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ANESTHESIA IN SHEEP: MAINTAINING ETHICAL STANDARDS IN EXPERIMENTAL RESEARCH

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Abstract

Sheep (Ovis aries) are frequently used as experimental models in various fields of biomedical research, necessitating the development of ethical and effective anesthetic protocols. This paper aims to review and evaluate current anesthetic protocols for sheep, focusing on their application in experimental research settings. Emphasis is placed on the importance of ethical treatment, including minimizing pain, distress, and physiological stress while maintaining adequate anesthesia levels for surgical procedures. Several approaches are discussed, including preanesthetic preparation, drug combinations, and monitoring techniques. Special attention is given to the balance between ensuring animal welfare and achieving scientific validity. This review also addresses key considerations for specific protocols, particularly those involving sheep, offering insights into sedation, induction, and maintenance strategies that reduce the risks of complications such as regurgitation and aspiration. The goal is to guide researchers in adopting practices that align with ethical standards and experimental objectives, ultimately enhancing the quality and reproducibility of research outcomes.

Key words*: anesthesia, ethical, research, sheep.*

INTRODUCTION

With the growing emphasis on animal welfare in research, it has become essential to ensure that anesthetic protocols provide effective anesthesia and align with ethical treatment standards. Sheep (*Ovis aries*) have long been used as models in experimental research due to their physiological similarities to humans, alongside other species like pigs (Moldovan et al., 2022). However, there is a limited body of scientific literature on anesthesia and analgesia in sheep (Stillman & Whittaker, 2019).

In veterinary anesthesia, the balance between achieving adequate sedation and maintaining physiological stability is critical. Previous studies have highlighted challenges in sheep anesthesia, such as regurgitation, aspiration, and respiratory depression, which can occur if protocols are not tailored to the sheep's unique anatomical and physiological characteristics (Costea et al., 2022).

Recent advancements in veterinary anesthesiology have led to the refinement of anesthetic protocols that prioritize both efficacy and the reduction of stress and discomfort in the animals, as seen in studies focusing on multimodal anesthesia approaches (Costea et al., 2024). However, further research is needed to assess their implementation in sheep-specific studies, ensuring that these protocols adhere to ethical principles.

This paper explores the anesthetic protocols used in sheep employed in experimental research. By examining preoperative preparation, induction, and maintenance strategies, the aim is to provide a comprehensive overview of current anesthesia practices while highlighting the importance of adhering to ethical guidelines. Additionally, the paper seeks to address the potential risks associated with anesthesia in sheep and how these can be mitigated through proper protocol selection and monitoring.

Ethical considerations play an important role in research involving live animals. When animals are used in experimental research, it is essential to strive to ‘replace’ them with non-sentient alternatives, ‘reduce’ the number of animals required, and ‘refine’ the procedures to ensure minimal pain and distress for the subjects (Richter, 2024). In line with these principles, this study complies with EU Directive 2010/63/EU on the Protection of Animals Used

for Scientific Purpose which explicitly recognizes the replacement of animal experiments as a long-term objective (Franco & Olsson, 2014), and Romanian Law no. 43/2014, which regulates the protection of animals used for scientific purposes (Romanian Parliament, 2014).
The overall objective is to provide researchers with a thorough understanding of the complexities involved in sheep anesthesia, ensuring that their protocols not only meet the needs of the research but also uphold the highest standards of animal welfare.

MATERIALS AND METHODS

The data presented in this paper derived from our scientific studies on 18 anesthetic protocols applied to adult sheep (*Ovis aries*), anesthetized for medical research, at the University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Veterinary Medicine.
The researches described in this study were approved by the Bioethical Committee of the Faculty of Veterinary Medicine, University of Agronomic Sciences and Veterinary Medicine of Bucharest, decision no. 33/1.02.2024.
The study adhered to national and European regulations on the care and use of animals for scientific purposes, following the 3R principles (Replacement, Reduction, and Refinement). The 18 cases that were part of a research project and some private cases presented in our clinic, including sheep of varying breeds, with ages between 2 to 4 years and weights ranging from 43 to 67 kg that underwent various experimental medical procedures and were anesthetized respecting the same protocol.
The standardized premedication protocol involved intramuscular (i.m.) administration of midazolam (0.2 mg/kg), ketamine (5 mg/kg), and butorphanol (0.1 mg/kg). Following premedication, anesthesia induction was achieved intravenously (i.v.) with either propofol (3-5 mg/kg) or alfaxalone (1-3 mg/kg). Endotracheal intubation was then performed, and anesthesia was maintained with isoflurane at concentrations between 1.0 to 3.0 vol % in 100% oxygen supplemented with ketamine via continuous rate infusion (0.5 mg/kg/h, i.v.).

RESULTS AND DISCUSSIONS

This study presents a comprehensive analysis of the procedures implemented within the anesthetic protocols for 18 sheep (*Ovis aries*) used for experimental research, aimed at ensuring adherence to and the application of ethical standards.
Our study examined five key criteria related to animal research ethics: adherence to the 3Rs Principles (Replacement, Reduction, and Refinement), compliance with ethical and legal standards, pain, and stress management during anesthesia, transparency in methodology and documentation, and oversight and ethics approval.
The first criterion assessed was adherence to the 3Rs Principles (Replacement, Reduction, and Refinement), which offer a structured framework for conducting research ethically, while ensuring that scientific objectives are met.
Although live animals were essential for obtaining accurate data in this study, alternatives were carefully considered, and future research should continue exploring alternative models that could replace the use of live animals. Alternative approaches can enhance and reinforce the generation of scientific knowledge while adhering to one of the 3R Principles, specifically Replacement (Andersen & Winter, 2019).
Reducing the number of animals used in our study was a priority. Our objective was to keep the number of animals at the minimum necessary to obtain valid results, without compromising the objectives of our research. We designed the studies to involve a minimum number of animals (*Ovis aries*), ensuring the highest standards of animal welfare while limiting the number of subjects (Russell & Burch, 1959).
Refinement was integral to our protocols as well. By prioritizing the Refinement principle of the 3Rs, researchers can improve animal welfare while maintaining scientific integrity, leading to more ethical and reliable research outcomes (Rinwa et al., 2024).
The second criterion assessed was compliance with ethical and legal standards. All procedures were conducted in strict accordance with national and international ethical guidelines to

safeguard animal welfare throughout the research. Specifically, our study adhered to the European Union Directive 2010/63/EU, which regulates the protection of animals used for scientific purposes, establishes guidelines to ensure ethical treatment, minimize suffering, and promote the application of the 3Rs Principles (Replacement, Reduction, and Refinement) in research (European Parliament & Council of the European Union, 2010), and Romanian Law no. 43/2014, which incorporates Directive 2010/63/EU and establishes measures to protect animals used for scientific or other experimental purposes (Romanian Parliament, 2014).

The third criterion focused on pain and stress management before, during, and post-anesthesia. Ethical research mandates effective strategies for managing pain and stress in all experimental animals, incorporating preoperative, intraoperative, and postoperative measures to safeguard animal welfare. Pain triggers behavioral, autonomic, and neuroendocrine responses, resulting in a negative affective state that can significantly compromise animal welfare (Steagall et al., 2021).

To minimize pain and distress in sheep, the anesthesia protocols were refined in strict accordance with ethical standards. This included the use of a multimodal approach combining appropriate anesthetic and analgesic agents, comprehensive pre- and post-anesthetic care measures, and continuous monitoring to promptly address any signs of discomfort or physiological imbalance.

Preoperative measures included thorough evaluations to ensure the animals' suitability for anesthesia. Each sheep underwent a physical assessment to identify potential risks, with a focus on the respiratory, cardiovascular, and digestive systems. The gastrointestinal tract was checked for any signs of distension or discomfort and hematological and biochemical tests were conducted. These evaluations were performed in low-stress conditions, avoiding harsh restraint methods or loud noises to minimize stress. Proper preanesthetic assessment is crucial for confirming the animals' health and readiness for procedures and is a fundamental aspect of ethical standards in animal research.

A key component of our study was the careful selection of preanesthetic medication. We utilized a combination of drug classes, including sedative-opioid combinations and anesthetic agents, to optimize analgesia while minimizing the risk of adverse effects. Comprehensive preanesthetic monitoring was performed to ensure the safety and stability of each sheep. Baseline measurements for heart rate, respiratory rate, body temperature, and pulse oximetry were recorded. Continuous monitoring of heart rate, respiratory rate, and oxygen saturation was maintained throughout the procedure to uphold the sheep's well-being (Costea et al., 2022). The use of a premedication combination of midazolam, ketamine, and butorphanol, alongside diligent monitoring and effective pain management, was designed to minimize distress and discomfort.

To prevent regurgitation due to excessive saliva production, the sheep were positioned in sternal recumbency (Somers, 1957). Their heads were carefully elevated, and protective measures were taken to prevent eye dryness and potential lesions. Since tear production decreases during anesthesia, eye protection was initiated from the premedication phase (Pavel et al., 2024). Mild ophthalmic ointment was applied to lubricate the eyes, followed by closing and taping them shut. Applying ophthalmic ointment and keeping the eyes closed is critical to reducing the risk of corneal damage (Thurmon & Benson, 1993).

Preanesthetic medication, including midazolam (0.2 mg/kg), ketamine (5 mg/kg), and butorphanol (0.1 mg/kg), was administered intramuscularly into the triceps muscle, adhering strictly to animal welfare protocols. A 20-gauge intravenous catheter was inserted into the cephalic vein under sterile conditions to ease anesthesia induction and allow for the administration of additional parenteral anesthetics, analgesics, emergency medications, or intravenous fluids. The sheep's condition was closely monitored to ensure safety and stability during the transition into anesthesia.

Intraoperative measures focused on supporting animal welfare and procedural efficiency. Anesthesia was induced intravenously with either propofol (3-5 mg/kg) or alfaxalone

(1-3 mg/kg) until the desired effect was reached. Intubation was carried out using soft ropes for access and a long laryngoscope blade. The sheep were placed in sternal recumbency with the neck in hyperextension to facilitate clear visualization of the laryngeal opening. Tracheal palpation and direct visualization were employed to select the appropriate tube size. Ethical standards were upheld throughout the process by minimizing stress and discomfort for the sheep. Positioning was carefully monitored to prevent injury, and the intubation was performed efficiently to limit any potential distress. Supplemental oxygen was provided via a flow-by technique, and continuous monitoring of vital signs ensured the animal's physiological stability.

After successful intubation, the cuff of the endotracheal tube was inflated and secured to the mandible using textile ropes, ensuring the tongue remained unobstructed. A textile roll was positioned under the submandibular region to elevate the head and reduce the risk of saliva regurgitation. Appropriate padding was applied to prevent nerve compression and excessive pressure on major muscle groups, aligning with ethical standards (Costea et al., 2015) to prioritize the sheep's comfort and welfare throughout the procedure (Figure 1).

Figure 1. Ensuring sheep's comfort and welfare (original)

Continuous monitoring of vital signs was conducted to ensure the animal's stability throughout the procedure. Anesthesia was maintained with isoflurane (1.0-3.0%) in 100% oxygen, delivered through a standard small animal circle circuit, and supplemented with a continuous rate infusion of ketamine (0.5 mg/kg/h, i.v.) for additional analgesia and sustained anesthetic depth. All sheep breathed spontaneously, which eliminated the need for assisted ventilation, even during deep anesthesia. Ethical standards were upheld by closely monitoring vital parameters and adjusting the anesthetic protocol as needed to maintain physiological stability and ensure animal well-being. This approach effectively minimized distress, adhering to the highest standards of animal care.

Intravenous fluid therapy was administered to prevent hypotension and hypoperfusion, with a maintenance rate of 5 ml/kg/h using Ringer's Lactate solution. Anesthetic depth was continuously monitored by evaluating the absence of the palpebral reflex, mandibular tone, cardiopulmonary stability, and any signs of movement or swallowing. Advanced monitoring techniques, including pulse oximetry, capnography, rectal temperature, blood pressure, and continuous electrocardiography, were employed. Arterial blood pressure was maintained within clinically acceptable ranges throughout the procedure to ensure the sheep's physiological stability.

Postoperative measures focused on ensuring a smooth recovery and minimizing stress for the sheep. Cuff deflation and extubation were delayed until the sheep exhibited sufficient clinical signs, such as the palpebral reflex, coughing, swallowing, and limb movements. Following extubation, vital signs were continuously monitored, and intravenous fluids were administered for an additional 10 minutes. During this period, the sheep remained calm and cooperative. Efforts were made to minimize stressors, such as loud noises, excessive light, and sudden movements, to enhance the sheep's comfort. The animals were then moved to a recovery area, placed in a warm, sheltered environment to prevent hypothermia, which could delay recovery. Anesthesia recovery was uneventful, and the sheep fully recovered after extubation. All animals resumed routine activities, including feeding and drinking, approximately 45 minutes after anesthesia. Improvements in anesthesia techniques, including rapid induction and recovery protocols, helped reduce potential suffering.

The fourth criterion assessed was transparency in methodology and documentation. Ethical research demands clear and thorough reporting of all methods. To ensure our study could be evaluated for its ethical standards, we provided comprehensive documentation of every aspect of the anesthesia protocol including the drugs and dosages administered, preanesthetic evaluations, and post-procedure care. This transparency enables replication of the study and assessment of the ethical implications of the techniques used, fostering accountability in the scientific community. Throughout the study, we meticulously monitored welfare indicators such as behavioral changes, physiological stress markers, and recovery rates, ensuring that any signs of distress or suffering were promptly addressed.

The final criterion focused on ethics approval and oversight. The entire group of animals was included in research procedures approved by the Bioethical Committee of the Faculty of Veterinary Medicine, University of Agronomic Sciences and Veterinary Medicine of Bucharest. This esteemed institution rigorously reviewed the research design, anesthesia protocols, and potential risks to the animals, ensuring that all procedures complied with ethical standards. The approval process serves as a safeguard to ensure the humane treatment of animals in research and underscores the importance of applying ethical frameworks in experimental studies. Measures taken to mitigate potential harms and improve animal welfare further contribute to the reproducibility of the research (Brill et al., 2021).

CONCLUSIONS

This study provides a comprehensive analysis of the anesthetic protocols used in experimental research involving sheep, focusing on the application of ethical standards in the management of animal welfare. By evaluating key criteria such as adherence to the 3Rs Principles, compliance with ethical and legal standards, pain and stress management, transparency in methodology, and ethics approval, we ensured that the research was conducted with the utmost respect for animal welfare.

Our findings highlight the importance of refining anesthetic protocols to minimize stress and pain, incorporating continuous monitoring, and applying rigorous preoperative, intraoperative, and postoperative care measures. By strictly adhering to established ethical frameworks, we were able to ensure the humane treatment of the sheep throughout the research process. Transparency in our methodology also ensures that our study can be replicated and evaluated, promoting accountability within the scientific community.

Furthermore, the approval of our study by the Bioethical Committee of the Faculty of Veterinary Medicine, University of Agronomic Sciences and Veterinary Medicine of Bucharest, underscores the importance of institutional oversight in safeguarding animal welfare in experimental research. Ultimately, this research reinforces the importance of adhering to ethical standards while achieving valid scientific outcomes and highlights the need for continued efforts to refine research protocols in anesthesia in sheep.

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