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Use and Efficacy of Analgesic Agents in Sheep (*Ovis aries*) Used in Biomedical Research

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Sheep (*Ovis aries*) are widely used as large animal models in biomedical research. However, current literature on the use of analgesics in sheep generally focuses on an industry or farm level of use. This structured review evaluates use and efficacy of analgesics administered to sheep in a biomedical research setting. Electronic databases were searched with terms related to analgesia in research sheep. After application of exclusion criteria, 29 peer-reviewed publications were evaluated from 1995 to 2018. Drugs used for analgesia in sheep include opioids, α_2 agonists, NSAID, local anesthetics, NMDA receptor antagonists, and calcium channel blockers. Opioid agonists have previously been considered short acting and of questionable efficacy in sheep, but newer modalities may provide effective analgesia. NSAID may exhibit an analgesic effect only when inflammatory pain is present and may not be beneficial for use in acute pain models. α2 agonists provide effective yet short-lived analgesia; however, side effects are of concern. Local anesthetics were previously widely used as stand-alone agents, as alternatives to the use of general anesthetics in sheep. These agents have since fallen out of favor as sole agents. Despite this, they provide a valuable analgesic effect when used as adjuncts to general anesthetic regimes. The NMDA antagonist ketamine provided good analgesia and is likely underutilized as an analgesic agent in sheep. Future controlled studies should further evaluate the analgesic properties of ketamine in sheep.

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Current regulatory requirements governing the care and use of animals in research frequently cite the need for provision of analgesics. For example, The Australian Code for the Care and Use of Animals for Scientific Purposes,[48] states that analgesic use must be considered as part of a plan to manage pain and distress. The choice of agent and administration route need to be appropriate for the species and life stage of the animal as well as compatible with the purpose and aims of the project.[30] Provision of pain relief for experimental animals is an important issue that is growing in light of public awareness and interest in animal welfare in animal-based research.[23]

Sheep (*Ovis aries*) are widely used as models in biomedical research; their large size simplifies surgery, they are easy to handle, and are physiologically similar to humans.[60] A considerable proportion of their use in biomedical research involves surgical intervention, for example, in orthopedic research, cardiovascular investigations, maternal–fetal medicine. These interventions can be expected to cause considerable pain. Evidence from research establishments in the United Kingdom indicates that detailed clinical records of signs of pain are frequently kept for large animals.[29] However, pain assessment in sheep is notoriously difficult due to their stoic nature. Furthermore, researchers have difficulty finding appropriate analgesic protocols for sheep, given the paucity of literature focusing on anesthesia and analgesia in this species.

This structured review therefore provides a summary of analgesics investigated in sheep undergoing procedures in a biomedical research setting. We also sought to provide general recommendations regarding analgesic effectiveness in this species and avenues for future research.

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Search Methods

The electronic databases PubMed, Google Scholar, and The Web of Science for the years 1995 through 2018 were searched by using terms related to analgesia in research sheep. Search terms included: sheep analgesia, sheep analgesic, ovine analgesia, ovine analgesic, sheep opioids, sheep α_2 agonists, sheep NSAID, and sheep local anesthetic. Only articles with full text available and that were English language publications, or those with translation, were included. Articles obtained through Google Scholar were checked to ensure that they were peer-reviewed, scholarly articles, and, as a final check, were cross-referenced in PubMed and The Web of Science. 'Gray' literature was included when retrieved through the described searches. Case reports were not included. After the database searches, the retrieved articles were evaluated on the basis of the title and abstract to ensure that the administered dose of analgesic agent was recorded, a method of pain assessment was included, controls or between-group comparisons were present, and that studies related to sheep in a biomedical research setting. All papers included for data extraction had a detailed methods section. In several studies, analgesics were administered as part of a balanced anesthesia protocol, but these agents were not the subject of investigation. In those cases, only data regarding the analgesic agent under study, as well as control data, were collected. This type of study design does cause difficulties in interpretation of the absolute analgesic effect of the agents studied. Pain assessment methods included those measuring nociceptive or effective pain response, provided outcomes were quantifiable in nature. Reference lists of included papers were also checked for further literature which met the inclusion criteria.

Search Results

Initial searches produced 494 results. After applying inclusion criteria and excluding duplicates, a total of 29 peer-reviewed

Table 1. Studies included in the current review.

Authors	Year of publication	Main drug class studied	Reference
Ahern and colleagues	2009	Opioid	2
Bigham and Shafiei	2008	Local anesthetic	3
Bortolami and colleagues	2015	Opioid	5
Dadafarid and Najafpour	2008	Local anesthetic, miscellaneous	10
DeRossi and colleagues	2006	α2 agonist, local anesthetic	14
DeRossi and colleagues	2012	Opioid, local anesthetic	12
DeRossi and colleagues	2015	Opioid, local anesthetic	11
DeRossi and colleagues	2017	Opioid, local anesthetic	13
Durej and colleagues	2012	Opioid	17
Ghadirian and Vesal	2013	α2 agonist, local anesthetic	21
Ghadirian and colleagues	2016	Opioid, local anesthetic	22
Grant and colleagues	2001	α2 agonist	23
Grant and Upton	2004	α2 agonist	24
Guedes and colleagues	2006	Miscellaneous	25
Habibian and colleagues	2011	Opioid, local anesthetic	26
Haerdi-Landerer and colleagues	2005	α2 agonist	27
Kania and colleagues	2009	Miscellaneous	31
Lizarraga and Chambers	2006	NSAID	36
Lucky and colleagues	2007	Local anesthetic	39
Ludbrook and colleagues	1995	Opioid, α2 agonist	40
Moolchand and colleagues	2014	α2 agonist	42
Murdoch and colleagues	2013	α2 agonist	44
Musk and colleagues	2014	Opioid, α2 agonist	45
Otto and colleagues	2000	Opioid	53
Otto and colleagues	2000	Opioid	54
Rostami and Vesal	2012	α2 agonist, local anesthetic	59
Shafford and colleagues	2004	Local anesthetic	62
Wagner and colleagues	2011	Local anesthetic	67
Walkowiak and Graham	2015	Opioid	68

publications were reviewed (Table 1). Analgesics used in these publications included opioids, α_2 agonists, NSAID, miscellaneous agents, and local anesthetics.

Assessment of Pain

In the studies reviewed, a variety of pain assessment techniques were described (Figure 1). Several papers that we reviewed used measures of nociception. These included: application of mechanical noxious stimuli,[5,22,36,42,45] electrical stimulus,[23,24,27,40] and thermal stimulation.[45,67] These studies recorded time to voluntary leg withdrawal from the stimulus. Other studies utilized superficial or deep muscle pinpricks,[3,11-14,17,21,22,26,39,42,59] as an alternative method of mechanical stimulation of so-called 'first-pain.'[9] This method tends to evaluate analgesia by using a scale based on reaction to the stimulus.[14] To assess diffusion or extent of analgesia, skin pricks and deep muscle pricks are made in adjacent dermatomic regions, beginning at the tail and moving cranially.[14] Noxious stimuli activate afferent nociceptors, which trigger multiple levels of information processing, not all of which go through higher brain centers. This higher-order brain processing creates the experience of pain, which is considered to influence animal wellbeing.[49] Therefore, these methods have been criticized because they do not provide information regarding the presence of affective pain. In contrast, affective pain assessment using measures of behavior or movement was used in 7 studies.[2,25,31,44,54,62,67] Observations paired with measurement of other parameters, for example, heart rate or cardiac output, were another commonly used assessment technique.

Opioids

Opioid agonists have frequently been considered as less effective analgesics in sheep than in other species,[32] with high doses causing excitation and behavioral changes.[51] Conversely, other authors suggest that opioids are effective analgesics, especially for visceral pain.[19] Table 2 summarizes the studies that utilized opioid agents.

Adverse side effects of opioids include pruritus, nausea, vomiting, urinary retention, and respiratory depression.[26] Opioids were used as analgesic agents in 13 of the 29 papers reviewed, with mixed findings concerning side effects. One group administered the opioids alfentanil and pethidine, both of which produced significant agitation in sheep.[40] This agitation manifested as irregular head and limb movement, nystagmus, and chewing. Agitation was believed to be secondary to excitatory effects and rendered pain observations unreliable.

Buprenorphine is a partial μ-opioid receptor agonist and κ antagonist. Studies in sheep have found this drug to induce thermal but no mechanical antinociception over a wide dose range.[50] Buprenorphine was used in 4 of the 29 articles reviewed. Buprenorphine provided effective postoperative analgesia after tibial osteotomy, determined through use of a numerical rating scale.[54] However, buprenorphine caused sedation and decreases in blood pressure, heart rate, and respiration when administered

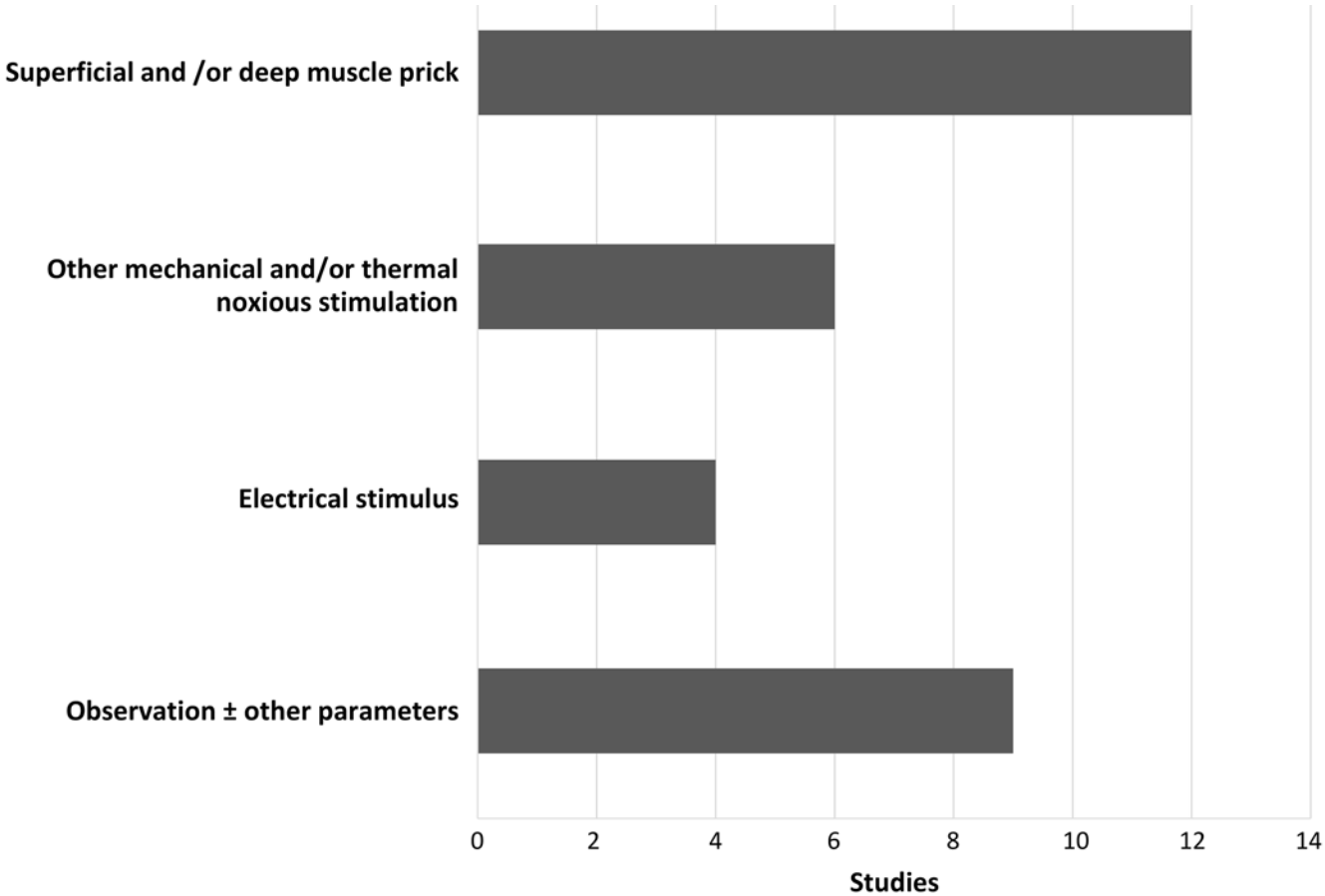

Figure 1. Methods of pain assessment in the studies reviewed. Note that several studies used multiple methods.

epidurally. These effects were attributed to buprenorphine's highly lipophilic nature, which allowed ready absorption by epidural vessels.[53] As such, the benefits of systemic side effect reduction, usually associated with epidural administration do not exist for buprenorphine. Similarly, benefits of epidural administration of other lipophilic opioids, including fentanyl, alfentanil, and sufentanil, might also be called into question.[53] Sustained-release buprenorphine had comparable magnitude of antinociceptive effect but longer duration of action than the standard formulation.[68]

Butorphanol, a κ agonist and partial μ agonist, was used in only one reviewed study.[17] Sheep given butorphanol intramuscularly required a 28% increase in isoflurane minimum alveolar concentration for maintenance of anesthetic depth, compared with sheep that received epidural morphine and bupivacaine. In addition, sheep given butorphanol had higher heart rates and an increased rate of bloating after recovery. Furthermore, butorphanol provided poorer muscle relaxation and analgesia during orthopedic surgery than epidural morphine and bupivacaine.

Fentanyl, a κ and μ agonist, is 80 to 100 times as potent as morphine. The use of a transdermal patch is beneficial since it reduces or eliminates multiple daily administrations by parenteral routes. Patches, therefore, provide reliable, minimally invasive, and extended analgesia.[2] Transdermally administered fentanyl provided superior analgesia in sheep undergoing unilateral tibial osteotomy, compared with intermittent IM administration of buprenorphine.[2] An observational pain score was used and included measures of respiratory rate, willingness to rise from a recumbent position, apparent level of comfort standing on the limb, ability to move, mental demeanor, appetite, and behavior on palpation of the surgery site. Pain assessment scores for fentanyl- and buprenorphine-treated sheep decreased linearly, but sheep treated with fentanyl had consistently lower pain scores. This effect was likely due to the ceiling effect of buprenorphine as a result of partial agonist activity.[2] Conversely, fentanyl patch application caused no change in thermal or mechanical nociceptive thresholds after laparotomy in pregnant sheep, although the authors noted that animals had received ketamine and buprenorphine during anesthesia.[45]

Caution is needed when using transdermal patches, because the rate of drug delivered is dependent on body temperature.[56] The dose may increase by as much as 1/3 if the body temperature at the site of application reaches 40 °C. Therefore, placement sites that the sheep are likely to lie on should be avoided, for example by placing patches on the lateral portion of the antebrachium[62] or the forelimb ipsilateral to the operated hindleg.[2]

Methadone was included in 2 studies.[11,13] In one study, when administered alone, epidural methadone produced moderate length analgesia and mild to moderate motor blockade, with minimal effect on physiologic parameters.[11] Combination with bupivacaine produced a reduced duration of analgesia.[11] This effect can be considered beneficial because reducing the dosage of each drug reduces the risk of side effects, especially those seen with epidural bupivacaine. In a later study by the same authors using the bupivacaine–methadone combination, moderate motor blockade was produced, but mild sedation was reported, likely as a result of the opioid component.[13]

Morphine, a full μ agonist, was administered epidurally to induce analgesia in one study.[12] Only mild analgesia resulted. However, the method of pain assessment used in the trial, which

Table 2. Opioids used as analgesics in the papers reviewed.

Opioid agent	Action at opioid receptors	Dose	Route	Summary	Reference
Alfentanil	μ and κ agonist	1 or 2 mg	IV	Produced agitation, as manifested by irregular movements of the head and limbs, nystagmus, and chewing. This rendered pain assessment method unreliable.	40
Buprenorphine	Partial μ and κ agonist, δ antagonist	0.01 mg/kg	IM	Less effective than fentanyl, according to pain scores	2
		0.005 mg/kg	IM, epidural	No clinical advantages of epidural administration compared with systemic in the provision of analgesic effect	53
		0.01 mg/kg	IM	Effective analgesic following tibial osteotomy based on behavioral score.	54
		0.27 mg/kg (sustained-release formulation)	IM, SC	Lack of response to thermal nociception for 72 h with one dose of sustained release formulation or 8 hourly dosing of standard formulation. Sustained release advantageous due to reduced handling required.	68
Butorphanol	μ antagonist to partial agonist,κ agonist	0.2 mg/kg	IM	Needed 27% more isoflurane and had shorter and less complete analgesia than epidural morphine–bupivacaine group (see below).	17
Fentanyl	μ agonist, κ agonist	0.002 mg/kg/h	Transdermal	Pain score was reduced (in comparison to buprenorphine)	2
		0.075 mg/h	Transdermal	No significant change in thermal or mechanical thresholds following laparotomy in pregnant sheep	45
Fentanyl + bupivacaine		0.002 and 0.25 mg/kg	Epidural	Reduced duration of analgesic effect compared with bupivicaine or bupivicaine–methadone (180 compared with 240 min). Produced moderate motor blockade and sedation.	13
Methadone, methadone + bupivacaine	μ agonist + NMDA antagonist	0.3 mg/kg	Epidural	Analgesia duration of 220 min, as determined by using deep muscle stimulation. No effect on physiologic parameters.	11
		0.15 and 0.25 mg/kg	Epidural	Analgesia duration of 180 min, as determined by using deep muscle stimulation. No effect on physiologic parameters	11
		0.25 and 0.3 mg/kg	Epidural	Similar duration of action to bupivacaine alone (240 min). Produced moderate motor blockade and sedation.	13
Morphine	μ agonist	0.1 mg/kg	Epidural	Mild analgesia	12
Morphine + bupivicaine		0.25 mg/kg	Epidural	Combination produced complete analgesia to thorax and forelimb: onset, 5 to 10 min; duration 140 min. Provided longer duration of analgesia than either morphine or bupivacaine alone.	12
		0.05 mg/kg	Epidural	Onset of analgesia, 13 min; duration, 195 min	17
		0.1 and 1 mg/kg			

Table 2. Continued.

Opioid agent	Action at opioid receptors	Dose	Route	Summary	Reference
Morphine + lidocaine		0.1 and 4.8 mg/kg	Nerve block	The addition of morphine to lidocaine did not alter the duration of antinociception compared with lidocaine alone during brachial plexus block.	22
Pethidine	μ agonist	100 and 300 mg	IV	Produced agitation rendering pain assessment unreliable	40
Piritramide	μ agonist	0.57 mg/kg	IM	Effective postoperative analgesia as determined by behavioral score. Not as effective as buprenorphine.	54
Tramadol	Weak μ agonist + serotonin reuptake inhibitor	1 mg/kg	Epidural	Onset of action, 14 min; duration of analgesia, 319 min	26
		4 mg/kg	IV	No mechanical antinociceptive effects produced during 12 h of measurement	5
		6 mg/kg			
Tramadol + lidocaine		1 mg/kg	Epidural	Onset of action, 6 min; duration of analgesia, 100 min	26
		2.46 mg/kg	Nerve block	The addition of tramadol to lidocaine did not alter the duration of antinociception compared with lidocaine alone during brachial plexus block.	22
		1 and 4.6 mg/kg			

comprised the application of superficial and deep noxious stimuli, only allowed for measurement of more profound analgesia in the sheep. Opioids act mainly on small unmyelinated afferent nerves through hyperpolarization of the axons. However, a sharp needle prick given as part of the pain assessment method used may still be transmitted by myelinated nociceptive fibers. This stimulation may have caused unmyelinated fibers to depolarize despite altered resting membrane potential. Therefore, superficial analgesia may have been present that was undetectable due to the nature of the pain perception test used.[12] Nevertheless, the authors concluded that the use of epidural morphine alone did not provide sufficient analgesia in sheep.

When tested in species other than sheep, morphine combined with bupivacaine produces superior analgesia to either agent used alone.[35,38] Two studies support this finding in sheep and further suggest that morphine alone provides unsatisfactory analgesia.[12,17] This combination provided an extended duration of analgesia compared with that of either bupivacaine or morphine alone. In addition, the demand for inhalant anesthetic was reduced relative to morphine alone.[17] However, prolonged sympathetic motor blockade may result due to the local anesthetic. When motor blockade extends into the cervical region, it can affect diaphragmatic function, through alteration of phrenic nerve stimulation.[4] Depressed ventilation with the combination was noted in one study reviewed but did not exceed limits considered clinically important.[12] Conversely, another study showed no depression in respiration.[17] In summary, epidural administration of morphine and bupivacaine provides adequate analgesia for sheep, through a technique that is relatively easy to perform by veterinarians. Side effects, such as muscle paralysis and depressed ventilation, may arise and require suitable management.

Piritramide is a synthetic full μ-opioid receptor agonist that was evaluated in one study in which sheep underwent tibial osteotomy.[54] The data obtained were in agreement with other research and determined the duration of analgesia to be between 6 and 8 h.[33] Pretreatment with piritramide provided effective postoperative analgesia, but the agent appeared to have less effect than buprenorphine.

Tramadol has 2 mechanisms of action: first as a μ-opioid receptor agonist and second as an inhibitor of the reuptake of norepinephrine and serotonin.[55] Studies in other species have suggested that epidural tramadol can provide prolonged postoperative analgesia without serious side effects.[47,70] These pharmacologic effects make tramadol an attractive drug for epidural administration in sheep. Tramadol had a duration of analgesia of 319 min when assessed by response to a pinprick test and pressure from a hemostatic clamp. However, increased heart rate and decreased respiratory rate occurred.[26] Conversely, Bartolome, and colleagues 2015 discovered no mechanical antinociceptive effects of tramadol, but this study involved IV administration.[5] Given the side effects seen with tramadol alone, further research is required to determine appropriate dose rates for epidural administration in small ruminants. To counteract these side effects, one group used a combination of tramadol and 2% lidocaine as an alternative analgesic regimen.[26] This combination not only eliminated the side effects seen with tramadol alone but reduced the ataxia associated with epidural lidocaine. However, in another study using this combination as part of a nerve block, the addition of tramadol to lidocaine offered no improved benefit over lidocaine alone, although, in light of the lack of information on optimal tramadol dose in sheep, dosing may have been too low for effect.[22]

α2 Agonists

α-adrenergic agonists bind to receptors on vascular smooth muscle, inducing contraction and vasoconstriction. Two types of adrenoceptors are contained in vascular smooth muscle: α1

and α_2. Therapeutic agents are generally selective for either of these receptors, with α_2 agents predominating in veterinary species because of their sedative effects.[58]

The first α_2 agonist used for veterinary species was xylazine, in the late 1960s. Since then α_2 agonists have become widespread sedatives, analgesics, and anesthetic adjuncts in animals.[32] Early studies on the use of α_2 agonists in sheep reported anesthetic deaths and pulmonary edema after administration of xylazine in various breeds.[58,66] Newer reports on α_2 agonists describe pulmonary edema and hypoxemia.[31] The mechanism of analgesic action is through the hyperpolarization of neurons due to the opening of K^+ channels after agonist binding at α_2-adrenergic receptors.[37] α-2 adrenoceptors are widely distributed throughout the body and are densely expressed in the laminae I–II in the sheep spinal cord.[6] Analgesia is mediated mainly in the spinal cord,[34] with cholinergic and nitric oxidergic mechanisms also contributing to the analgesic effect.[15,69] The purported low analgesic efficacy of opioid agonists in sheep[32] and the finding that high doses of opioids cause excitation and behavioral change[51] have resulted in α_2 agonists becoming the drug of choice for effective sedation and analgesia in sheep.[32] However, careful management of side effects is required through evidence-based dose selection and administration route and close clinical monitoring.

Clonidine (Table 3) produces analgesia by α_2-adrenergic action in the dorsal horn of the spinal cord. In the one study in which clonidine was administered via the subarachnoid space, an analgesic duration of 99 min was acheived, as assessed through superficial and deep muscle pricks.[14] This finding was consistent with data from previous sheep studies.[49] The administration produced a decrease in heart rate and arterial blood pressure,[14] as reported previously after intrathecal and extradural administration.[7] Clonidine produced effective analgesia in the tail, perineum, hind limbs, flank, and caudodorsal rib areas, whereas administration of lidocaine through the same route produced analgesic effects limited to the perineal and upper hindlimbs.[14] However, side effects of increased salivation and frequent urination were evident after clonidine use.

Detomidine is a lipophilic α_2 agonist that was used intrathecally in one study reviewed.[27] An increase in pain threshold was produced. However, this increase was less than 50% of that produced by xylazine when similarly administered. The intrathecal route of administration was selected to minimize the risk of misinjection, but the dose rate was selected in light of sedative and analgesic effects seen after intravenous injection in other studies. However, detomidine is lipophilic; after intrathecal injection, it is taken up into extradural fat and plasma. In contrast, systemic administration enables more effective and rapid penetration of the blood–brain barrier. As such, the dose rate used in the study[27] was likely inadequate and rendered comparison between detomidine and xylazine misleading. In addition, side effects of pollakisuria and diuresis were noted, in accord with other findings.[64] Interestingly, in light of the previous discussion on dose rates, a later study failed to show an analgesic effect of detomidine when given IV, despite a 4-fold increase in dosage.[42]

Medetomidine has the highest selectivity for the α_2 receptor subtype of all the α_2 agonists currently used. Many adverse side effects, including hypoxemia, peripheral vasoconstriction, bradycardia, and uterine muscle contraction, are associated primarily with α1 adrenoreceptor stimulation. It would thus be assumed that the use of medetomidine negates these side effects.[44] However, many side effects are still of concern, with hypoxemia being the biggest risk.[44] When administered by using an osmotic pump in the abdominal cavity, pregnant sheep receiving medetomidine had lower, more stable pain scores than a control group.[44] Administration through an osmotic pump reduces the peaks, and subsequent troughs, associated with repeat doses and prevents other side effects that result from high plasma concentrations, including increased systemic and pulmonary vascular resistance, heart rate, and cardiac output.[44] In a later follow-up study using nociceptive tests, medetomidine use led to an increase in the thermal nociceptive threshold but caused no change in mechanical threshold, thus providing some suggestion of analgesic effectiveness, and was superior to the fentanyl patch tested in the same study.[45]

Xylazine is 10 to 20 times more potent in ruminants than in other species.[24] One group compared the analgesic effects of xylazine when administered through intravenous, intramuscular, and subcutaneous routes, as assessed by response to an electrical stimulus. When administered intravenously, xylazine caused a rapid but brief increase in antinociception, which lasted 25 mins.[24] The sedative effects of xylazine increase with dose, with doses above 50 µg/kg IM causing sedation. Xylazine given by the intramuscular route resulted in a longer analgesic response with a duration of 40 min. This effect is likely due to the relatively rapid peak analgesic affect and slow washout phase. In addition, fewer cardiovascular side effects were produced after intramuscular than intravenous administration.[24] Subcutaneous administration produced more variable drug effects, which the authors assumed to result from the dependence on the rate of local skin perfusion.[24]

NSAID

NSAID have antiinflammatory and analgesic effects. They also reduce fever and inhibit platelet aggregation. These effects are mediated through their inhibition of cyclooxygenase, thus decreasing prostaglandin production. In sheep, some NSAID provide longer lasting analgesia in the absence of inflammation than others.[36] For example, flunixin, dipyrone, ketoprofen and tolfenamic acid have a more extensive duration of action in the absence of inflammation compared to salicylic acid and phenylbutazone. This outcome may be a drug-dependent effect due to additional mechanisms of action for some NSAID. Other proposed mechanisms include activation of opioidergic and α2-adrenergic descending inhibitory systems and inhibition of the serotoninergic descending excitatory system. These systems converge on the spinal cord rather than in the periphery.[37] Table 4 presents a summary of the reviewed studies that used NSAID. Only one of the reviewed studies had an experimental design specifically focused on evaluation of NSAID,[36] therefore, limited information is available regarding the use of this class of drugs in sheep in biomedical research studies. Furthermore, the inclusion criteria for this review required a focus on studies in biomedical research and are likely to have excluded some NSAID studies in sheep. However, the type of procedures for which these drugs are administered in agricultural animals is likely quite different from the surgical models for which sheep are used often in biomedical research.

Side effects of NSAID include the prolongation of clotting times due to the inhibition of platelet thromboxane production and gastric ulceration due to the reduced production of abomasally protective prostaglandins. These side effects are more commonly associated with COX1-selective drugs.[19]

Carprofen is a member of the proprionic acid class of NSAID. It is a moderately potent inhibitor of phospholipase A2 and a weak inhibitor of COX, with preferential activity for COX2. No included studies examined carprofen. In a case study not included here for review, sheep that received caprofen had

Table 3. α2 agonists used as analgesics in the papers reviewed.

α2 agonist	Dose	Route	Summary	Reference
Clonidine	0.003 mg/kg	Subarachnoid	Time to analgesia onset 10 min, duration of action 99 min, as determined by using deep muscle stimulation. Moderate sedative effect	14
Clonidine + lidocaine	0.003 and 1.2 mg/kg	Subarachnoid	Longer duration of analgesia (187 min) than clonidine alone	14
Detomidine	0.01 mg/kg	Intrathecal	Pollakisuria, diuresis, slower onset and shorter duration than xylazine (drug was administered with atipamezole, which antagonized some side effects)	27
	0.04 mg/kg	IV	No analgesic effect was seen, according to deep needle pricking and incision	42
Medetomidine	0.003 mg/kg/h	Abdominal osmotic pump	Lower and more stable pain score than saline controls without sedation (study performed in pregnant sheep).	44
	0.003 mg/kg/h	Abdominal osmotic pump	Caused an increase in the thermal nociceptive threshold during the immediate postoperative period in pregnant sheep. No change in mechanical threshold. Superior to fentanyl patch, according to these outcomes.	45
	0.006 mg/kg	IV	No analgesic effect was seen, according to deep needle pricking and incision	42
Xylazine	0.05 mg/kg	Intrathecal	Caused pollakisuria, diuresis, and incontinence. Increased pain threshold with faster onset and longer duration of analgesic action than detomidine (drug was administered with atipamezole, which antagonized some side effects).	27
Xylazine + lidocaine	2 mg/h (5 mg loading dose, IM)	IV	Loading dose + constant rate infusion provides effective, predictable steady state analgesia	23
	2.5 mg	IV, SC, IM	IV rapid onset and highest peak analgesic values. Short duration of action of 25 min. IM and SC provide longer duration of analgesic action of 40 min and greater total analgesic response	24
	0.2 mg/kg	IV	Fairly rapid onset of action (6 min), short duration of 10 min, according to response to needle prick and incision.	42
	2.5, 5, and 10 mg	IM	Higher threshold current to produce limb withdrawal when given IV in comparison to similar dose IM	40
	2.2 mg	IV	Onset of action 7 min and duration of action 186m. Xylazine prolonged motor and sensory block	21
	0.05mg/kg and 5 mg/kg	Nerve Block	Onset of action 3 min, duration of action 148 min. Xylazine prolonged analgesia.	59
	0.05 and 4.8 mg/kg	Epidural		

secondary hyperalgesia and allodynia that persisted for 3 d after maxillofacial surgery, and haptoglobin concentrations were significantly increased.[1] Therefore carprofen did not prevent an acute inflammatory response and postsurgical hypersensitivity in this model.

Ketoprofen is a member of the proprionic acid class of NSAID, which has been shown to inhibit the cyclooxygenase and lipooxygenase pathways.[63] When administered using an intrathecal route with cumulative doses, ketoprofen did not increase the threshold of limb withdrawal due to noxious stimuli.[36] This result implied the lack of a direct spinal cord effect in sheep. However, the cumulative doses administered were within the range shown to inhibit COX1 and COX2 activity and produce analgesia in inflammatory pain models in other species.[16] Therefore, a direct spinal analgesic effect may only occur in the presence of inflammation. However, when ketoprofen was administered intravenously, pain threshold showed a 2-fold increase. The hypoalgesic effects of intravenous administration were reversed through combination with intrathecal opioid or α_2-adrenergic receptor antagonists. This finding implies that endogenous inhibitory mechanisms mediate the central analgesic effects of ketoprofen and other NSAID.[36]

Meloxicam preferentially inhibits COX2. This mechanism leads to potent antiinflammatory effects yet reduces the risk of side effects on the gastric mucosa , because the drug only weakly inhibits the biosynthesis of prostaglandins, which are protective in this region.[18] Although meloxicam does not seem to have been widely investigated in sheep from a biomedical research perspective, a recent study did evaluate its pain and inflammation-relieving properties in Merino sheep, with a focus on the use of meloxicam in production.[8] Pain was assessed through measures of weight-bearing, lameness score, forelimb raises, and noxious mechanical stimulus application after the application of a validated pain model, created through injection of turpentine into the proximal phalanx. All pain-related variables were decreased with escalating meloxicam dosages

Table 4. NSAID used as analgesics in the papers reviewed.

NSAID	Mechanism	Dose	Route	Summary	Reference
Ketoprofen	Proprionic acid, COX2 inhibitor	0.375–200 µM	Intrathecal	Thresholds were not raised	36
		3 mg/kg	IV	Pain threshold doubled. Hypoalgesic effect of intravenous ketoprofen was prevented by intrathecal naloxone or atipamezole.	
Phenylbutazone	Pyrazolone class- non selective COX inhibitor	0.375–200 µM	Intrathecal	Pain thresholds were not increased	36
		8 mg/kg	IV	Pain thresholds were not increased	
Salicylic acid	Monohydroxybenzoic acid, nonselective COX inhibitor	0.375–200 µM	IT	Pain thresholds were not increased	36
		10 mg/kg	IV	Pain thresholds were not increased	
Tolfenamic acid	Anthranilic acid derivative, nonselective COX inhibitor	0.375–200 µM	IT	Pain thresholds were not increased	36
		2 mg/kg	IV	Pain thresholds were increased and returned to baseline by 210 min	

to a maximum of 1 mg/kg, in a dose-dependent fashion. Of potential interest to researchers is the finding that the inflammation parameters measured were unaffected.[8]

Phenylbutazone is a member of the pyrazolone class of NSAID and is a nonselective COX inhibitor. No significant increase in threshold values to a mechanical noxious stimulus were recorded when phenylbutazone was administered intrathecally or intravenously.[36]

Salicylic acid is a slightly less potent inhibitor of COX2 than of COX1 and can uncouple oxidative phosphorylation.[61] Salicyclic acid had no analgesic effect when administered intrathecally in healthy sheep,[36] and intravenous salicyclic acid likewise had no analgesic effect in sheep.[36] However, aspirin has been shown to inhibit COX in sheep, as assessed by decreased serum thromboxane B2.[52] This result may be due to an action on the platelets. Studies of aspirin in cattle have similarly failed to show benefits to salicylic acid use.[57]

Tolfenamic acid is an anthranilic acid NSAID that preferentially inhibits COX2. When administered intrathecally to sheep, no analgesic effect occurred.[36] However, the model used an acute rather than inflammatory measure of pain, and the pain threshold was raised by using the intravenous route.[36] Because no peripheral inflammation was present in the sheep, this analgesic effect cannot be attributed to inhibition of COX enzymes in the periphery. Therefore, a central COX inhibitory mechanism must occur. Tolfenamic acid has been proposed to inhibit prostanoid synthesis in the dorsal horn of the spinal cord after the blood–brain barrier has been crossed.[46]

Local Anesthetics

Local anesthetics produce anesthesia by inhibiting excitation of nerve endings or blocking conduction in peripheral nerves. This effect is achieved through reversibly blocking voltage-gated sodium channels, thus reducing the sodium ion influx. Local and regional anesthetic techniques were once commonly used for ovine surgeries, because general anesthesia was deemed unsafe due to complications including tympanitis, regurgitation, and cardiopulmonary alteration.[17] The use of local anesthesia has since fallen out of favor.[66] Therefore, the focus has shifted toward the benefits of using local and regional anesthetics as analgesic adjuncts to general anesthesia (Table 5).

Bupivacaine was used in 8 of the 29 studies reviewed. The administration of a bupivacaine nerve block provided no clear analgesic benefit over the use of sham blocks after femorotibial joint surgery.[67] However, a comparatively low bupivacaine dose rate and the use of other analgesics may have confounded interpretation of the efficacy of the block.[67] Epidural bupivacaine decreased general anesthetic requirements, improved the quality of recovery, and prevented the central sensitization of nociceptive pathways after surgical procedures thus reducing the need for postoperative analgesia.[59] Duration of analgesic effect in comparison with the commonly used local anesthetic drug lidocaine was controversial. One study[22] suggested a longer duration with bupivacaine but another[39] found the converse. However, this difference may have arisen due to study design and the use of different dose rates, thereby complicating comparison of the studies.

Combinations of bupivacaine with other drugs may confer benefits. Bupivicaine in combination with morphine produced a longer duration of analgesia than either drug alone.[12] However, the addition of methadone to bupivacaine did not extend the analgesic effect.[13] Ketamine–bupivacaine, using a lower dose of bupivacaine compared to that used as a sole agent, resulted in a comparable state of analgesia to bupivacaine alone.[10]

Lidocaine was used in 11 of the 29 papers reviewed. In one study, lumbosacral epidural administration of lidocaine produced cutaneous analgesia that was limited to the perineal and upper hindlimbs.[14] This restricted effect was not observed in other reports, in which analgesia extended throughout the tail, perineum, hindlimbs, flank, and caudodorsal rib areas.[3,39] This limited effect may be attributed to the dose used (1.2 mg/kg), which was less than half that of the next lowest epidurally administered dose.

Large or repeated doses of local anesthetics may induce systemic toxicity, with clinical signs including opisthotonos, convulsions, hypotension, apnea, and death in severe cases. It is generally accepted that the total dose of lidocaine should not exceed 6 to 10 mg/kg.[19] A range of drugs has been combined with lidocaine to increase analgesic effectiveness, without increasing the local anesthetic dose.

The addition of epinephrine to lidocaine increased the duration of analgesia but prolonged the time to onset.[59] The

Table 5. Local anesthetic agents used as analgesics in the papers reviewed

Local anesthetics	Dose	Route	Summary	Reference
Bupivacaine, bupivacaine + lidocaine	0.5 mg/kg	Epidural	Complete analgesia to thorax and forelimb; onset, 5–10 min; duration of action, 70 min	12
	0. 5 mg/kg	Epidural	184 min of analgesia in caudal areas, which was longer than ketamine alone or bupivacaine–ketamine but did increase heart rate	10
	2.5 mL of 0.5%	Epidural	Mildly reduced duration of action (30 min) compared with lidocaine (34 min). Drowsiness, tympany, and shivering were observed.	39
	1.2 mg/kg	Epidural	Analgesia achieved postoperative period, onset – 8 min, duration of action 170 min. Greater duration than lidocaine.	59
	1 mg/kg	Nerve block	No clear benefit of femoral or sciatic nerve block in stifle surgery	67
	0.5 mg/kg	Epidural	Analgesia duration of 240 min as determined by deep muscle stimulation. No effect on physiologic parameters.	11
	1.25 mg/kg	Nerve block	Duration of sensory and motor blocks were considerably longer than for lidocaine and lidocaine-opioid combinations. No adverse effects noted.	22
	0.5mg/kg	Epidural	Duration of action of 240 min assessed by using superficial and deep muscle pricks. Limited cardiovascular and respiratory concern.	13
	10 and 40 mg	Intraarticular	Intraarticular analgesia present 5 to 7 h postoperatively, Data support use of this combination to provide additional anesthesia in joint surgery. Administered in addition to standard analgesic protocol of phenylbutazone and fentanyl patch.	62
Lidocaine (lignocaine)	2% (1 mL/7 kg)	Epidural	Faster onset than when combined with $MgSO_4$, but the duration of action was reduced (53 min).	3
Lidocaine + epinephrine	1.2 mg/kg	Subarachnoid	Cutaneous analgesia limited to the perineal and upper hind limbs. Duration of action, 55 min.	14
Lidocaine + $MgSO_4$	5 mg/kg (2% solution)	Nerve block	Onset of action, 11 min; duration, 100 min (shorter than with epinephrine or xylazine), determined according to superficial and deep pinprick	21
	2% (2.86 mg/kg)	Epidural	Onset of action, 4 min; duration, 54 min	26
	2.5 mL of 2% (50 mg)	Epidural	Prolonged analgesia (34 min) compared with bupivacaine.	39
	5.0 mg/kg	Epidural	Onset of action, 7 min; duration, 108 min.	59
	5 mg/kg	Nerve block	Duration of sensory block, 100 min	22
	5 mg/kg and 0.005 mg/mL	Nerve block	Analgesia onset, 11 min; duration, 133 min	21
	2% and 0.0005% (2.5 mL)	Epidural	Incorporation of epinephrine did not prolong analgesic duration.	39
	5.1 mg/kg and 0.005 mg	Epidural	Onset of action, 8 min; duration, 190 min.	59
	2% (1 mL/7 kg)	Epidural	Slower onset of action but longer duration of action (174 min) than lidocaine alone	3
	1mL of 10%			

delayed onset may have been caused by the resulting decreased pH of the anesthetic solution, reducing the amount of nonionized local anesthetic. Epinephrine produces vasoconstriction at the injection site slowing the rate of systemic absorption. The drug is therefore bound to sodium channels in the area for longer, increasing the analgesic effect.[65] Conversely, the addition of epinephrine to lidocaine in another report decreased the duration of analgesia.[39] This contradicts literature in other species.

The addition of the α2 agonists clonidine[14] and xylazine[21,59] increased the duration of analgesia compared with lidocaine alone. Two theories have been proposed for this prolongation: first, that the α_2 agents induce vasoconstriction, thus interfering with vascular absorption of local anesthetic, resulting in higher concentrations at nerves;[21,59] and second, that α_2 agonists may induce local anesthetic-like effects when combined with a local anesthetic.[36] Although analgesic duration may be increased by the addition of α_2 agonists, the addition of xylazine to epidural or nerve blocks is generally contraindicated due to potential adverse effects. Previously reported concerns include cardiopulmonary depression[43] and neurotoxicity after neuraxial administration.[20] Therefore, xylazine should be administered through the intramuscular or intravenous route rather than in combination epidurally, given that intramuscular and intravenous methods allow for dose adjustment based on the animal's reaction to local anesthetic, condition, and the surgical procedure.[59]

Magnesium sulfate has been combined with lidocaine for epidural administration. The $MgSO_4$ combination increased the duration of analgesia compared with lidocaine alone, but prolonged the time to onset of analgesia.[3] $MgSO_4$ blocks Ca^{2+} influx and noncompetitively antagonizes NMDA excitatory receptors, thus preventing central sensitization.[3] The combination appears to be an effective single-dose epidural analgesic

Table 6. Miscellaneous agents used as analgesics in the papers reviewed.

Drug	Mechanism	Dose	Route	Summary	Reference
Ketamine	NMDA receptor antagonist	2.5 mg/kg	Epidural	Caused some sedation and decrease in respiratory rate.	10
		1 mg/kg	Epidural	Decreased pain postoperatively as determined through pain behavior assessment and improved use of limb after orthopedic surgery.	25
Ketamine + bupivacaine		0.25 and 1.25 mg/kg	Epidural	Combination produced faster onset than each agent alone. Analgesic duration longer than ketamine alone and shorter than bupivacaine alone. Analgesia was determined by using pinprick and pinch of caudal areas.	10
Nifedipine	Ca^{2+} channel blocker	1 or 2 mg total	Lateral ventricle of brain	When given 10 min prior to a visceral pain insult of duodenal distension, nifedipine decreased behavioral signs of pain. Ca^{2+} channel blockade by the drug was thought to have an antinociceptive effect.	31

for sheep undergoing prolonged procedures. Cardiovascular and respiratory side effects are few.

Miscellaneous Drugs

Ketamine, an NMDA receptor antagonist (Table 6), has potent analgesic effects at subanesthetic doses. Although the resulting analgesic effect has been proposed to be more profound in somatic rather than visceral pain, some authors purport that ketamine is underutilized as an analgesic in sheep.[37] NMDA receptors are normally inactive because they are blocked by magnesium ions. However, when neuronal transmission changes, such as during nociceptive signaling, the magnesium blockade is removed, allowing an inflow of Ca^{2+} ions. Under these circumstances, nonpainful stimuli can become painful, and painful stimuli are more intense and prolonged.[25] The binding of ketamine to NMDA receptors blocks the movement of calcium, sodium, and potassium. Long-lasting analgesic effects were produced in sheep undergoing orthopedic procedures.[25]

Nifedipine is a voltage-gated calcium channel blocker that has antinociceptive properties.[41] When nifedipine was administered at least 10 min before exposure to the acute visceral pain associated with duodenal distension in sheep, it counteracted all pain symptoms.[31] Although these data are promising, we do not recommend the use of nifedipine as an analgesic until effective dose rates and routes have been determined in future studies.

Conclusions

We undertook this review to collate the information within the scientific literature regarding the use, dosage, and regimes of analgesic agents used in sheep in biomedical research. Hopefully this information will assist scientists who are reluctant to use analgesics for fear of introducing a confounding factor to studies.[28]

Opioid agonists have traditionally been considered short acting and of questionable efficacy in sheep. However, newer modalities such as transdermal patches and drug combinations may provide effective analgesia in sheep. NSAID may exhibit analgesic effect only when inflammatory pain is present and not be beneficial for use in acute pain models. Additional controlled study of these agents as used in biomedical research would benefit the field, especially regarding the effects of these agents on inflammation and research parameters of interest. α2 agonists provide effective but short-lived analgesia and are associated with side effects of pulmonary edema and hypoxemia. When combined with anesthesia, local anesthetics provide a valuable analgesic effect. Too few studies of $Ca2^{+}$-channel blockers in sheep are available to support useful recommendations regarding efficacy, dose rate, and route. The NMDA antagonist ketamine provides good analgesia and may be underutilized in sheep. In the future, additional study should be devoted to investigating the analgesic effects of ketamine in sheep.

References

1. **Abu-Serriah M, Nolan AM, Dolan S.** 2007. Pain assessment following experimental maxillofacial surgical procedure in sheep. Lab Anim **41:**345–352. https://doi.org/10.1258/002367707781282794.
2. **Ahern BJ, Soma LR, Boston RC, Schaer TP.** 2009. Comparison of the analgesic properties of transdermally administered fentanyl and intramuscularly administered buprenorphine during and following experimental orthopedic surgery in sheep. Am J Vet Res **70:**418–422. https://doi.org/10.2460/ajvr.70.3.418.
3. **Bigham A, Shafiei Z.** 2008. Comparison of caudal epidural anaesthesia with lidocaine-distilled water and lidocaine magnesium sulfate combination in sheep. BJVM **11:**125–130.
4. **Bonnet F, Derosier JP, Pluskwa F, Abhay K, Gaillard A.** 1990. Cervical epidural anaesthesia for carotid artery surgery. Can J Anaesth **37:**353–358. https://doi.org/10.1007/BF03005590.
5. **Bartolome E, Della Rocca G, Di Salvo A, Giorgi M, Kim T, Isola M, De Benedictis G.** 2015. Pharmacokinetics and antinociceptive effects of tramadol and its metabolite O-desmethyltramadol following intravenous administration in sheep. Vet J **205:**404–409. https://doi.org/10.1016/j.tvjl.2015.04.011.
6. **Brandt SA, Livingston A.** 1990. Receptor changes in the spinal cord of sheep associated with exposure to chronic pain. Pain **42:**323–329. https://doi.org/10.1016/0304-3959(90)91145-9.
7. **Castro MI, Eisenach JC.** 1989. Pharmacokinetics and dynamics of intravenous, intrathecal, and epidural clonidine in sheep. Anesthesiology **71:**418–425. https://doi.org/10.1097/00000542-198909000-00018.
8. **Colditz IG, Paull DR, Lloyd JB, Johnston L, Small AH.** 2019. Efficacy of meloxicam in a pain model in sheep. Aust Vet J **97:**23–32. https://doi.org/10.1111/avj.12779.
9. **Curatolo M, Petersen-Felix S, Arendt-Nielsen L.** 2000. Sensory assessment of regional analgesia in humans: a review of methods and applications. Anesthesiology **93:**1517–1530. https://doi.org/10.1097/00000542-200012000-00025.

10. **Dadafarid H, Najafpour A**. 2008. Epidural analgesia with bupivacaine, ketamine, and the combination of bupivacaine and ketamine in sheep. Iran J Vet Surg **3:**19–27.
11. **DeRossi R, Jardim PH, Hermeto LC, Pagliosa RC.** 2015. Comparison of analgesic and systemic effects of bupivacaine, methadone, or bupivacaine/methadone administered epidurally in conscious sheep. Aust Vet J **93:**164–169. https://doi.org/10.1111/avj.12313.
12. **DeRossi R, Pagliosa R, Módolo TC, Maciel FB, Macedo GG.** 2012. Thoracic epidural analgesia via the lumbosacral approach using multiport catheters with a low concentration of bupivacaine and morphine in sheep. Vet Anaesth Analg **39:**306–314. https://doi.org/10.1111/j.1467-2995.2011.00689.x.
13. **DeRossi R, Pagliosa RC, de Carvalho AQ, Macedo GG, Hermeto LC.** 2017. Fentanyl and methadone used as adjuncts to bupivacaine for lumbosacral epidural analgesia in sheep. Vet Rec **180:**96. https://doi.org/10.1136/vr.103860.
14. **DeRossi R, Righetto FR, Almeida RG, Medeiros U Jr, Frazílio FO.** 2006. Clinical evaluation of clonidine added to lidocaine solution for subarachnoid analgesia in sheep. J Vet Pharmacol Ther **29:**113–119. https://doi.org/10.1111/j.1365-2885.2006.00722.x.
15. **Detweiler DJ, Eisenach JC, Tong CY, Jackson C.** 1993. A cholinergic interaction in α 2 adrenoceptor-mediated antinociception in sheep. J Pharmacol Exp Ther **265:**536–542.
16. **Dolan S, Nolan AM.** 1999. N-methyl D-aspartate induced mechanical allodynia is blocked by nitric oxide synthase and cyclooxygenase-2 inhibitors. Neuroreport **10:**449–452. https://doi.org/10.1097/00001756-199902250-00002.
17. **Durej M, Ledecký V, Michalák D, Hluchý M, Kòazovický D.** 2012. Efficacy of epidural administration of morphine with bupivacaine for orthopaedic surgery in sheep. Acta Vet Brno **62:**111–119. https://doi.org/10.2298/AVB1201111D.
18. **Engelhardt G.** 1996. Pharmacology of meloxicam, a new non-steroidal anti-inflammatory drug with an improved safety profile through preferential inhibition of COX2. Br J Rheumatol **35 Suppl 1:**4–12. https://doi.org/10.1093/rheumatology/35.suppl_1.4.
19. **Galatos AD.** 2011. Anesthesia and analgesia in sheep and goats. Vet Clin North Am Food Anim Pract **27:**47–59. https://doi.org/10.1016/j.cvfa.2010.10.007.
20. **George LW.** 2003. Pain control in food animals. p 1–13. In: Steffey EP, editor. Recent advances in anesthetic management of large domestic animals. Ithaca (NY): International Veterinary Information Service
21. **Ghadirian S, Vesal N.** 2013. Brachial plexus block using lidocaine/epinephrine or lidocaine/xylazine in fat-tailed sheep. Vet Res Forum **4:**161–167.
22. **Ghadirian S, Vesal N, Maghsoudi B, Akhlagh SH.** 2016. Comparison of lidocaine, lidocaine-morphine, lidocaine-tramadol or bupivacaine for neural blockade of the brachial plexus in fat-tailed lambs. Vet Anaesth Analg **43:**109–116. https://doi.org/10.1111/vaa.12280.
23. **Grant C, Summersides GE, Kuchel TR.** 2001. A xylazine infusion regimen to provide analgesia in sheep. Lab Anim **35:**277–281. https://doi.org/10.1258/0023677011911606.
24. **Grant C, Upton RN.** 2004. Comparison of the analgesic effects of xylazine in sheep via 3 different administration routes. Aust Vet J **82:**304–307. https://doi.org/10.1111/j.1751-0813.2004.tb12712.x.
25. **Guedes AGP, Pluhar GE, Daubs BM, Rudé EP.** 2006. Effects of preoperative epidural administration of racemic ketamine for analgesia in sheep undergoing surgery. Am J Vet Res **67:**222–229. https://doi.org/10.2460/ajvr.67.2.222.
26. **Habibian S, Bigham AS, Aali E.** 2011. Comparison of lidocaine, tramadol, and lidocaine-tramadol for epidural analgesia in lambs. Res Vet Sci **91:**434–438. https://doi.org/10.1016/j.rvsc.2010.09.023.
27. **Haerdi-Landerer MC, Schlegel U, Neiger-Aeschbacher G.** 2005. The analgesic effects of intrathecal xylazine and detomidine in sheep and their antagonism with systemic atipamezole. Vet Anaesth Analg **32:**297–307. https://doi.org/10.1111/j.1467-2995.2005.00214.x.
28. **Harris J, Flecknell P, Thomas A, Warrell DA.** 2013. On the use of analgesia in experimental toxicology. Toxicon **64:**36–37. https://doi.org/10.1016/j.toxicon.2012.12.008.
29. **Hawkins P.** 2002. Recognizing and assessing pain, suffering and distress in laboratory animals: a survey of current practices in the UK with recommendations. Lab Anim **36:**378–395. https://doi.org/10.1258/002367702320389044.
30. **Institute for Laboratory Animal Research.** 2011. Guide for the care and use of laboratory animals, 8th ed. Washington (DC): National Academies Press.
31. **Kania BF, Kowalczyk M, Brytan M, Tomaszewska D, Przekop F.** 2009. The inhibition of experimentally induced visceral hyperalgesia by nifedipine—a voltage-gated $Ca2^{+}$ channels blocker (VGCCs) in Sheep. Res Vet Sci **86:**285–292. https://doi.org/10.1016/j.rvsc.2008.04.010.
32. **Kästner SBR.** 2006. α_2 agonists in sheep: a review. Vet Anaesth Analg **33:**79–96. https://doi.org/10.1111/j.1467-2995.2005.00243.x.
33. **Kietzmann D, Bouillon T, Hamm C, Schwabe K, Schenk H, Gundert-Remy U, Kettler D.** 1997. Pharmacodynamic modelling of the analgesic effects of piritramide in postoperative patients. Acta Anaesthesiol Scand **41:**888–894. https://doi.org/10.1111/j.1399-6576.1997.tb04805.x.
34. **Kyles AE, Waterman AE, Livingston A.** 1993. The spinal antinociceptive activity of the α_2-adrenoceptor agonist, xylazine in sheep. Br J Pharmacol **108:**907–913. https://doi.org/10.1111/j.1476-5381.1993.tb13485.x.
35. **Liu SS, Carpenter RL, Mackey DC, Thirlby RC, Rupp SM, Shine TS, Feinglass NG, Metzger PP, Fulmer JT, Smith SL.** 1995. Effects of perioperative analgesic technique on rate of recovery after colon surgery. Anesthesiology **83:**757–765. https://doi.org/10.1097/00000542-199510000-00015.
36. **Lizarraga I, Chambers JP.** 2006. Involvement of opioidergic and α_2-adrenergic mechanisms in the central analgesic effects of non-steroidal antiinflammatory drugs in sheep. Res Vet Sci **80:**194–200. https://doi.org/10.1016/j.rvsc.2005.06.001.
37. **Lizarraga I, Chambers JP.** 2012. Use of analgesic drugs for pain management in sheep. N Z Vet J **60:**87–94. https://doi.org/10.1080/00480169.2011.642772.
38. **Logas WG, el-Baz N, el-Ganzouri A, Cullen M, Staren E, Faber LP, Ivankovich AD.** 1987. Continuous thoracic epidural analgesia for postoperative pain relief following thoracotomy: a randomized prospective study. Anesthesiology **67:**787–791. https://doi.org/10.1097/00000542-198711000-00026.
39. **Lucky NS, Hashim MA, Ahmed JU, Sarker K, Gazi NM, Ahmed S.** 2007. Caudal epidural analgesia in sheep by using lignocaine hydrochloride and bupivacaine hydrochloride. Bangladesh Journal of Veterinary Medicine **5:**77–80.
40. **Ludbrook G, Grant C, Upton R, Penhall C.** 1995. A method for frequent measurement of sedation and analgesia in sheep using the response to a ramped electrical stimulus. J Pharmacol Toxicol Methods **33:**17–22. https://doi.org/10.1016/1056-8719(94)00051-5.
41. **Michaluk J, Karolewicz B, Antkiewicz-Michaluk L, Vetulani J.** 1998. Effects of various Ca2+ channel antagonists on morphine analgesia, tolerance and dependence, and on blood pressure in the rat. Eur J Pharmacol **352:**189–197. https://doi.org/10.1016/S0014-2999(98)00373-2.
42. **Moolchand M, Kachiwal A, Soomro S, Bhutto Z.** 2014. Comparison of sedative and analgesic effects of xylazine, detomidine, and medetomidine in sheep. Egypt Journal of Sheep and Goat Sciences **65:**1–6.
43. **Muir WW, McDonell WN, Kerr CL.** 2011. Anesthetic physiology and pharmacology. p 15–81. In: Grimm KA, Tranquilli WJ, Lamont, LA editors. Essentials of small animal anesthesia and analgesia. West Sussex (United Kingdom): John Wiley and Sons.
44. **Murdoch FR, Maker GL, Nitsos I, Polglase GR, Musk GC.** 2013. Intraperitoneal medetomidine: a novel analgesic strategy for postoperative pain management in pregnant sheep. Lab Anim **47:**66–70. https://doi.org/10.1177/0023677212473712.
45. **Musk GC, Murdoch FR, Tuke J, Kemp MW, Dixon MJ, Taylor PM.** 2014. Thermal and mechanical nociceptive threshold testing in pregnant sheep. Vet Anaesth Analg **41:**305–311. https://doi.org/10.1111/vaa.12103.
46. **Muth-Selbach US, Tegeder I, Brune K, Geisslinger G.** 1999. Acetaminophen inhibits spinal prostaglandin E2 release after peripheral noxious stimulation. Anesthesiology **91:**231–239. https://doi.org/10.1097/00000542-199907000-00032.

47. **Natalini CC, Polydoro ADS, Crosignani N.** 2007. Antinociceptive effects of epidural tramadol administration in dogs as an analgesic for experimental stifle surgery. Acta Sci Vet **35:**189–195. https://doi.org/10.22456/1679-9216.15969.
48. **National Health and Medical Research Council**. [Internet]. 2013. Code for the care and use of animals in scientific procedures. [Cited 01 October 2019]. Available at: https://www.nhmrc.gov.au/about-us/publications/australian-code-care-and-use-animals-scientific-purposes
49. **Nolan A, Livingston A, Waterman A.** 1987. Antinociceptive actions of intravenous α2-adrenoceptor agonists in sheep. J Vet Pharmacol Ther **10:**202–209. https://doi.org/10.1111/j.1365-2885.1987.tb00530.x.
50. **Nolan A, Livingston A, Waterman AE.** 1987. Investigation of the antinociceptive activity of buprenorphine in sheep. Br J Pharmacol **92:**527–533. https://doi.org/10.1111/j.1476-5381.1987.tb11353.x.
51. **Nolan A, Waterman A, Livingston A.** 1986. The analgesic activity of α_2 adrenoceptor agonists in sheep: a comparison with opioids. Vet Anaesth Analg **14:**14–15.
52. **Nolan AM, Callingham BA, Evans RJ.** 1990. Effects of aspirin on xylazine-induced hypoxaemia in sheep. Res Vet Sci **48:**386–388. https://doi.org/10.1016/S0034-5288(18)31034-8.
53. **Otto KA, Gerich T, Volmert C.** 2000. Hemodynamic and electroencephalographic effects of epidural buprenorphine during orthopedic hindlimb surgery in sheep: a comparison with intramuscular buprenorphine and epidural saline. J Exp Anim Sci **41:**121–132. https://doi.org/10.1016/S0939-8600(00)80004-8.
54. **Otto KA, Steiner KHS, Zailskas F, Wippermann B.** 2000. Comparison of the postoperative analgesic effects of buprenorphine and piritramide following experimental orthopaedic surgery in sheep. J Exp Anim Sci **41:**133–143. https://doi.org/10.1016/S0939-8600(00)80005-X.
55. **Pang WW, Huang PY, Chang DP, Huang MH.** 1999. The peripheral analgesic effect of tramadol in reducing propofol injection pain: a comparison with lidocaine. Reg Anesth Pain Med **24:**246–249.
56. **Pettifer GR, Hosgood GJ.** 2003. The effect of rectal temperature on perianesthetic serum concentrations of transdermally administered fentanyl in cats anesthetized with isoflurane. Am J Vet Res **64:**1557–1561. https://doi.org/10.2460/ajvr.2003.64.1557.
57. **Plummer PJ, Schleining JA.** 2013. Assessment and management of pain in small ruminants and camelids. Vet Clin North Am Food Anim Pract **29:**185–208. https://doi.org/10.1016/j.cvfa.2012.11.004.
58. **Read MR.** 2003. A review of α_2 adrenoreceptor agonists and the development of hypoxemia in domestic and wild ruminants. J Zoo Wildl Med **34:**134–138. https://doi.org/10.1638/1042-7260(2003)034[0134:AROAAA]2.0.CO;2.
59. **Rostami M, Vesal N.** 2012. The effects of adding epinephrine or xylazine to lidocaine solution for lumbosacral epidural analgesia in fat-tailed sheep. J S Afr Vet Assoc **83:**1–7. https://doi.org/10.4102/jsava.v83i1.1.
60. **Scheerlinck JPY, Snibson KJ, Bowles VM, Sutton P.** 2008. Biomedical applications of sheep models: from asthma to vaccines. Trends Biotechnol **26:**259–266. https://doi.org/10.1016/j.tibtech.2008.02.002.
61. **Schrör K, Voelker M.** 2016. Part III. Aspirin. NSAIDS and aspirin: recent advances and implications for clinical management. p 107–122. In: Lanas A, editor. NSAIDs and aspirin. Recent advances and implications for clinical management. Zaragoza (Spain): Springer. https://doi.org/10.1007/978-3-319-33889-7_7.
62. **Shafford HL, Hellyer PW, Turner AS.** 2004. Intra-articular lidocaine plus bupivacaine in sheep undergoing stifle arthrotomy. Vet Anaesth Analg **31:**20–26. https://doi.org/10.1111/j.1467-2995.2004.00126.x.
63. **Sigurdsson GH, Youssef H.** 1994. Amelioration of respiratory and circulatory changes in established endotoxic-shock by ketoprofen. Acta Anaesthesiol Scand **38:**33–39. https://doi.org/10.1111/j.1399-6576.1994.tb03833.x.
64. **Singh JT, Singh AP, Peshin PK, Sharifi D, Patil DB.** 1994. Evaluation of detomidine as a sedative in sheep. Indian J Anim Sci **64:**237–238.
65. **Skarda RT, Tranquilli WJ.** 2011. Local anesthetics and regional analgesic techniques, p 326–377. In: Grimm KA, Tranquilli WJ, Lamont LA, editors. Essentials of small animal anesthesia and analgesia. West Sussex (United Kingdom): John Wiley and Sons.
66. **Uggla A, Lindqvist A.** 1983. Acute pulmonary oedema as an adverse reaction to the use of xylazine in sheep. Vet Rec **113:**42. https://doi.org/10.1136/vr.113.2.42.
67. **Wagner AE, Mama KR, Ruehlman DS, Pelkey S, Turner AS.** 2011. Evaluation of effects of sciatic and femoral nerve blocks in sheep undergoing stifle surgery. Lab Anim (NY) **40:**114–118. https://doi.org/10.1038/laban0411-114.
68. **Walkowiak KJ, Graham ML.** 2015. Pharmacokinetics and antinociceptive activity of sustained-release buprenorphine in sheep. J Am Assoc Lab Anim Sci **54:**763–768.
69. **Xu Z, Li P, Tong C, Figueroa J, Tobin JR, Eisenach JC.** 1996. Location and characteristics of nitric oxide synthase in sheep spinal cord and its interaction with α_2-adrenergic and cholinergic antinociception. Anesthesiology **84:**890–899. https://doi.org/10.1097/00000542-199604000-00017.
70. **Yaddanapudi LN, Wig J, Singh B, Tewari MK.** 2000. Comparison of efficacy and side effects of epidural tramadol and morphine in patients undergoing laminectomy: a repeated dose study. Neurol India **48:**398–400.