this scenario, detecting an appreciable difference would be more challenging and
293 possibly require a larger sample size. Unfortunately, the use of a suboptimal analgesic
294 treatment, namely a subclinical ropivacaine dose or even epidural saline, would have
295 raised some ethical concerns.

296

297 Serum magnesium concentrations were not measured. Although mild increases in
298 ionised magnesium concentration might have gone undetected, it is reasonable to
299 assume that a clinically relevant hypermagnesaemia would have been accompanied by
300 cardiac arrhythmias and, possibly, persistent hypotension, none of which were observed
301 in this study population. Moreover, one study found that 2.5 mg kg^{-1} of epidural
302 magnesium did not result in clinical signs of hypermagnesaemia in dogs (Bahrenberg et
303 al. 2015).

304

305 **Conclusions**

306 In conclusion, the addition of 2 mg kg⁻¹ magnesium sulphate to epidural ropivacaine did
307 not result in considerable improvement of quality and duration of perioperative
308 analgesia, nor did it prolong the motor block. Further trials are needed to determine
309 whether a higher dose of magnesium administered via the epidural route would increase
310 the analgesic effect in dogs undergoing orthopaedic surgery.

311

312 **Conflict of interest statement**

313 None of the authors have financial or personal relationships with individuals or
314 organisations that could inappropriately influence or bias the content of the paper.

315

316 **References**

317 Adami C, Bergadano A, Bruckmaier RM et al. (2011) Sciatic-femoral nerve block with
318 bupivacaine in goats undergoing elective stifle arthrotomy. *Vet J* 188, 53- 57.

319 Adami C, Casoni D, Spadavecchia C et al. (2016) Addition of magnesium sulphate to
320 ropivacaine for spinal analgesia in dogs undergoing tibial plateau levelling osteotomy.
321 *Vet J* 209, 163-168.

322 Adami C, Veres-Nyéki K, Bergadano A et al. (2012) Evaluaton of peri-operative
323 epidural analgesia with ropivacaine, ropivacaine and sufentanil, and ropivacaine,
324 sufentanil and epinephrine in isoflurane anesthetized dogs undergoing tibial plateau
325 levelling osteotomy. *Vet J* 194, 229-234.

326 Arcioni R, Palmisani S, Tigano S (2007) Combined intrathecal and epidural magnesium
327 sulfate supplementation of spinal anesthesia to reduce post-operative analgesic

- 328 requirements: A prospective, randomized, double-blind, controlled trial in patients
329 undergoing major orthopaedic surgery. *Acta Anaesthesiol Scand* 51, 482-489.
- 330 Bahrenberg A, Dziki BT, Rioja E et al. (2015) Antinociceptive effects of epidural
331 magnesium sulphate alone and in combination with morphine in dogs. *Vet Anaesth*
332 *Analg* 42, 319-328.
- 333 Bilir A, Gulec S, Ozcelik A et al. (2007) Epidural magnesium reduces postoperative
334 analgesic requirement. *Br J Anaesth* 98, 519-523.
- 335 Bodian CA, Freedman G, Hossain S et al. (2001) The Visual Analog Scale for Pain:
336 Clinical Significance in Postoperative Patients. *Anesthesiology* 95, 1356-1361.
- 337
338 Busselberg D, Pekel M, Platt B et al. (1994) Mercury (Hg²⁺) and zinc (Zn²⁺): two
339 divalent cations with different actions on voltage-activated calcium channel currents.
340 *Cell Mol Neurobiol* 14, 675-687.
- 341 Buvanendran A, McCarthy R, Tuman KJ et al. (2002) Intrathecal magnesium prolongs
342 fentanyl analgesia: A prospective, randomized, controlled trial. *Anesth Analg* 95, 661-
343 666.
- 344 Conzemius M, Evans R, Wagner S et al. (2005) Effect of surgical technique on limb
345 function after rupture of the cranial cruciate ligament in dogs. *J Am Vet Med Assoc*
346 226, 232-236.
- 347 Conzemius M, Hill C, Perkowski S et al. (1997) Correlation between subjective and
348 objective measures used to determine severity of postoperative pain in dogs. *J Am Vet*
349 *Med Assoc* 210, 1619-162.

- 350 Crociolli GC, Cassu RN, Nicácio GM et al. (2015) Gabapentin as an adjuvant for
351 postoperative pain management in dogs undergoing mastectomy. *J Vet Med Sci* 77,
352 1011-1015.
- 353 Holton L, Reid J, Nolan A et al. (2001) Development of a behaviour-based scale to
354 measure acute pain in dogs. *Vet Rec* 148, 525-531.
- 355 KuKanich B (2013) Outpatient oral analgesics in dogs and cats beyond nonsteroidal
356 anti-inflammatory drugs: An evidence-based approach. *Vet Clin North Am Small Anim*
357 *Pract* 43, 1109-1125.
- 358 Ladha A, Alam A, Idestrup, Chol S et al. (2013) Spinal haematoma after removal of a
359 thoracic epidural catheter in a patient with coagulopathy resulting from unexpected
360 vitamin K deficiency. *Anaesth* 68, 856-860.
- 361 Madden M, Gurney M, Bright S (2014) Amantadine, an N-Methyl-D-Aspartate
362 antagonist, for treatment of chronic neuropathic pain in a dog. *Vet Anaesth Analg* 41,
363 440-441.
- 364 Murphy J, Paskaradevan J, Wu C et al. (2013) Analgesic efficacy of continuous
365 intravenous magnesium infusion as an adjuvant to morphine for postoperative
366 analgesia: a systematic review and meta-analysis. *Middle East J Anaesthesiol* 22, 11-20.
- 367 Norkus C, Rankin D, KuuKanich B et al. (2015) Pharmacokinetics of oral amantadine
368 in greyhound dogs. *J Vet Pharmacol Ther* 38, 305-308.
- 369 Oezalevli M, Cetin T, Isik G et al. (2005) The effect of adding intrathecal magnesium
370 sulphate to bupivacaine-fentanyl spinal anaesthesia. *Acta Anaesthesiol Scand* 49, 1514-
371 1519.

- 372 Pumberger M, Memtsoudis S, Hughes A et al. (2013) An analysis of the safety of
373 epidural and spinal neuraxial anesthesia in more than 100,000 consecutive major lower
374 extremity joint replacements. *Reg Anesth Pain Med* 38, 515-519.
- 375 Reid J, Nolan AM, Scott EM et al. (2007) Development of the short-form Glasgow
376 Composite Measure Pain Scale (CMPS-SF) and derivation of an analgesic intervention
377 score. *Animal Welfare* 16, 97-104.
- 378 Rioja E, Dziki B, Schoeman JP (2012) Effects of a constant rate infusion of
379 magnesium sulphate in healthy dogs anaesthetized with isoflurane and undergoing
380 ovariohysterectomy. *Vet Anaesth Analg* 39, 599-610.
- 381 Sammarco J, Conzemius M, Smith GK et al. (1996) Postoperative analgesia for stifle
382 surgery: A comparison of intra-articular bupivacaine, morphine, or saline. *Vet Surg* 25,
383 59-69.
- 384 Serlin RC, Mendoza TR, Nakamura Y et al. (1995) When is cancer pain mild, moderate
385 or severe? Grading pain severity by its interference with function. *Pain* 61, 277-284.
- 386 Simpson J, Eide T, Koski G et al. (1994) Intrathecal magnesium sulfate protects the
387 spinal cord from ischemic injury during thoracic aortic cross-clamping. *Anesthesiol* 81,
388 1493-1499.
- 389 Valverde A, Morey T, Davies W et al. (2003) Validation of several types of noxious
390 stimuli for use in determining the minimum alveolar concentration for inhalation
391 anesthetics in dogs and rabbits. *Am J Vet Res* 64, 957-962.
- 392

Appendix 2

Modified Tarlov's scale (Buvanendran et al., 2002; Adami et al., 2016) to evaluate the neurological function of the hind limbs and the degree of motor blockade in 20 dogs undergoing total hip replacement.

The same observer who was blind to the treatment evaluated the dogs as soon as they were awake enough to respond to stimulation (vocal call and incitement to sit or stand up) and then 60, 120, 180, 240, 300 minutes and 24 hours after surgery.

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

Figure legends

Figure 1 Intraoperative physiological variables recorded from 20 dogs anaesthetized for total hip replacement and assigned to one of two treatment groups: group C (Control, epidural ropivacaine; $n = 10$) and group M (Magnesium, epidural combination of magnesium and ropivacaine; $n = 10$). Data are presented as mean \pm standard deviation. 0: values recorded as baseline in the anaesthetized dogs prior to surgical stimulation; 1: values recorded immediately after skin incision; 2: values recorded after femoral head osteotomy.

Footnotes:

Mean arterial pressure

†Significantly different from baseline for Group M (p value < 0.05 , q value = 8.80)

‡Significantly different from baseline for Group C (p value < 0.05)

Respiratory rate

†Significantly different from baseline for Group M ($p < 0.05$, $q > 8.00$)

‡Significantly different from baseline for Group C ($p < 0.05$, $q = 8.40$)

Figure 2 Postoperative pain scores recorded from 20 dogs anaesthetized for total hip replacement and assigned to one of two treatment groups: group C (Control, epidural ropivacaine; $n = 10$) and group M (Magnesium, epidural combination of magnesium and ropivacaine; $n = 10$). Data are presented as medians and interquartile ranges (25%-75%). 1: values recorded after recovery, as soon as the patients were able to sit and respond to vocal call; 2, 3, 4, 5 and 6 are 60, 120, 180, 240, 300 minutes and 24 hours after recovery.

Footnotes

Sammarco score

†Significantly different from baseline for Group M (p value < 0.05)

‡Significantly different from baseline for Group C (p value < 0.05)

Visual Analogue Scale score

†Significantly different from baseline for Group M (p value < 0.05, q value = 12.16)

‡Significantly different from baseline for Group C (p value < 0.05, q value = 11.65)

Tarlov's score

†Significantly different from baseline for Group M (p value < 0.05)

‡Significantly different from baseline for Group C (p value < 0.05)











