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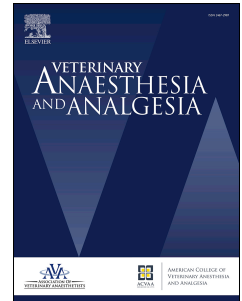
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Running Head: Ketamine or alfaxalone for cat ovariectomy

RESEARCH PAPER

Dexmedetomidine-methadone-ketamine versus dexmedetomidine-methadone-alfaxalone for cats undergoing ovariectomy.

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Authors' contributions

1 **Abstract**

2 **Objective** To compare the duration, quality of anaesthesia and analgesia and quality of
3 recovery of dexmedetomidine and methadone combined with either ketamine or
4 alfaxalone.

5 **Study design** Randomized prospective clinical trial.

6 **Animals** Forty-four healthy client-owned cats presenting for ovarioectomy.

7 **Methods** Cats were randomly assigned to one of two treatment groups: DAM (n=22),
8 which were administered intramuscular (IM) dexmedetomidine ($15 \mu\text{g kg}^{-1}$), methadone
9 (0.3 mg kg^{-1}) and alfaxalone (3 mg kg^{-1}), and DKM (n=22), which were administered
10 IM dexmedetomidine ($15 \mu\text{g kg}^{-1}$), methadone (0.3 mg kg^{-1}) and ketamine (3 mg kg^{-1}).
11 During anaesthesia, heart rate, respiratory rate and systolic arterial pressure were
12 measured every 5 minutes. Cats that moved or had poor muscle relaxation were
13 administered an additional 1 mg kg^{-1} intravenously (IV) of either alfaxalone (DAM) or
14 ketamine (DKM). In cases of increased autonomic responses to surgical stimulation,
15 fentanyl ($2 \mu\text{g kg}^{-1}$) was administered IV. At the end of the surgery, atipamezole ($75 \mu\text{g}$
16 kg^{-1}) was administered intramuscularly and the times to both sternal recumbency and
17 active interaction were recorded. Quality of recovery was evaluated with a Simple
18 Descriptive Scale. The UNESP-Botucatu multidimensional composite pain scale and a
19 Visual Analogue Scale (VAS) were used to evaluate post-operative analgesia at the
20 return of active interaction and 1, 2 and 3 hours later.

21 **Results** The additional anaesthesia and rescue fentanyl requirements were similar
22 between groups. The quality of recovery was better in the DAM group than the DKM
23 group (SDS scores: 0[0-1] and 1[0-3], respectively; $p = 0.002$). Postoperative pain
24 scores decreased progressively over time in both groups with no significant differences

25 ($p = 0.08$) between them.

26 **Conclusions and clinical relevance** Both protocols provided comparable quality of
27 anaesthesia and analgesia that were suitable for cats undergoing ovarioectomy. In
28 combination with methadone and dexmedetomidine, alfaxalone and ketamine showed
29 comfortable and reliable recoveries.

30

31 **Introduction**

32 Ovarioectomy is one of the most common reasons for anaesthesia in young female cats in
33 Europe. Due to the fractious nature of some cats and the limited anaesthesia equipment
34 availability of many small veterinary clinics, an intramuscular (IM) anaesthetic protocol
35 offers distinct advantages. However, the anaesthetic drugs should be safe, well-absorbed
36 by IM route and provide reliable unconsciousness, muscle relaxation and analgesia.

37 In cats, alpha-2 agonists are commonly used anaesthetic agents because they
38 provide reliable sedation and short-term analgesia (Cullen et al. 1996; Murrell et al.
39 2005; Nagore et al. 2013). Furthermore, opioid and alpha-2 agonist combinations have a
40 synergistic analgesic effect (Meert et al. 1994; Slingsby et al. 2014) and provide deeper
41 sedation compared with the effect of either agent alone (Girard et al. 2010).

42 Ketamine is often used in combination with opioids and alpha-2 agonists
43 because it is inexpensive and offers the advantage of producing predictable dissociative
44 and analgesic effects (Ko et al. 2011; Harrison et al. 2011; Carbone 2012). However,
45 repeated dosing of ketamine during anaesthesia has been associated with drug
46 accumulation and delayed recovery in cats (Baggot et al. 1976; Liu et al. 2006).

47 Furthermore, ketamine stimulates the cardiovascular system (increase heart rate (HR),
48 blood pressure and cardiac output) because of central stimulation of the sympathetic

49 system. This leads to an increase in myocardial work that increases the myocardial
50 oxygen demand leading to impaired cardiovascular function in cats with underlying
51 cardiac disease (Clutton 2007). This effect potentially endangers fractious cats in which
52 preanaesthetic examination is not feasible.

53 Alfaxalone is a neurosteroid anaesthetic available in Europe in a cyclodextrin
54 based formulation (Alfaxan, Jurox, Australia). It has excellent cardiovascular stability
55 (Muir et al. 2009) and fast clearance from the body, making it suitable for repeated
56 dosing during anaesthesia (Whittem et al. 2008). Consequently, alfaxalone offers some
57 advantages over ketamine when it is used as part of a balanced anaesthetic protocol.
58 Alfaxalone has been used at different dosages to induce anaesthesia intravenously (IV)
59 (Pinelas et al. 2014) and IM (Grubb et al. 2013). Alfaxalone may have analgesic
60 properties, resulting from its blockade of T-type Ca^{2+} channels and potentiation of
61 GABA_A ligand-gated channels (Pathirathna et al. 2005). However, a beneficial
62 analgesic benefit has not been observed clinically (Winter et al. 2003; Murison &
63 Martinez Taboada 2010).

64 The aim of this study was to compare the anaesthetic, cardiorespiratory,
65 analgesic and recovery quality effects of ketamine or alfaxalone in combination with an
66 alpha-2 agonist (dexmedetomidine) and an opioid (methadone), in cats undergoing
67 ovariectomy.

68 **Materials and methods**

69 The study was approved according to Directive 2010/63/EU by the Chair of the
70 Veterinary University Hospital Ethics Approval Board and informed consent was
71 obtained from all owners.

72

73 Animals

74 The sample size was calculated using a commercial software program (SigmStat and
75 SigmaPlot 12) to detect a Visual Analogue Scale (VAS) difference between groups of
76 10 mm with a standard deviation of xx using a T-test with 80% power and 5%
77 significance.

78 Forty-nine clients owned female cats undergoing elective ovarioectomy were included in
79 the study (Fig. 1). Cats underwent routine preanaesthetic physical examination in order
80 to assess their health status according to the American Society of Anesthesiologists
81 (ASA) classification. Exclusion criteria were $ASA \geq II$, fractious personality and age
82 greater than eight years.

83

84 Anaesthesia and surgery

85 The cats were fasted by the owners for 12 hours before being admitted to the university
86 hospital of Veterinary Medicine of Alfort, France, on the scheduled surgery day. On
87 arrival, a preanaesthetic physical examination was performed. Study-eligible cats were
88 then individually housed in single cages in a dedicated cat room and were randomly
89 assigned, based on drawing numbered pieces of paper from an envelope, to one of two
90 treatment groups. Group DAM (n=22) were administered IM dexmedetomidine ($15 \mu\text{g}$
91 kg^{-1} ; Dexdomitor; Orion Pharma, Finland), methadone (0.3 mg kg^{-1} ; Comfortan;
92 Eurovet, Belgium) and alfaxalone (3 mg kg^{-1} ; Alfaxan; Jurox, Australia) and Group
93 DKM (n=22) were administered IM dexmedetomidine ($15 \mu\text{g kg}^{-1}$), methadone, (0.3 mg
94 kg^{-1}) and ketamine (3 mg kg^{-1} Imalgene 1000; Merial, France).

95 All cats were injected IM with one of the two anaesthetic combinations prepared
96 by a veterinarian not directly involved in the study. This individual also equalized the

97 volume of the DKM solution to that of the DAM solution using sterile saline so the
98 anaesthetist could not discern which treatment combination was being administered.
99 When the injection volume exceeded 1 mL, the anaesthetic combination was
100 administered into two injection sites (right and left lumbar muscles). Times to sternal
101 and lateral recumbency, quality of induction and adverse effects such as vomiting,
102 hypersalivation, distress, tremors, myoclonus and increased muscle tone were recorded.
103 Sternal recumbency was defined as a position in which the legs were tucked under the
104 body and the cat has a decreased responsiveness to its surroundings. Lateral
105 recumbency was defined as a position in which the cat lay on its side and was
106 unresponsive to its surroundings. General anaesthesia was considered induced when the
107 cats were shifted from lateral to dorsal recumbency, and did not attempt to reposition
108 themselves. If general anaesthesia was not induced within 30 minutes after the injection,
109 the cats were reinjected IM with half of the initial doses of both dexmedetomidine and
110 alfaxalone for the DAM group, or dexmedetomidine and ketamine for the DKM group,
111 without methadone and were excluded from the study. Once anaesthesia was induced, a
112 22-gauge catheter (Delta Med, Italy) was placed in the cephalic vein. All cats were then
113 administered $7 \text{ mL kg}^{-1} \text{ hour}^{-1}$ of sterile saline (NaCl 0.9%, B. Braun, Germany) IV
114 during the procedure.

115 An IV injection of 20 mg kg^{-1} of amoxicillin (Clamoxyl, GlaxoSmithKline, UK)
116 was administered as soon as the catheter was placed, and then repeated at the end of the
117 surgery. Eye lubricant (Ocrygel; TVM, France) was applied at the beginning of
118 anaesthesia and then every 45 minutes until recovery. For the surgery, cats were
119 positioned in dorsal recumbency. Time from the beginning (first incision of the
120 abdominal wall, coeliotomy) to the end of surgery (last suture knot) was recorded.

121 Surgeries were performed by final year veterinary students under the direct supervision
122 of in-house surgeons. A multiparametric monitor (Cardiocap II, Datex, IL, USA) was
123 used during anaesthesia. Heart rate and rhythm were monitored by electrocardiography,
124 respiratory rate (f_R) was assessed by visual observation of chest movements, pulse rate
125 and arterial oxygen saturation (SpO_2) were detected by pulse oximetry, and systolic
126 arterial pressure (SAP) was intermittently measured using a Doppler (Doppler Vet BP;
127 Sonomed, Poland) placed over the ulnar artery. The animals were allowed to breathe
128 room air. Cats showing signs of hypoventilation ($f_R < 6$ breaths $minute^{-1}$) or severe
129 hypoxemia ($SpO_2 < 90\%$) were intubated, manually ventilated and excluded from the
130 study. Animals with arterial saturation values less than 94% SpO_2 , were supplemented
131 with oxygen (FIO_2 100%) at a rate of 2 L $minute^{-1}$ via a mask. In the event that oxygen
132 supplementation did not result in normalization of SpO_2 , the cats were intubated to
133 permit manually assisted ventilation with 100% oxygen and excluded from the study.
134 Animals were maintained at a body temperature above 36.5° C by a forced air warmer
135 (Warm Touch; Mallinckrodt Medical, Ireland).

136 During surgery the depth of anaesthesia was evaluated every 5 minutes, based
137 on the following descriptors: occurrence of spontaneous blinking (yes/no), occurrence
138 of movements during surgical stimulation (yes/no), and inadequate muscle relaxation
139 (yes/no). If two of the above parameters were observed (i.e. yes) then the patient
140 received either alfaxalone 1 mg kg^{-1} IV (DAM) or ketamine 1 mg kg^{-1} IV (DKM).

141

142 **Intraoperative nociceptive evaluation**

143 For each cat, baseline values for HR, f_R and SAP were determined prior to surgical
144 stimulation. When two of these three parameters increased by 30% above the baseline, 2
145 $\mu\text{g kg}^{-1}$ fentanyl (Fentanyl; Mylan, France $50 \mu\text{g ml}^{-1}$) was administered IV.

146 **Postoperative pain assessment and quality of recovery assessment**

147 At the end of the surgery (defined as time of tying last suture knot), but not earlier than
148 30 minutes after the last anaesthetic (ketamine or alfaxalone) supplemental dose, all
149 animals received atipamezole $75 \mu\text{g kg}^{-1}$ IM (Alzane, Zoetis, NJ, USA). Time to sternal
150 recumbency and active interaction (defined as responsiveness to voices, alertness and
151 interest in the surroundings) were recorded. Quality of recovery was evaluated after
152 atipamezole injection until the cat regained sternal recumbency. A simple descriptive
153 scale (SDS) indicated by (0) very smooth recovery, (1) smooth recovery, (2) poor
154 recovery and (3) very poor recovery requiring rescue sedation (dexmedetomidine, $2 \mu\text{g}$
155 kg^{-1} IV), was used.

156 Postoperative pain was evaluated, at the same time points, using two different
157 scoring systems. Firstly, a modified version of the UNESP-Botucatu multidimensional
158 composite pain scale (MCPS) (Brondani et al. 2013), where the maximum total score
159 was 24 instead of 30, because of the exclusion of the subscale “physiological change”,
160 which was incompatible with the drug used in our study. Secondly, a Visual Analogue
161 Scale (VAS) was used where 0 mm was labelled as “no pain” and 100 mm as “worst
162 possible pain” (Jensen et al. 2003). The same anaesthetist performed the pain
163 assessments starting at the first spontaneous cat interaction (T0), and then at 1 (T1), 2
164 (T2) and 3 (T3) hours later. Buprenorphine (Vetergesic; Sogeval, France) $20 \mu\text{g kg}^{-1}$ IV
165 was administered as postoperative rescue analgesia when a score greater than “two” for
166 the subscale “expression of pain”, or a score greater than “three” for the subscale

167 “psychomotor changes” was recorded on the UNESP-Botucatu MCPS, and/or when the
168 VAS score exceeded 40 mm of the maximum value of 100 mm. At the end of the pain
169 assessment, all cats were administered 0.2 mg kg⁻¹ meloxicam (Metacam; Boehringer-
170 Ingelheim, Germany) subcutaneous (SC) and 20 µg kg⁻¹ buprenorphine SC, unless
171 buprenorphine had been administered earlier as postoperative rescue analgesia. The
172 same anaesthetist evaluated intraoperative nociception, all assessments of postoperative
173 pain and quality of recovery.

174

175 **Statistical analysis**

176 Descriptive statistics were performed to assess the normal distribution of data. To
177 compare the intraoperative physiological variables (HR, f_R and SAP) between the two
178 treatment groups, a repeated measures ANOVA (A) followed by a Bonferroni multiple
179 comparison test were used. The time for the first supplemental bolus and the duration of
180 surgery followed a normal distribution. For this reason, a t-test (T) was used. To
181 compare the total dose of intraoperative rescue fentanyl, postoperative rescue
182 buprenorphine and rescue sedation by each group, a Fisher’s test (F) was used. Total
183 dose of alfaxalone or ketamine administered to each group, time to active interaction
184 and SDS scores for assessment of recovery quality were analysed with non-parametric
185 tests. For this reason, a Mann-Whitney test was used (MW). The composite pain,
186 UNESP-Botucatu MCPS and VAS scores achieved by each group over time were
187 analysed by repeated measures ANOVA followed by a Bonferroni multiple comparison
188 test.

189 Statistical analysis was performed using commercially available software
190 (NCSS, 2007; SigmaPlot 12). Values of $p < 0.05$ were considered significant. Data are
191 reported as mean \pm standard deviation or median (range).

192

193 **Results**

194 **Animals**

195 Data were normally distributed only for the duration of anaesthesia and the time to
196 anaesthetic induction. Five cats were excluded because of their fractious nature (Fig. 1).
197 The remaining 44 animals were classified as ASA I, and none were rejected after
198 preanaesthetic physical examination. These 44 cats were randomly allocated to the two
199 anaesthetic combination groups. The treatment groups did not differ statistically with
200 respect to age [7 (6-74) months] and body weight [2.8 (1.8-4.1) kg]. Anaesthetic
201 induction was smooth in all animals, additional doses were not required to achieve a
202 surgical plane of anaesthesia, and apnoea, vomiting or emergence reactions were not
203 observed. The average time from IM injection to sternal recumbency and the time to
204 sternal and lateral recumbency are summarized in Table 1.

205

206 **Anaesthesia and intraoperative nociceptive evaluation**

207 The duration of surgery was 75 ± 16 minutes for DAM and 69 ± 15 minutes for DKM
208 ($p = 0.22^T$). Time to the first supplemental dose after the initial IM injection was
209 different between groups ($p = 0.046^T$) (Table 1). There was no difference ($p = 0.44^{MW}$)
210 between groups in the number of alfaxalone doses administered during surgery (Table
211 1).

212 There was no difference in HR ($p = 0.23^A$) and SpO₂ ($p = 0.26^A$) between groups
213 (Table 1). None of the animals required endotracheal intubation, but 18 cats, nine from
214 each group, were administered 100% oxygen supplementation by mask. In these
215 animals, SpO₂ increased to values higher than 94% after a few minutes, at which point
216 the oxygen was disconnected and additional oxygen supplementation was not required
217 again during the study.

218 The f_R was higher in DAM compared with DKM ($p = 0.013^A$) (Table 1).
219 However, the mean SAP was higher in DKM compared to DAM ($p = 0.025^A$) (Table 1).
220 Although rescue analgesia with fentanyl was necessary for three cats (14%) in DKM
221 and none in DAM, these proportions were not significant between groups ($p = 0.20^F$).

222

223 **Postoperative pain assessment and quality of recovery assessment**

224 Rescue analgesia with buprenorphine was administered to 9 cats in group DAM and to
225 8 cats in group DKM ($p = 0.76^F$) (Table 2). There was no difference between groups in
226 postoperative pain UNESP-Botucatu MCPS ($p = 0.20$) or VAS scores ($p = 0.63$) at T0,
227 T1, T2 and T3. Repeated measures ANOVA showed an increase pain score from active
228 interaction to 1 hour, after which all pain scores decreased over time in both groups
229 (UNESP-Botucatu MCPS ($p = 0.078$) and VAS ($p = 0.07$), see Table 2).

230 Rescue sedation was administered to four cats in DKM and no cats in DAM ($p =$
231 0.107^F). Time from IM atipamezole injection to active interaction was 4 (0-28) minutes
232 for DAM and 6 (0-50) minutes for DKM ($p = 0.22^{MW}$). For recovery, SDS scores were
233 better in the DAM group ($p = 0.002^{MW}$), see Table 2.

234

235 **Discussion**

236 In the present study, both IM protocols showed comfortable and reliable recoveries for
237 ovariectomies. The duration of these teaching-surgeries (72 ± 15 minutes) required
238 multiple supplemental doses that are unlikely to be necessary in a shorter general
239 practice ovariohysterectomy (21 ± 7 minutes, Case et al. 2015).

240 In our study, both groups were administered a drug mixture containing
241 dexmedetomidine and methadone. After IM injection, no adverse effects such as
242 excitement-dissociation or vomiting were observed. Alpha-2 and μ receptors are found
243 in similar anatomical regions (i.e. in the brain and spinal cord) and they have common
244 signal transduction pathways (G proteins) and mechanisms of action, such as activation
245 of potassium channels in the postsynaptic neuron, making the cell insensitive to
246 excitatory input (Sinclair 2003). This association can provoke synergistic effects if used
247 simultaneously (Ossipov et al. 1990) and could be at the origin of the excitement-free
248 recoveries.

249 Contrarily to some publications where dexmedetomidine administered alone
250 provoked some emesis (McSweeney et al. 2012; Nagore et al. 2013), no animal
251 presented with these symptoms in this study. It is possible that combination with
252 methadone, which has antiemetic effects (Robertson & Taylor 2004) at sedating doses,
253 blocked the emetic action of dexmedetomidine (Blancquaert et al. 1986). Also, the
254 recent study of Papastefanou et al. (2015) demonstrated that administration of
255 dexmedetomidine and butorphanol together prevented emesis and reduced the incidence
256 and severity of nausea compared with dexmedetomidine alone.

257 The time to the first supplemental dose was shorter in the DKM group compared
258 to the DAM group. This observation is in contrast to the pharmacokinetics of ketamine
259 and alfaxalone, where the former has a longer half-life compared to the latter in cats

260 (Whittem et al. 2008). The dilution of ketamine, performed to adjust the DKM solution
261 to an equal injectable volume as the DAM solution, could have affected the
262 redistribution kinetics of ketamine and subsequently the need for an earlier
263 supplemental dose. In addition, palpebral reflex was maintained constantly in the DKM
264 group, unlike the DAM group and could have affected the anaesthetist's perception of
265 the deep plane of anaesthesia, making them more prone to administer a supplemental
266 dose of ketamine.

267 To maintain a plane of anaesthesia suitable for ovariectomy, it was necessary to
268 reinject alfaxalone every 8-10 minutes following the first IV supplemental dose of 1.0
269 mg kg⁻¹. These results are in accordance with the Food and Drug Administration's
270 recommendations for alfaxalone (1.1 to 1.3 mg kg⁻¹ every 7-8 minutes, NADA, 2012).
271 In our study, the total alfaxalone dose used for the maintenance of anaesthesia was 0.23
272 (0.10-0.35) mg kg⁻¹ minute⁻¹. In the study of Schwarz et al. (2014), total intravenous
273 anaesthesia (TIVA) with alfaxalone after premedication with medetomidine and
274 butorphanol was 0.17 ± 0.02 mg kg⁻¹ minute⁻¹ IV. This difference in effective alfaxalone
275 dose might be because of the different routes of administration (intermittent doses
276 *versus* TIVA) rather than an over-estimation of the anaesthesia requirements in our
277 study. Supplemental doses may require a larger total dose of drug compared with TIVA
278 to maintain a similar plane of anaesthesia. In addition, the extended duration of our
279 teaching-ovariectomies could have influenced the anaesthetic requirements.

280 Intraoperative rescue analgesia was indirectly used to estimate the absence or
281 presence of nociception. Both combination groups were equivalent for intraoperative
282 analgesia requirements. As ketamine has analgesic effects, in contrast to the
283 questionable clinical analgesic effects of alfaxalone, we were expecting an analgesic

284 superiority in the DKM group. We believe the analgesic equivalence of both groups is
285 likely the result of the addition of methadone and dexmedetomidine to both protocols.
286 Their strong analgesic properties could have masked differences between the DKM and
287 DAM groups. Moreover, the doses of dexmedetomidine and methadone used produce
288 bradycardia that could have masked tachycardia resulting from pain, and produced
289 profound sedation that could have masked blinking and movement resulting from pain.
290 In order to minimize this possible confounding factor, our physiological baseline values
291 (HR, f_R and SAP), were determined after the dexmedetomidine and methadone
292 administration at the moment of induction and before any surgical stimulation.

293 Overall intraoperative respiratory rate was significantly lower in DKM
294 compared with DAM, but no difference was seen in arterial oxygen saturation (SpO_2).
295 Even though DKM showed a lower respiratory rate, it did not cause respiratory
296 depression. Respiratory depression has been reported with the use of ketamine alone or
297 in combination with an alpha-2 agonist (e.g. medetomidine; Harrison et al. 2011).
298 Likewise, alfaxalone has been also associated, during intravenous induction, with a
299 dose-dependent decrease in respiratory rate and minute volume (Whittem et al. 2008;
300 Beths et al. 2014). However, Grubb et al. (2013) showed no respiratory decrease when
301 alfaxalone was administered intramuscularly to cats, which is in accordance with the
302 results of our study. It is our opinion that the decreased respiratory rate might result
303 from the 1 mg kg^{-1} dose of IV alfaxalone administered during anaesthesia. This dose is
304 close to alfaxalone's induction dose. This remains to be investigated.

305 The DKM group had higher systolic blood pressure compared with the DAM
306 group, but there were no differences in HR between the two groups. The similar heart
307 rates in both groups likely results from the bradycardic effect of dexmedetomidine plus

308 methadone. The higher SAP in the DKM group is expected because of the greater
309 cardiac sympathetic action of ketamine (Peck et al. 2008). Unfortunately, the scientific
310 literature is incomplete concerning the sympathetic effects of alfaxalone and therefore
311 we cannot compare the mechanism on systolic blood pressure.

312 To evaluate postoperative pain, we used a VAS because it has been widely
313 employed in veterinary research for its ease, rapidity reliability and general assessment
314 of trends (Mich & Hellyer 2009). Nonetheless, VAS can be subjective and moderately
315 imprecise (Mich & Hellyer 2009). As we used a dissociative drug (ketamine), the ideal
316 cut-off point was modified, because of the residual dissociation interference to 40 mm.
317 Moreover, the VAS is not a very precise way to define an “ideal” pain score. To
318 overcome these limitations, we opted for the parallel use of a multidimensional
319 composite UNESP-Botucatu MCPS validated for the cat. This combination of two pain
320 scales offered the best compromise of ease, speed, reliability and objectivity. We did
321 not see any significant difference between groups for postoperative pain assessments.
322 Recently, in a similar study comparing post ovariectomy pain in cats after alfaxalone-
323 alone or ketamine-medetomidine anaesthesia, Kalchofner-Guerrero et al. (2014)
324 reported that anaesthesia with ketamine-medetomidine provided better post-surgical
325 analgesia than alfaxalone alone, but in this study opioids were not used during the
326 surgical procedure. Probably, our pain scales were not sensitive enough to detect slight
327 differences in analgesia between the two groups because the combination of
328 dexmedetomidine plus methadone was efficacious enough to prevent any analgesic
329 difference, if any, being revealed between ketamine and alfaxalone.

330 Ketamine has also been associated with a confounding effect on the
331 psychomotor subscale of the UNESP-Botucatu MCPS (Buisman et al. 2015). In our

332 attempt to reduce this interference, we assessed pain after an active interaction with
333 each animal, while in the study of Buisman et al. (2015) pain scale evaluations were
334 performed hourly post-extubation. Similar postoperative pain studies in cats after
335 ovariectomy have included meloxicam administration before (Benito-de-la-Víborá et al.
336 2008) or at completion of surgery to assure postoperative analgesia. To avoid
337 interference with the pain score assessments we administered meloxicam, only at the
338 end of the study.

339 We did not observe any statistical difference between groups in recovery values,
340 which were overall of good quality. Dysphoric recoveries are well documented with
341 ketamine (Baggot 1976) but have also been reported after administration of alfaxalone
342 (Zaki et al. 2009; Grubb et al. 2013; Rodrigo-Mocholi D et al. 2015). In the DAM
343 group none of the cats required rescue sedation compared to four animals in the DKM
344 group. This is probably because of the faster pharmacokinetics of alfaxalone (Whittem
345 et al. 2008), and the use of atipamezol to reverse the sedative effects produced by
346 dexmedetomidine. Further investigation is necessary to understand the mechanism of
347 alfaxalone emergence reactions.

348 Additionally, there were others limitations to this study. First, the large volume
349 of the anaesthetic agents required for IM injection (after equivalency between groups)
350 necessitated administering the drugs into two injections. These lumbar IM injections
351 increased the level of pain and stress. Second, we have included all animals that were
352 administered rescue analgesia and sedation in the final statistics study. This could have
353 lead to bias in the results. Third, learning students performed the ovariectomies, so time
354 of surgery was prolonged. Consequently, multiple additional doses were required. If the

355 study were transposed to clinical practice supplemental doses would unlikely be
356 necessary, making it a simple protocol.

357

358 **Conclusion and clinical relevance**

359 In this randomized prospective clinical trial, both anaesthesia protocols were suitable
360 for cats undergoing ovarioectomy and were comparable in quality of anaesthesia and
361 analgesia. When combined with methadone and dexmedetomidine, alfaxalone and
362 ketamine showed comfortable and reliable recoveries.

363

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- 477

478 **Tables**

479 **Table 1** Data after injection of intramuscular (IM) dexmedetomidine ($15 \mu\text{g kg}^{-1}$),
 480 methadone (0.3 mg kg^{-1}) and alfaxalone (3 mg kg^{-1})(DAM, n=22) or dexmedetomidine
 481 ($15 \mu\text{g kg}^{-1}$), methadone (0.3 mg kg^{-1}) and ketamine (3 mg kg^{-1}) (DKM, n=22) to cats
 482 undergoing ovarioectomy. Cardiorespiratory measurements were taken every 5 minutes
 483 during surgery. The number of supplemental doses were the number administered after
 484 the first supplemental dose (1 mg kg^{-1} of either alfaxalone or ketamine).

Parameter	Group		<i>p</i> -value
	DAM	DKM	
Time to sternal recumbency (minutes)	1 ± 1	2 ± 1	N/A
Time to lateral recumbency (minutes)	2 ± 1	4 ± 2	N/A
Time to first supplemental dose (minutes)	58 ± 18	47 ± 16	0.046*
Number of supplemental doses (n°)	4 (1-6)	3 (1-7)	0.44
HR (beats minute^{-1})	128 ± 29	138 ± 21	0.23
SpO ₂ (%)	94 ± 3	94 ± 1	0.26
f_R (breaths minute^{-1})	30 ± 7	25 ± 6	0.013*
SAP (mmHg)	125 ± 16	141 ± 27	0.025*

485 *Statistically significant between groups. Data are reported as mean \pm standard
 486 deviation or median (range).

487 N/A, non-applicable; HR, heart rate; SpO₂, haemoglobin oxygen saturation; f_R ,

488 respiratory rate; SAP, systolic arterial pressure

489

490 **Table 2** Medians and percentiles [10th – 90th] of recovery quality, assessed with a
 491 simple descriptive scale (SDS) and postoperative pain assessed with a Visual Analogue
 492 Scale (VAS) and the UNESP-Botucatu multidimensional composite pain scale (MCPS)
 493 and recorded from 43 cats undergoing elective ovarioectomy. Pain assessments were
 494 carried out at various time points: as soon as the cats were observed to interact actively
 495 with the investigator (T0), and then 1 (T1), 2 (T2) and 3 (T3) hours after that.
 496

Parameter	Group	
	DAM (n=21)	DKM (n=22)
Recovery score	0 (0-1)	1(0-3)
VAS T0	40 (0-60)	20 (0-58)
VAS T1	20 (0-60)	40 (0-78)
VAS T2	20 (0-60)	20 (0-58)
VAS T3	0 (0-40)	20 (0-40)
MCPS T0	2 (0-5)	1 (1-6)
MCPS T1	1 (0-13)	2 (0-10)
MCPS T2	1 (0-5)	1(0-8)
MCPS T3	0 (0-4)	1 (0-7)

497 The SDS ranged from 0) very smooth recovery to 3) very poor recovery; the VAS
 498 ranged from 0) no pain to 100) worst possible pain and the MCPS ranged from 0) no
 499 pain to 24) worst possible pain.

500

501 DAM, dexemedetomidine, methadone and alfaxalone; DKM (dexemedetomidine,
 502 methadone and ketamine.

503 **Figure 1** Consort Flow Diagram

504

