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Fracture repair in a sheep. Ethical dilemma: is it possible to provide adequate intra-operative pain relief within the law?

Lampaan olkaluun murtumaleikkaus. Onko tuotantoeläimen intraoperatiivinen kivunlievitys mahdollista lain puitteissa?

SUMMARY

A 16-year-old, female Suffolk sheep weighing 46.3 kg was referred for humeral fracture repair. The anaesthetic protocol was managed using either drugs licensed for sheep (European Regulation 37/2010) or administered under the 'cascade' (Directive 2004/28/EC) with the appropriate withdrawal period applied. Multimodal balanced analgesia was provided with an electrostimulation-guided cervical paravertebral block using a combination of lidocaine and detomidine and with intravenous ketamine infusion, meloxicam and butorphanol. The local block was successful, but after 80 minutes a sympathetic response to surgical stimulation was observed, most likely due to waning effects of lidocaine. A paravertebral block with lidocaine was not adequate for the whole duration of the fracture repair and detomidine did not prolong its duration of effect. The shortage of licensed analgesic drugs in food-producing animals make the provision of efficient and long-lasting analgesia challenging in sheep.

YHTEENVETO

16-vuotias uuhi tuli lähetteellä olkaluun murtumaleikkaukseen. Anestesiasuunnitelmaan valittiin joko lääkeaineita, joilla on Euroopan komission asetuksen N:o 37/2010 mukainen myyntilupa lampaille, tai kaskadisäännöstä soveltaen ja vaadittavia varoaikoja noudattaen lääkeaineita, joilla on myyntilupa toiselle tuotantoeläinlajille. Multimodaalinen intraoperatiivinen kivunlievitys koostui sekä kaulan paravertebraaliaulueen puudutuksesta lidokaiinin ja detomidiinin yhdistelmällä että suonensisäisestä ketamiini-infuusiosta, meloksikaamista ja butorfanolista. Puudutus onnistui, mutta sen vaikutus alkoi hälvetä 80 minuuttia myöhemmin. Paravertebraalipuudutus lidokaiinia käyttäen ei ollut tarpeeksi pitkäkestoinen riittääkseen koko leikkauksen ajan eikä detomidiini pidentänyt lidokaiinin vaikutusta. Tuotantoeläimille tarkoitettuja kipulääkkeitä on rajoitetusti, mikä vaikeuttaa murtumaleikkausten kivunlievitystä.

KEY POINTS

- Veterinarians have an ethical and moral obligation to relieve and prevent animal suffering caused by diseases and minor or major procedures.
- Few anaesthetic and analgesic drugs are licensed for sheep or could be administered under the 'cascade', making the provision of anaesthesia and analgesia challenging.
- This case report highlights the difficulties of providing adequate analgesia in an ovine fracture repair, while accomplishing the regulation on drugs and their maximum residue limits in foodstuffs of animal origin.

INTRODUCTION

In the European Union, drugs for ovine species must be administered under the European Commission Regulation (EU) 37/2010 of allowed and prohibited substances. Pharmacologically active substances are classified according to their maximum residue limits (MRL) in foodstuff of animal origin in order to protect public health. Allowed substances may have an established or provisional MRL, or they may not require one. Drugs for which MRL cannot be established should never be administered to food-producing animals. By law, a veterinarian may only prescribe drugs listed in the table of allowed substances to a food-producing animal. Target species, indications for use and MRL of a veterinary medicinal product are outlined in the table and are under regulation of the National Competent Authorities. In the event that there is no authorised veterinary medicinal product in a EU Member State for a condition affecting a food-producing species, the veterinarian can, in exceptional circumstances, and under his direct personal supervision, treat the animal "under the cascade", i.e. administer either a drug from table of allowed substances licensed for a different condition in the same species or for the same condition in another species (Directive 2004/28/EC).

Due to the limited number of anaesthetic and analgesic drugs licensed for food-producing animals, it is difficult to effectively provide anaesthesia and analgesia during the peri-anaesthetic period. Nonetheless, veterinarians have an ethical and professional obligation to relieve and prevent animal suffering to guarantee welfare, while legally and morally ensuring that animal derivatives are free from harmful drug residues. In addition, pain assessment is difficult in food-producing animals, both because they have developed to hide signs of pain in order to preserve themselves from predation[1] and because a pain scale has not been validated in these species.[2]

Various nerve blocks (sacrococcygeal and lumbosacral epidural, intravenous regional anaesthesia, paravertebral, cornual and retrobulbar blocks) have been used to perform local anaesthesia in sheep.[3] Recently, a successful cervical paravertebral block in a sheep using bupivacaine was described,[4] which is reported to have a duration of action of 6–8 hours.[5] Cervical paravertebral block aims to anaesthetise the 6th (C6), 7th (C7), 8th (C8) cervical nerves and the 1st (T1) thoracic nerve and is indicated for surgical procedures of shoulder and brachium.

We describe the difficulties encountered when attempting to provide adequate multi-modal analgesia during fracture repair in a food-producing species. The aim of multimodal analgesia is to provide better pain relief, minimise the side effects of a single drug, reduce both the requirement of maintenance agent and post-operative rescue analgesia and decrease the risk of post-operative hyperalgesia.

CASE

A 16-year-old Suffolk sheep, weighing 46.3 kg, was referred to University College Dublin's Veterinary Hospital for non-weight bearing lameness of the right forelimb following a severe trauma. Radiographs showed a mid-diaphyseal simple oblique humeral fracture. Therapy with flunixin (Flunixin Injection, Norbrook Laboratories, Northern Ireland; 2.2 mg/kg) SC and amoxicillin (Bimoxyl LA, Bimeda Animal Health, Ireland; 15 mg/kg) IM was started immediately. On the following day the animal was scheduled for internal fixation of the fracture with a compression plate. A 16-gauge catheter was placed into the right jugular vein without prior sedation. Anaesthetic drugs were chosen to comply with either EU 37/2010 or Directive 2004/28/EC with the appropriate withdrawal periods applied. The sheep was premedicated with butorphanol (Alvegesic, Alvetra, Vienna, Austria; 0.2 mg/kg) IV, but developed signs of excitement and thus additional sedation with three consecutive doses of detomidine (Domosedan, Virbac, UK; 0.001 mg/kg each) was administered IV until slight sedation was achieved. Anaesthesia was induced with ketamine (Narketan-10, Vetoquinol, Ireland; 6 mg/kg) and thiopental sodium (Pentothal Sodium, Intervet, Italy; 1 mg/kg) IV. Lidocaine (Lidocaine Hydrochloride, B Braun Medical, Ireland; 0.4 mg/kg) was instilled onto the larynx and the trachea was intubated with an 8.0 mm internal diameter cuffed endotracheal tube with the help of a flexible introducer. The cuff was immediately inflated. Anaesthesia was maintained with isoflurane (Iso-Vet, Piramal Healthcare, UK) in 100% oxygen through a small-animal circle system. Mechanical ventilation in volume-controlled mode (Mallard 2800 C-P, Mallard Medical/AB Technologies, USA) was started immediately: tidal volume was set at 10 to 15 ml/kg and adjusted to maintain end-tidal carbon dioxide tension ($P_E'CO_2$) between 5.0–

6.2 kPa and peak inspiratory pressure between 10 and 15 cmH_2O; respiratory rate (f_R) and inspiratory–expiratory ratio were set at 10 bpm and 1:2, respectively. Hartmann's solution (Hartmann's Lactated Ringer's solution; B Braun, Germany; 10 ml/kg/h) was infused throughout the procedure. A 22-gauge intravenous catheter was placed in the right auricular artery for the measurement of invasive arterial blood pressure (IBP) and the transducer was zeroed to atmospheric pressure and placed at the level of the right atrium. A multiparameter monitor (B40, GE Medical Systems, Germany) was used to continuously measure heart rate (HR), electrocardiogram, f_R, IBP, $P_E'CO_2$, end-tidal isoflurane (F_E'Iso), and haemoglobin oxygen saturation (SpO_2). Palpebral reflex, eye position and lacrimation were also monitored. Data were recorded at 5-minute intervals.

Multimodal analgesia was provided with a constant rate infusion (CRI) of ketamine (10 μg/kg/min), butorphanol 0.2 mg/kg IV every 2 hours and a cervical paravertebral nerve block. The limb to be anaesthetised was positioned uppermost, and after aseptic preparation of the site, the nerve block was performed with a nerve stimulator (Plexygon Nerve Stimulator, Vygon Italia Srl,). The scapula was displaced caudally and the transverse processes of the 6th cervical vertebra and the head of 1st rib were palpated and identified. To block C6 and C7, the tip of a 22-gauge 50 mm long insulated nerve stimulator needle (Echoplex, Vygon, France) was inserted in a transverse plane and walked off both the cranial and caudal borders of 6th cervical vertebra and directed medially until the ventral branches of the nerves were encountered. To block C8 and T1, the same procedures were repeated at the level of the cranial and caudal borders of the first rib, respectively. The nerve stimulator's electrical current was initially set at 1 mA, duration of the stimulus at 0.15 ms, and frequency at 1 Hz. The current was reduced stepwise when the expected motor responses were seen, and the local anaesthetic solution was injected when the response was present at 0.4 mA but absent at 0.2 mA. The expected motor responses were outward-inward rotation of the shoulder and brachium, and flexion of the elbow (C6); inward-outward rotation of the brachium, flexion-extension of the elbow, and extension of the carpus and digits (C7); inward rotation of the brachium, flexion-extension of the elbow, and flexion-extension of the carpus and digits (C8); and flexion of the carpus and digits (T1).[6]

The nerve stimulator was able to elicit inward-outward rotation of the shoulder and the brachium, but not any other movements. A total of four injections with a solution of lidocaine 2% (0.4 mg/kg per site) and detomidine 0.002 mg/ml were made either in a guided manner where correct movement was present, or blindly where movements were absent. A total dose of lidocaine 1.6 mg/kg and detomidine 0.16 μg/kg were injected. Before injecting the drugs, negative aspiration verified that the needle was not located intravascularly, and the compressed air injection technique[7] was used to ensure excessive pressure was not generated during drug injection. Before incision HR and mean blood pressure

(MAP) were 70–75 bpm and 65–75 mmHg, respectively. Initially, no sympathetic stimulation was detected, but 80 minutes into the surgery a sudden increase in HR (85–105 bpm) and MAP (85–98 mmHg) were recorded. Anaesthetic depth was increased by changing F_E'Iso from 0.8 to 1.2% and by administering butorphanol 0.2 mg/kg IV. However, these interventions had little effect on the sympathetic response. Hypercarbia and hypoxaemia as causes of increased HR and MAP were ruled out by taking an arterial blood gas sample (table 1). Once the surgical manipulation stopped, HR and MAP returned to their initial values.

Anaesthesia and surgery lasted 225 and 151 minutes, respectively. Recovery was uneventful, rapid and smooth. Meloxicam (Metacam 20 mg/ml, Boehringer Ingelheim, UK; 0.5 mg/kg) was given IV in the immediate post-operative period and was continued SC daily until discharge, as the IV catheter was removed 24 hours after surgery. Pain was assessed subjectively every 8 hours by evaluating teeth grinding, food intake and lameness. The sheep was discharged from the hospital 10 days after surgery.

DISCUSSION

In this clinical case, analgesia for forelimb fracture repair in a sheep was provided with a lidocaine–detomidine cervical paravertebral nerve block, intravenous butorphanol, meloxicam and ketamine CRI, complying with the legislation concerning substances permitted for the treatment of food-producing animals.

The forelimb is innervated by the brachial plexus, which arises from the ventral branches of C6, C7 C8 and T1. These branches give rise to the suprascapular, subscapular, axillary, radial, median, ulnar, thoracodorsal and musculocutaneous nerves. With the nerve stimulator, only the inward-outward rotation of shoulder and brachium could be elicited, making it likely that the radial, median and ulnar nerves were damaged, which was also suggested by the severity of the trauma observed intraoperatively. Landmarks were easily palpated, making inaccurate position of the needle unlikely. The local block was considered successful judging from the lack of sympathetic response during the first 80 minutes of surgery, which allowed a low concentration of isoflurane to maintain anaesthesia (F_E'Iso 0.8 %, below the minimum alveolar concentration of isoflurane). Eighty minutes correspond with the expected duration of action of lidocaine (60–120 minutes).[8] In Ireland, no local anaesthetics are authorised in sheep. Thus, another veterinary medical product authorised in Ireland in another animal species for an equivalent condition was used, according to Article 11 of Directive 2004/28/CE. From the short-acting (30–60 minutes) procaine (authorised in cattle for minor procedures) and the intermediate-acting (60–120 minutes) lidocaine (authorised in *Equidae* for loco-regional anaesthesia) the latter was chosen, based on the type and anticipated duration (120 minutes) of the procedure. A

solution of procaine and adrenaline is commercially available in Ireland. Adrenaline is intended to slow the absorption of the local anaesthetic and prolong its duration of action to 45–90 minutes, but this was still considered too short for this procedure. A solution of lidocaine and adrenaline is also commercially available, but not in Ireland. In hindsight, adrenaline, which is an allowed substance, could have been added to the 2% lidocaine solution, potentially prolonging the period of peri-operative analgesia. However, detomidine was used as an adjuvant in an attempt to prolong lidocaine's action. Perineural alpha$_2$-agonists significantly prolong the duration of analgesia when used as an adjuvant to local anaesthesia both in human[9] and veterinary medicine.[10] In particular, dexmedetomidine doubles the duration of the block by blocking the hyperpolarization-activated cation current in a concentration-dependent manner.[11,12] Xylazine has also been shown to prolong the sensory and motor blockade of lidocaine when administered epidurally in sheep.[13] However, bradycardia, sedation and increased urination have also been observed, pointing to systemic xylazine absorption from the injection site.[14] Of the alpha$_2$-agonists, xylazine has the greatest potential to cause severe negative respiratory effects in sheep, namely hypoxaemia, increased pleural pressure, increased respiratory resistance, decreased lung compliance and pulmonary oedema.[15] The degree of hypoxaemia appears to be dose-dependent[16] and more severe in elderly than juvenile sheep.[17] Moreover, the degree of hypoxaemia caused by xylazine in sheep is potentially more severe than that caused by detomidine.[18] To avoid potential serious side effects in this old sheep, a relatively conservative concentration of detomidine both as a premedicant and as an adjuvant was administered. To our knowledge, the use of detomidine as an adjuvant in peripheral nerve blocks has not been described. However, detomidine did not prolong the action of lidocaine, either because the concentration used was too small or detomidine was ineffective for this purpose.

In the veterinary literature, only one case report describes a successful paravertebral brachial plexus block in a sheep.[4] However, the authors used the local anaesthetic bupivacaine, which is not listed in the table of allowed substances. Therefore, it was not possible to follow their protocol in a clinical setting.

Butorphanol (mu-antagonist and kappa-agonist) and levomethadone (mu-agonist) are opioids listed in the table of allowed substances, and although only licensed for *Equidae*, they can be administered to other food-producing species under the cascade, provided that the minimum withdrawal period is employed (28 days for meat and 7 days for milk). Unfortunately, levomethadone is not available in Ireland. In sheep, butorphanol provides visceral more than somatic analgesia and its duration of action is short (up to 120 minutes).[19]

There are no non-steroidal anti-inflammatory drugs licenced for sheep in Ireland, however they are commonly administered to provide pain relief following orthopaedic surgery.[20] In accordance with the cascade, flunixin was started when the sheep was admitted into the hospital, and the last dose was given 3 hours before the operation. Flunixin has a half-life of less than 4 hours in sheep.[21] Meloxicam, which is licenced in cattle, has a long mean residence time (15.13±1.67 hours) in sheep[22] and can potentially provide analgesia for several hours. Therefore, meloxicam was administered under the cascade on the day of surgery and daily thereafter for 10 days. Although according to some reports meloxicam may cause a delay in bone healing,[23] the results are contradictory,[24,25] and its administration was based on a risk–benefit ratio.

Ketamine exerts its analgesic effect by antagonizing NMDA receptors, preventing both acute and chronic pain.[26] Moreover, a ketamine CRI has a sparing effect on minimum alveolar concentration (MAC) of isoflurane in dogs,[27] cats[28] and rabbits.[29] This sparing effect has not been studied in sheep. The MAC of isoflurane in sheep is 1.53%,[30] but in this case report anaesthesia was maintained with a F_E'Iso ≤1.2% throughout, even after the local block wore off. It is likely that ketamine contributed to intraoperative analgesia and reduced anaesthetic requirements. Although the pharmacokinetics of ketamine have not been characterised in sheep, it accumulates in humans when given at a rate greater than 14 μg/kg/min, leading to dysphoria.[31] In order to avoid any possible excitement that could affect the quality of recovery and outcome of surgery, we decided against increasing the rate or administering a bolus of ketamine when the effect of the local anaesthetic wore off. Isoflurane, licensed for *Equidae* and piglets, was given under the cascade.

Anaesthesia and pain management in food-producing animals, especially sheep, can be challenging due to the limited number of authorised drugs available, the potential for severe adverse effects and the limited duration of action of authorised local anaesthetics. Moreover, sheep are stoic animals[32] and it is difficult to recognise signs of distress and discomfort when they are in pain.[33] Methods used to assess pain in sheep are either subjective (identification of behavioural changes, degree of lameness) or objective (measurement of stress response, cerebral evoked potentials and nociceptive threshold).[2] Grimace scales have been used in sheep both in clinical[34] and experimental[35] conditions. While they seem to correlate well with the presence of pain, stress or fear, these scales are not validated and no other validated pain scales exist for this species.[1] In this sheep, pain was assessed by evaluating its food intake and the presence of teeth grinding and lameness. Post-operative analgesia was provided with meloxicam. No pain was observed – or recognised – and therefore no additional analgesia was administered.

In summary, very few analgesic drugs are authorised for food-producing animals. In this case report analgesia for a fracture repair in a sheep was provided with a cervical paravertebral nerve block using the drugs legally available. Unfortunately, the detomidine dose added to the regional block solution failed to prolong the duration of action of lidocaine, leading to insufficient duration of intra-operative analgesia. Further work is required to develop techniques by which the duration of regional anaesthesia in sheep can be extended within the confines of licenced drugs.

TABLE 1 TAULUKKO

Arterial blood gas values measured 80 minutes after start of surgery at the time of a sudden increase in heart rate and blood pressure.

Verikaasuanalyysin tulokset sykkeen ja verenpaineen äkisti noustessa 80 minuuttia ortopedisen leikkauksen alkamisesta.

pH	PaO_2 (kPa)	$PaCO_2$ (kPa)	B.E.	Bicarbonate (mEq L^{-1})	SaO_2 (%)	FiO_2 (%)	$P_E'CO_2$ (kPa)
7.40	49.9	5.7	1.7	26.1	99.7	84	5.7

PaO_2 = partial pressure of arterial oxygen; $PaCO_2$ = partial pressure of arterial carbon dioxide; B.E. = base excess; SaO_2 = arterial oxygen saturation; FiO_2 = inspired fraction of oxygen; $P_E'CO_2$ = end-tidal carbon dioxide tension.

PaO_2 = valtimoveren happiosapaine; $PaCO_2$ = valtimoveren hiilidioksidiosapaine; B.E. = emäsylimäärä; SaO_2 = valtimoveren happisaturaatio; FiO_2 = sisäänhengitysilman happiosuus; $P_E'CO_2$ = uloshengitysilman hiilidioksidiosapaine.

REFERENCES

1. McLennan K. Why pain is still a welfare issue for farm animals, and how facial expression could be the answer. Agriculture 2018;8:127.
2. Lizarraga I, Chambers JP. Use of analgesic drugs for pain management in sheep. New Zealand Vet J. 2012;60:87-94.
3. Hodgkinson O, Dawson L. Practical anaesthesia and analgesia in sheep, goats and calves. In Pract. 2007;29:596-603.
4. Rodrigo-Mocholí D, Schauvliege S. Paravertebral brachial plexus blockade as part of a balanced anaesthesia in a sheep undergoing thoracic limb amputation. Vet Anaesth Analg. 2016;43:239-40.
5. Swerdlow M, Jones R. The duration of action of bupivacaine, prilocaine and lignocaine. Br J Anaesth. 1970;42:335-9.
6. May NDS. The Anatomy of the sheep: The University Of Queensland Press; Brisbane; 1955.

7. Ip VH, Tsui BC. Practical concepts in the monitoring of injection pressures during peripheral nerve blocks. Int Anesth Clin. 2011;49:67-80.
8. Atanassoff PG, Kelly DJ, Ayoub CM, Brull SJ. Electromyographic assessment of ulnar nerve motor block induced by lidocaine. J Clin Anesth. 1998;10:641-5.
9. Hussain N, Grzywacz VP, Ferreri CA, Atrey A, Banfield L, Shaparin N, et al. Investigating the efficacy of dexmedetomidine as an adjuvant to local anesthesia in brachial plexus block: a systematic review and meta-analysis of 18 randomized controlled trials. Reg Anesth Pain Med. 2017;42:184-96.
10. Bartel AKG, Campoy L, Martin-Flores M, Gleed RD, Walker KJ, Scanapico CE, et al. Comparison of bupivacaine and dexmedetomidine femoral and sciatic nerve blocks with bupivacaine and buprenorphine epidural injection for stifle arthroplasty in dogs. Vet Anaesth Analg. 2016;43:435-43.
11. Brummett CM, Hong EK, Janda AM, Amodeo FS, Lydic R. Perineural dexmedetomidine added to ropivacaine for sciatic nerve block in rats prolongs the duration of analgesia by blocking the hyperpolarization-activated cation current. Anesth J Am Soc Anesth. 2011;115:836-43.
12. Ouchi K, Koga Y, Nakao S, Sugiyama K. Dexmedetomidine dose-dependently enhances local anesthetic action of lidocaine. J Oral Maxillofac Surg. 2014;72:474-80.
13. Rostami M, Vesal N. The effects of adding epinephrine or xylazine to lidocaine solution for lumbosacral epidural analgesia in fat-tailed sheep. J South Afr Vet Assoc. 2012;83:7-14.
14. Ghadirian S, Vesal N. Brachial plexus block using lidocaine/epinephrine or lidocaine/xylazine in fat-tailed sheep Vet Res Forum 2013;4:161.
15. Kästner SBR. A_2-agonists in sheep: a review. Vet Anaesth Analg. 2006;33:79-96.
16. Talke PO, Traber DL, Richardson CA, Harper DD, Traber LD. The effect of α_2 agonist-induced sedation and its reversal with an α_2 antagonist on organ blood flow in sheep. Anesth Analg. 2000;90:1060-6.
17. Ko JC, McGrath CJ. Effects of atipamezole and yohimbine on medetomidine-induced central nervous system depression and cardiorespiratory changes in lambs. A J Vet Res. 1995;56:629-32.
18. Nolan A, Livingston A, Waterman A. The effects of alpha$_2$ adrenoceptor agonists on airway pressure in anaesthetized sheep. J Vet Pharmacol Therap. 1986;9:157-63.
19. Waterman AE, Livingston A, Amin A. Analgesic activity and respiratory effects of butorphanol in sheep. Res Vet Sci. 1991;51:19-23.
20. Taylor PM. Anaesthesia in sheep and goats. In Pract. 1991;13:31-6.
21. Welsh EM, McKellar QA, Nolan AM. The pharmacokinetics of flunixin meglumine in the sheep. J Vet Pharmacol Therap. 1993;16:181-8.
22. Shukla M, Singh G, Sindhura BG, Telang AG, Rao GS, Malik JK. Comparative plasma pharmacokinetics of meloxicam in sheep and goats following intravenous administration. Comp Biochem Physiol Part C Toxicol Pharmacol. 2007;145:528-32.
23. Simon AM, Manigrasso MB, O'Connor JP. Cyclo-oxygenase 2 function is essential for bone fracture healing. J Bone Miner Res. 2002;17:963-76.
24. Pablos AB, Ramalho SA, König Jr B, Furuse C, de Araújo VC, Cury PR. Effect of meloxicam and diclofenac sodium on peri-implant bone healing in rats. J Periodontol. 2008;79:300-6.
25. Al-Mashhadane AM, Taqa GAA, Al-Sandook TA. Effects of meloxicam on serum level of bone alkaline phosphatase, osteocalcin and bone density in a sheep model. IJERSTE. 2014;3:57-63.
26. Sleigh J, Harvey M, Voss L, Denny B. Ketamine – More mechanisms of action than just NMDA blockade. Trends Anaesth Crit Care 2014;4:76-81.
27. Gutierrez-Blanco E, Victoria-Mora JM, Ibancovichi-Camarillo JA, Sauri-Arceo CH, Bolio-González ME, Acevedo-Arcique CM, et al. Evaluation of the isoflurane-sparing effects of fentanyl, lidocaine, ketamine, dexmedetomidine, or the combination lidocaine-ketamine-dexmedetomidine during ovariohysterectomy in dogs. Vet Anaesth Analg. 2013;40:599-609.

28. Pascoe PJ, Ilkiw JE, Craig C, Kollias-Baker C. The effects of ketamine on the minimum alveolar concentration of isoflurane in cats. Vet Anaesth Analg. 2007;34:31-9.
29. Gianotti G, Valverde A, Sinclair M, Dyson DH, Gibson T, Johnson R. Prior determination of baseline minimum alveolar concentration (MAC) of isoflurane does not influence the effect of ketamine on MAC in rabbits. Can J Vet Res. 2012;76:261-7.
30. Bernards CM, Kern C, Cullen BF. Chronic cocaine administration reversibly increases isoflurane minimum alveolar concentration in sheep. Anesthesiol J Am Soc Anesth. 1996;85:91-5.
31. Bowdle AT, Radant AD, Cowley DS, Kharasch ED, Strassman RJ, Roy-Byrne PP. Psychedelic effects of ketamine in healthy volunteers: relationship to steady-state plasma concentrations. Anesth J Am Soc Anesth. 1998;88:82-8.
32. National Research Council. Recognition and alleviation of pain, stress and distress. In: Recognition and alleviation of pain and distress in laboratory animals. Washington, DC: National Academy Press, 1992;35–52.
33. Underwood WJ. Pain and distress in agricultural animals. J Am Vet Med Assoc. 2002;221;208-11.
34. McLennan KM, Rebelo CJ, Corke MJ, Holmes MA, Leach MC, Constantino-Casas F. Development of a facial expression scale using footrot and mastitis as models of pain in sheep. Appl Anim Behav Sci. 2016;176:19-26.
35. Häger C, Biernot S, Buettner M, Glage S, Keubler LM, Held N, et al. The Sheep Grimace Scale as an indicator of post-operative distress and pain in laboratory sheep. PloS One, 2017;12:1-15.

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