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Critical key points for anesthesia in experimental research involving sheep (*Ovis aries*)

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ABSTRACT
Anesthesia and analgesia have a major impact on ensuring animal welfare and safety, resulting in reduced stress response and effective pain control, ensuring the comfort of the animal, promoting faster recovery, and reducing the risk of complications associated with various research procedures. Each stage of anesthesia in sheep is vital for maintaining the animal's welfare, ensuring procedural success, minimizing stress, risks, and complications, and optimizing the quality of research data. Proper attention to detail and adherence to best practices at each stage contribute to the overall success of anesthesia management in sheep. Anesthesia protocols should suit individual requirements for each sheep, in light of factors such as health status, procedure duration, and desired anesthesia depth. Meticulous monitoring, adherence to best practices, and prompt intervention are essential for minimizing the risks of complications during sheep anesthesia and ensuring the safety and welfare of the animals undergoing anesthesia for research purposes. This article presents the main key points that can improve anesthetic management for sheep involved in experimental research to minimize stress response and complications, enhancing animal safety, welfare, and comfort during and after procedures. Multimodal anesthesia approaches ensure effective pain relief, tailored to the specific needs of individual animals or procedures, optimizing outcomes, and minimizing risks. Anesthesia management contributes to improved research data collection under conditions that enhance the validity and reliability of results. Sheep's impressive capacity to maintain homeostasis even during extended periods of anesthesia highlights the critical importance of upholding data quality in alignment with the universally accepted principles of replacement, reduction, and refinement for ethical animal research. By adhering to these principles, researchers can minimize the number of animals used, reduce any potential discomfort or distress experienced by the animals, and refine procedures to optimize animal welfare while still achieving scientific objectives.
Keywords: Anesthesia, Experimental research, Ruminants, Sheep.

Introduction

When selecting an animal model for experimental use, it is crucial to carefully evaluate its similarity to humans, adhere to international guidelines to minimize animal usage, and prevent the wastage of resources (Ionescu *et al*., 2024). Researchers employ specialized knowledge and techniques to ensure safe and effective anesthesia administration tailored to the specific needs of each animal species, minimize stress, maintain physiological stability, and support ethical standards in biomedical research involving animals (Moldovan *et al*., 2022). With appropriate monitoring and support, the recognition of perianesthetic complications can serve as a model for building an adequate protocol, to avoid any morbidity and advancement to mortality during experimental research (Mee *et al*., 1998; Bille *et al*., 2012; Costea *et al*., 2023; Varkoulis *et al*., 2023). Morbidity and mortality associated with general anesthesia have been studied for different ruminants; however, large multicentre studies investigating this subject for sheep are lacking (Ringer and Bettschart-Wolfensberger, 2023). There are not many studies for small ruminants and in the absence of specific data for sheep, we can only compare with the data presented for goats. The mortality rate during anesthesia for goats, as determined by a single cohort retrospective research was 7.3% overall, and 3.4% specifically among elective procedures (Steen *et al.,* 2023). According to the same study, the factors associated with anesthesia and involved in calculating the percentages of morbidity and mortality included hypothermia, bradycardia, hypotension, hypoxemia, regurgitation and aspiration, acute renal failure, myopathies, neuropathies, and fever of unknown origin (Steen *et al.,* 2023). Based on the authors' experience with various research procedures involving sheep under general anesthesia using inhalational agents, sheep exhibit excellent tolerance to general anesthesia and demonstrate prompt full recovery. These attributes are critical advantages that enhance their use in research (Costea *et al.,* 2022).

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Critical Key Points During the Preanesthetic Phase

Preanesthetic physical examination and patient preparation

A careful, stress-free preanesthetic physical examination including an assessment of respiratory and cardiovascular frequency, temperature, and hydration status should be performed. Furthermore, blood samples can be obtained from the jugular vein and analyzed to assess health status and establish baseline values for research procedures. Body weight should be measured and depending on breed, sex, and age, corrections will be applied in correlation with the amount of wool. Without considering the weight of the wool, there is a risk of overdose, especially for anesthetic medication, with implications for the level of homeostasis during the procedures.

In correlation with the establishment of preanesthetic fasting, different complications such as rumen distension, regurgitation, or aspiration pneumonia may occur during ruminant anesthesia. Preparation of the adult patients involves an ideal fast of 12–24 hours with a maximum pause of 48 hours (Trimmel *et al.,* 2022), while for young individuals fasting periods should not exceed 12 hours (Flecknell *et al.,* 2015). Water restriction depends on the procedure and is limited to 6–8 hours (Lin, 2014). Regurgitation occurs especially when animals are not fasted preoperatively. Fasting before anesthesia helps reduce the gas produced by fermentation and rumen bloating. This results in an increased intraabdominal pressure that compresses the diaphragm and major vessels, like the posterior vena cava, leading to compromised cardiopulmonary function (Lin, 2014). However, preoperative fasting in sheep may have a downside as it can disrupt electrolyte balance, potentially possibly establishing a negative foundation for prolonged anesthesia, particularly if the animal is fed predominantly a pelleted diet (Jasmin *et al.,* 2011).

A venous catheter can be inserted at the jugular vein level, which is considered the optimal site for catheter placement and secured, as it can slide easily due to the lanolin impact on the skin surface. In the absence of a venous catheter during anesthesia, additional doses of parenteral anesthetic and analgesics, emergency medication, or IV fluids will not be administered effectively.

Premedication and sedation

Premedication determines the sedation of the patient, facilitating contention and significantly reducing the doses further required for anesthesia. Intramuscular (IM) and intravenous (IV) routes are typically preferred as they yield more predictable effects.

Acepromazine can be used with caution, facilitating restraint before anesthesia induction, and not being recommended in protocols for cachectic or hypovolemic unstable animals. Administering acepromazine alone to sheep (0.02–0.05 mg/kg IV, 0.05–0.2 mg/kg IM) resulted in sedation levels comparable to those achieved when combined with opioids without causing any noticeable changes in cardiorespiratory function (Nishimura *et al.,* 2017). The sedative effect of acepromazine with buprenorphine was found to be similar to that of combining acepromazine with morphine in sheep (Musk and Wilkes, 2018).

Alpha-2 agonists are frequently utilized to achieve effective sedation and analgesia in sheep, with less analgesic efficacy than in other species, along with excitation and behavioral changes at high doses (Kästner, 2006). Xylazine induces sedation with central nervous depression (0.02–0.15 mg/kg IV, 0.05–0.3 mg/kg IM). For pregnant animals, the use of detomidine (0.03–0.5 mg/kg IV) is preferred over xylazine, as it increases the risk for abortion through its ecbolic effect, determining an early onset of parturition and placental retention (LeBlanc et al., 1984). Pulmonary edema has been identified as a significant contributor to mortality in ovine under anesthesia following the administration of xylazine (Lin *et al.,* 1993). Xylazine (0.075 mg/kg) and dexmedetomidine (0.005 mg/kg) both cause splenic erythrocyte trapping and significantly reduced ruminal motility when given IV (Debiage *et al.,* 2022). A dose of romifidine 50 µg/kg IV was reported to induce comparable sedation along with sternal recumbency as dosages of 0.15 mg/kg IV xylazine, 30 µg/ kg IV detomidine, or 10 µg/kg IV medetomidine (Kästner, 2006). Alpha-2 agonists are commonly used in sheep in combination with ketamine in protocols of induction or for total IV anesthesia techniques (White and Taylor, 2000).

Benzodiazepines are commonly used for sedation either alone or in combination with a variety of other drugs (opioids, ketamine, α2-agonists), with minimal and temporary effects on the cardiopulmonary systems. Diazepam (0.25–0.5 mg/kg IV) or midazolam (0.1–0.5 mg/kg, IV/IM) can be administered safely to small ruminants (Flecknell *et al.,* 2015). Midazolam is noted to be suitable for short-duration sedatives for sheep, with high bioavailability after IM administration (Simon *et al*., 2017). Benzodiazepine effects can be reversed using flumazenil or sarmazenil. Reversal of benzodiazepines is typically unnecessary unless there is a significant overdose. Tiletamine-zolazepam (Telazol®) is effective for providing medium-duration anesthesia in sheep. Due to its impact on cardiac and pulmonary function, the use of tiletamine-zolazepam is not recommended in studies requiring minimal impact on heart and lung function (Lagutchik *et al.,* 1991). Ruminants experience a gradual yet smooth recovery from Telazol® anesthesia due to the slower metabolism and the prolonged effects of zolazepam.

Ketamine (5 to 15 mg/kg) can be used in different combinations with an α2 agonist (xylazine 0.1–0.2 mg/kg) and an opioid (butorphanol 0.01–0.1 mg/kg) for sedation and analgesia. Anesthesia can be induced for a synergistic effect with a smaller dose of ketamine

Fig. 1. Sternal positioning during anesthesia with the head in an elevated position.

(5–7 mg/kg) and a benzodiazepine administered IV (Seddighi and Doherty, 2016).

Induction and intubation

Due to the high production of saliva of approximately 6–16 L/24 hours (Somers, 1957) and to prevent regurgitation, support (textile roll, sandbag) can be placed under the cervical region, to slightly elevate it and to allow drainage (Fig. 1). Induction can be achieved most frequently IV (propofol 4–6 mg/kg, alfaxalone 1–3 mg/kg, tiletamine-zolazepam 4 mg/kg, ketamine 20–30 mg/kg, and diazepam 0.2 mg/kg) and followed by inhalation maintenance in the case of complex, long procedures that benefit from endotracheal intubation (White and Taylor, 2000).

Intubation is performed under visual laryngoscopy with the patient ideally in sternal recumbency with the head and neck elevated (Desmecht *et al.,* 1995). Two soft ropes or similar will be used to facilitate the access. Due to the patient's weight, maintaining the head in this position is challenging and it becomes difficult to sustain it elevated to perform the intubation. The maximum opening of the oral cavity, even in the conditions of a reduced mandibular tone is limited, which makes it difficult to visualize and access the larynx. For intubation, it is necessary to use a laryngoscope with a long straight blade (Fig. 2). Sheep do not tend to exhibit profound laryngospasm but the administration of a local anesthetic on the larynx facilitates intubation. Lidocaine can be applied onto the larynx or sprayed. Due to the limited space in the oral cavity and abundant salivary secretions, the syringe or the nozzle of the spray must be handled with great care. The authors reported an incident in which during the administration of lidocaine by a spray with a nozzle, the nozzle detached from the bottle and slipped to the larynx. The extraction was done quickly with the help of forceps, but there was a risk of an endotracheal foreign body case. Endotracheal tubes (ETs) with cuffs, sized between 7 and 10 mm can be used for adult sheep. During the intubation sequence, sponge

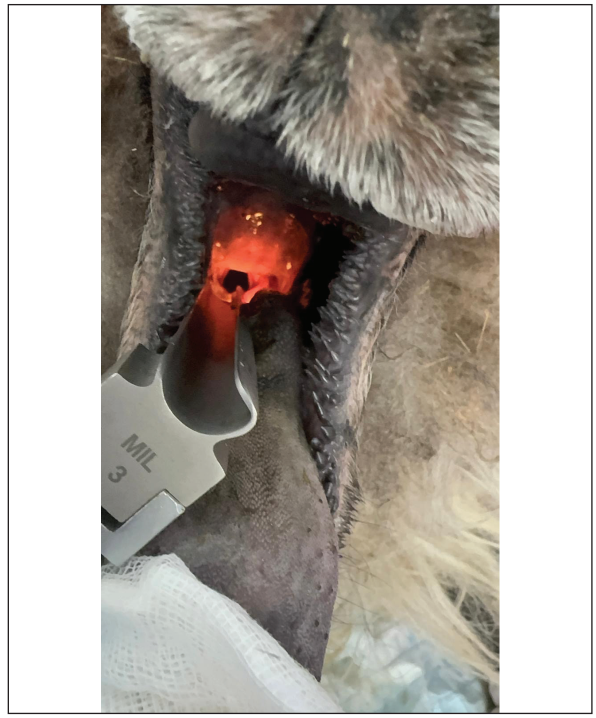


Fig. 2. Intubation using a laryngoscope, and larynx visualization.

forceps to clear debris from the oropharynx can be used if needed. If orotracheal intubation is not possible, nasotracheal intubation can be a useful alternative in extraordinary circumstances and for small animals, for maintaining airway patency. To aid in the passage of the nasotracheal tube, it is helpful to first pass a smaller tube as a guide (Seddighi and Doherty, 2016).

Physiological postprandial gas production in sheep is approximately 30 L/hour. During anesthesia, the motility of the digestive tract and belching are limited. It is also recommended to mount a rumen probe or, if this is not possible, the rumen can be decompressed by centesis, to prevent or reduce bloating. The sheep must be placed in such a way that its weight is uniformly distributed, ideally on a soft surface, to prevent the possible myopathies or neuropathies associated with anesthesia. Heat pads or heated surgery tables can be used to prevent sheep from becoming hypothermic during anesthesia. Once the sheep is positioned for surgery the intubation must be rechecked to be sure that the ET is still cuffed and in the original position. To decrease any risk of corneal ulceration, it is essential to protect the eyes by administering ophthalmic ointment and ensuring eye closure (Thurmon and Benson, 1993).

Maintenance

Maintenance of anesthesia can be achieved with a minimum alveolar concentration of commonly used inhalation anesthetic as isoflurane (1.5–1.9), sevoflurane (2.7), and oxygen (12–15 ml/kg/minute) (Pacharinsak *et al.,* 2023). Total intravenous anesthesia protocols for maintenance for experimental research procedures can be suitable for short anesthesia (<30 minutes). A mixture of ketamine (1 mg/ml), xylazine (0.1 mg/ml), and guaifenesin (50 mg/ml) in 5% dextrose can be infused at a mean dose of 1.2 ml/kg for induction and 2.6 ml/kg/h during maintenance (Seddighi and Doherty, 2016). Inhalation anesthesia has also the advantage of minimizing the common complication represented by hypoxemia and reducing the recovery time.

Loco-regional anesthetic methods can be used as alternatives to general anesthesia, or in conjunction with general anesthesia for optimal results. Lidocaine 2% provides anesthesia within 5 to 20 minutes, for 45 to 90 minutes. Mepivacaine 2% has a more rapid onset and can produce anesthesia for 1.5 to 3 hours. In contrast, bupivacaine 0.5% has a slower onset but a longer effect for 4–8 hours (Galatos, 2011).

Unless the sheep is dehydrated or experiencing hypovolemia, a rate of 5–10 ml/kg/h of electrolyte solution is typically adequate to maintain homeostasis during anesthesia. It is important to carefully consider any hemorrhagic events during the procedures and also the frequency and volume of blood sampling to minimize any potential impact on the reduction of hematocrit and hemoglobin concentrations in sheep (Trimmel *et al.*, 2022), since the estimated blood volume is approximately 63 ml per kilogram of body weight (Gillett and Halmagyi, 1966).

Recovery

Recovery from anesthesia can be associated with inherent risks. Certain anesthetics can affect cardiovascular function, potentially causing hypotension, arrhythmias, or other cardiac disturbances. Anesthesia can disrupt metabolic processes, leading to electrolyte imbalances or acid-base disturbances. Monitoring blood gas analysis with electrolyte measurement and providing support throughout the entire procedure and in the recovery time, can help mitigate metabolic complications (Trimmel *et al.,* 2022).

Sheep should be positioned into sternal recumbency in this phase, to facilitate the re-expand of possible collapsed lung areas due to the recumbency. Ruminal tympany, regurgitation, and aspiration pneumonia are common complications associated with general anesthesia in ruminants. Regurgitation happens more often for left lateral recumbency compared to right lateral recumbency (Lin, 2014). Yohimbine or atipamezole can be used as reversal agents for alpha2-agonists. The endotracheal cuffed tube will be removed only if swallowing spontaneously and chewing motions are present.

Some animals may have difficulty recovering during the awakening phase, experiencing complications such as prolonged recovery, muscle weakness, or difficulty maintaining the quadrupedal position. To minimize risks and complications during recovery, it is essential to maintain continuous monitoring of the vital signs along with appropriate postanesthetic care.

Myopathy, and neuropathy during recovery are important complications for sheep subjected to long recumbency, on inadequate surfaces, especially if the animal cannot be repositioned (Pavel, 2024).

Addressing anesthetic complications

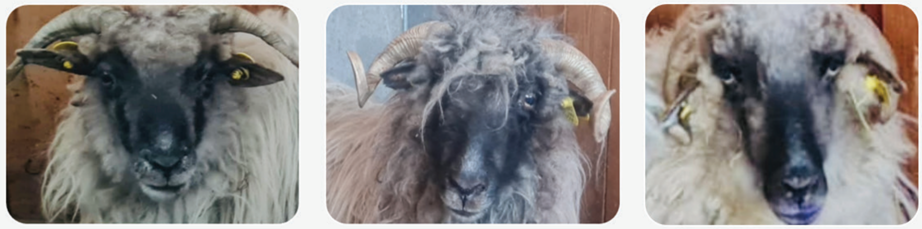
Assessing the depth of anesthesia in sheep based on physical signs can be challenging. Active regurgitation, often accompanied by swallowing motions or explosive discharge of large amounts of ruminal materials, serves as a dependable indicator. In contrast, passive regurgitation, marked by a steady flow of rumen fluid, suggests a deeper level of anesthesia. Passive regurgitation is related to an anesthetic-induced esophageal muscle relaxation (Izer *et al.,* 2023). Deep anesthesia is often signaled by a centrally positioned eye and an absent palpebral reflex (Malik, 2014).

Monitoring heart rate, blood pressure, and cardiac rhythm is essential to detect and manage cardiovascular complications promptly. In inhalation anesthesia, blood pressure tends to decrease as the anesthesia depth increases (Pavel, 2024). During general anesthesia, hypotension characterized by a mean blood pressure <65 mmHg should be managed (Clarke *et al.,* 2014). Blood pressure in sheep can be assessed non-invasive through various arteries (auricular, saphenous, digital artery), by the Doppler technique with a selected cuff approximately 40% of the limb's circumference, or by continuously invasive technique (Almeida, 2014). Maintaining a mean pressure (MAP) above 60 mmHg is essential during anesthesia, to ensure adequate tissue perfusion. To increase MAP in hypotensive sheep reduce the delivery of inhaled anesthetic, increase intravenous fluid rate, and administer inotropic and vasopressor medication.

Dobutamine is typically administered for the inotrope effect at a dosage range of 1–4 mg /kg/ min. Phenylephrine 1 to 2 mg/kg IV should be reserved for treating hypotension when excessive vasodilation is suspected. Hypertension, although uncommon, may arise during particularly painful procedures or in the presence of hypoxemia or severe hypercapnia.

Bradycardia with a MAP => 70 mm Hg, does not require treatment. However, if the heart rate falls below 40 bpm, administering 0.002 to 0.003 mg/kg of atropine intravenously is usually adequate to restore the heart rate. Tachycardia is not frequent for anesthetized sheep and does not necessarily suggest a low depth of anesthesia. If tachycardia persists, 0.1–0.2 mg/kg of xylazine may help.

Tissue perfusion can be evaluated subjectively through mucous membrane color. Placing the pulse oximeter probe on the tongue can display continuous pulse signals and hemoglobin saturation values (SpO_2), with efforts aimed at maintaining SpO_2 above 90%. However, excessive salivation in anesthetized sheep may hinder probe placement.



Action of the Sheep Grimace Scale			
Orbital tightening	Open eyes	Eyes half-closed	Eyes completely closed
Ear position	Ears in normal position	Flattened ears	Hanging ears
Head position	Head in normal position	Slanted head	Hanging head, Flehmen response

Fig. 3. Pain assessment actions for sheep Grimace Scale, adapted from [44].

Sheep may experience decreased respiratory function under anesthesia, leading to hypoventilation or respiratory arrest. This can be exacerbated by factors such as the choice of anesthetic agents, underlying respiratory disease, or prolonged anesthesia duration. The respiratory rate and tidal volume in sheep during anesthesia are approximately 40 breaths per minute and 8 to 10 ml/ kg, respectively (Davis and Musk, 2014). Ideally, capnography and/or arterial blood gas analysis should be performed to objectively assess ventilation, especially during prolonged anesthesia.

Hypoxemia is typically defined as an arterial PO_2 < 60 mmHg, and is more likely to occur in sheep anesthetized with injectable drugs, without supplemental oxygen. Factors such as dorsal recumbency and ruminal distension can increase the risk of hypoxemia. Oxygen supplementation at a rate of a maximum of 15 l/ minutes, is recommended after prolonged anesthesia, particularly for cases that experienced hypoxemia during the procedure (Seddighi and Doherty, 2016).

Temperature regulation is crucial, as sheep undergoing long anesthesia for research studies, can become hypothermic despite their fleece and body size. Therefore, it is important to employ standard measures to prevent hypothermia. Heat lamps, blankets, or external heat sources should be used during anesthesia and in the early recovery phase.

In the event of an anesthetic incident leading to a change in vital parameters and an emergency, immediately cease the administration of anesthetics, antagonize if feasible, and provide first aid measures (Davis and Musk, 2014). If the patient is not already intubated, urgently perform intubation. If already intubated, check the patency and location of the tube. Ensure oxygenation and ventilation and provide adequate cardiovascular support, considering that chest compressions may be challenging due to the patient's anatomy. Administer emergency medication as appropriate to the situation. For severe bradycardia, administer Atropine 0.1 mg/ kg intravenously. In cases of cardiac arrest, administer Adrenaline 0.01 mg/kg intravenously.

Managing analgesia effectively

Pain control in farm animals is typically assessed by changes in clinical status along with behavioral disorders (Abrahamsen, 2008). Animals may show signs of expiratory grunt, teeth grinding, decreased appetite and rumination, vocalization, increased lying, or reduced grooming. Increased respiratory rate and heart rate is a sensitive, non-invasive method to assess mild to moderate pain in sheep (Cohen *et al.,* 2024). Farm animals that feel pain can remain isolated from the group, dull, depressed, or lethargic. The lack of analgesia management can cause a reduction in food and water intake, with a decrease in daily weight gain, milk production, reduced locomotor activity, endocrine changes, catabolic state, fever, leukocytosis, and behavior problems. Preemptive administration of analgesia is ideal in sheep. Intense pain necessitates the administration of a potent pain-relieving medication, that should be given proactively to manage symptoms effectively (Stubsjøen *et al.*, 2009).

The pain recognition domain evolved with the development of the pain scoring validated systems including data such as facial expression changes (Fig. 3) used for pain assessment Grimace Scale (Kania *et al.*, 2021). A validated sheep composite scale for the assessment of abdominal pain has also been published (Häger *et al.*, 2017). These tools are very useful in the management of analgesia and the verification of the effectiveness of the treatment of sheep undergoing research procedures. The use of artificial intelligence for pain assessment offers a promising and objective alternative for evaluating pain in sheep (Silva *et al.*, 2020).

Various classes of analgesic drugs are used for pain control, including opioids, α2-adrenergic agonists, NSAIDs, and local anesthetics (McLennan and Mahmoud, 2019). Opioids, although highly effective and commonly used for treating severe pain in other species, exhibit limited effectiveness in sheep. Opioids can be included as part of the preoperative anesthesia regimen and continued postoperatively to provide additional pain relief.

The most used opioid for sheep is Butorphanol (0.01–0.5 mg/kg), being included in protocols for short-term analgesia and sedation, respectively. Its efficacy in providing visceral analgesia is questionable. It can cause side effects in the central nervous system (agitation, euphoria, dysphoria) or gastrointestinal stasis (Stillman and Whittaker, 2019). Buprenorphine (0.005–0.01 mg/kg) intravenously, intramuscularly, or subcutaneously, primarily produces analgesia lasting for 3–4 hours. It can be readministered at 4–6-hour intervals via the intramuscular or subcutaneous route. The onset of action is slow, and abnormal behavior, such as agitation, may manifest (McLennan and Mahmoud, 2019).

For major surgical interventions and with an increased duration, stronger opioids can be used. Fentanyl 0.01 mg/kg intravenously provides rapid-onset but short-duration analgesia (less than 1 hour). Transdermal fentanyl, such as fentanyl patches (100 μg/60 kg), could be considered for prolonged analgesia, potentially lasting up to 3 days. Care should be exercised when employing transdermal patches, as the rate of drug delivery depends on body temperature. If the body temperature at the application site reaches 40°C, the dose may increase by up to 1/3. Hence, it is advisable to avoid application sites where sheep are likely to lie (McLennan and Mahmoud, 2019).

α2-adrenergic agonists offer both analgesia and sedation in sheep, regardless of whether they are administered systemically or epidurally. While sedation typically lasts longer than analgesia, these drugs can lead to cardiopulmonary changes and hypoxemia. In sheep, α2-adrenergic agonists are more likely to achieve analgesia compared to opioids. Medetomidine 0.005 mg/kg IM, and Xylazine 0.05 mg/kg IV can provide sedation, recumbency, and effective analgesia lasting approximately 45-60 minutes (Kästner, 2006). Xylazine and lidocaine can be injected subarachnoid for analgesia (DeRossi *et al.*, 2005). Intrathecal xylazine has a rapid onset (around 20 minutes) and a longer effect (about 100 minutes) compared to detomidine (Haerdi-Landerer, 2005). Balanced analgesia, involving multiple classes of drugs like an alpha-2 agonist and an N-methyl-D-aspartate antagonist (low-dose ketamine 2–5 mg/kg IV), likely offers superior pain relief for severe pain (Lin, 2014).

NSAIDs offer analgesia through peripheral anti-inflammatory effects, but in sheep, they also appear to have centrally mediated analgesic properties (Lizarraga and Chambers, 2012). Analgesia protocols can be supplemented with non-steroidal anti-inflammatory medication, thus ensuring multimodal pain control. NSAIDs are particularly useful for orthopedic and visceral pain, although they can induce gastrointestinal ulcerations. Due to their extended duration and lack of sedative effects, NSAIDs are well-suited for preemptive administration. Flunixin, given intravenously at a dose of 2.2 mg/kg, is an effective analgesic in sheep, although it is less potent than α2-adrenergic agonists. Its duration of action is approximately 3–6 hours, and re-administration of 2.3 mg/kg every 12 hours or 1.1 mg/kg every 8 hours has been recommended (Lin, 2014). Phenylbutazone, administered intravenously or orally at a dose range of 2–6 mg/kg, can be used in sheep and, similar to cattle, can likely be readministered once daily. Carprofen 4.0 mg/kg administered either SC or IM 90 minutes before the procedure was found to provide a reduction of the cortisol and the behavioral response to a surgical stimulus for mulesing (Small *et al.*, 2021). Ideally, NSAIDs should be administered preoperatively and continued postoperatively, given their effectiveness in managing pain and inflammation.

Local and regional anesthesia techniques should be utilized whenever feasible to target specific areas of pain. Starting from the type of procedure and the individual particularities, different techniques can be chosen, to be able to control the pain in a multimodal manner (Steagall *et al.*, 2021). Lidocaine exhibits a rapid onset, typically within minutes, and has a short duration of action lasting between 60 and 90 minutes. In contrast, bupivacaine has a slower onset, taking about 15 to 20 minutes to take effect, but it has a longer duration of action, lasting approximately 4 to 6 hours. The toxic dose of lidocaine, if administered intravenously, ranges from 3 to 7 mg/kg, while for bupivacaine, it is lower, ranging from 1 to 2 mg/kg. To increase the efficiency of loco-regional anesthesia, respectively of blockages, local anesthetics can be used in high concentrations, anesthetic substances (lidocaine and bupivacaine) can be combined with adrenaline (Colditz, 2009), they can be diluted in alkalizing substances (1 ml lidocaine 2% + 0.1 ml of sodium bicarbonate 8.4%). The administered solutions can be heated causing a faster onset of blockage and a reduction in the burning/pain sensation on injection. The efficiency of the administered local anesthetic can also be increased by decreasing the speed of transmission, respectively by cooling the nerve (Lidocaine 2%/bupivacaine 0.5%, 0.1–0.15 ml/kg can be injected subarachnoid for lumbosacral anesthesia (Collins *et al.*, 2013).

Discussion

Sheep share numerous anatomical and physiological similarities to humans, making them suitable subjects for research procedures. Effective anesthesia is crucial not only to minimize pain and distress but also to ensure accurate data collection in a safe environment. Sheep provide a degree of consistency and reproducibility in experiments that could be harder to achieve with smaller animals. Recognizing potential complications and taking proactive steps to avoid them is paramount to ensuring the safety and welfare of laboratory animals and adherence to best practices in anesthesia management. The sheep's impressive capacity to maintain homeostasis during extended anesthesia highlights the accordance with the globally recognized 3R principles of replacement, reduction, and refinement, established for ethical animal research. Future research efforts should prioritize evaluating the safety and effectiveness of novel drugs, as well as documenting supplementary pain-relieving substances to improve the welfare of sheep used in biomedical research.

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Conflict of interest

The authors declare that there is no conflict of interest.

Authors' contributions

Conceptualization, R.C., T.I., and R.P.; Methodology, R.C., T.I., N.C., A.D., L.F., and R.P.; Draft preparation, R.C., T.I., N.C., A.D., L.F., and R.P. All authors have read and agreed to the published version of the manuscript.

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Data availability

All data that support the findings of this study are included in the manuscript.

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