Scientific Papers. Series D. Animal Science. Vol. LXVIII, No. 1, 2025
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

PREANESTHETIC GUIDELINES IN SHEEP: ENSURING WELFARE AND SAFETY IN EXPERIMENTAL RESEARCH

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Abstract

The aim of this study was to identify the important preanesthetic factors that could improve anesthesia management in research sheep, focusing on reducing critical complications like regurgitation and aspiration of ruminal contents during general anesthesia, which can lead to serious respiratory risks and potential fatality. Our study was conducted on a group of 13 sheep (Ovis aries), 2-4 years old, selected for different surgical procedures, during September 2022-June 2024 at the Faculty of Veterinary Medicine of Bucharest. For the entire group the protocol involved a 24-hour fasting period, allowing water access until the premedication stage to ensure hydration. A comprehensive clinical evaluation was performed to identify any underlying health issues, and sedatives, along with prokinetic agents, were administered to reduce stress and encourage gastric emptying. Continuous monitoring of vital signs and behavior ensures the stability of the animals during surgical procedures. By following the comprehensive 7-step preanesthetic guidelines, the risk of regurgitation and aspiration, along with other risks can be significantly reduced, thereby improving sheep welfare and safety during general anesthesia.

Key words: *guidelines, preanesthesia, research, sheep.*

INTRODUCTION

Several factors come into play when considering the suitable animal model for experimental research, including anatomy, physiology, and genetic similarity to humans. Each animal model has specific strengths and limitations depending on the area of research.

Pigs have remarkably anatomical and physiological similarity to humans, especially in terms of the gastrointestinal tract, cardiovascular system, skin, and kidneys and are widely used in heart disease studies and surgical research, including organ transplantation, wound healing studies or metabolic studies (Moldovan et al., 2022). Pigs are not easy animals to handle, especially large ones (Costea et al., 2023). While rats are valuable in understanding basic biological mechanisms, their small size raises many anesthetic and surgical risks (Costea et al., 2015) and translating findings to human medicine sometimes requires validation in larger models like pigs or sheep.

Sheep (*Ovis aries*) are a valuable animal model for experimental studies, especially those involving surgical procedures. Their body weight, bone size, and bone-healing potential are similar to humans (Reinholz et al., 2004), making them useful for assessing biomaterials in bone regeneration and the integration of dental implant systems in dentistry, orthopedics, cardiovascular and respiratory system studies (Sartoretto et al., 2016). Since these procedures can cause anxiety and pain in the animals, they should be performed under general anesthesia.

Regurgitation and aspiration of ruminal contents represent significant complications during general anesthesia in sheep. These events can result in severe respiratory problems and even death. It is essential to implement appropriate preanesthetic protocols to minimize these risks. Due to the scarcity of scientific research on pain relief that involves sheep as an animal model in experimental studies, researchers frequently face challenges in determining appropriate analgesic protocols for these animals (Stillman & Whittaker, 2019). The discussion will center on commonly used anesthetic agents, including combinations of ketamine, midazolam, and butorphanol, which have been shown to be effective in minimizing stress and physiological disturbances in sheep.

This article provides detailed steps and measures for preanesthetic protocols to ensure that sheep undergo a safe and effective anesthesia experience, ensuring optimal pain management during experimental surgical procedures.

MATERIALS AND METHODS

The information presented in this article is based on scientific data obtained from the evaluation of 13 general anesthetic protocols. The research was carried out at the University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Veterinary Medicine, in compliance with the ethical guidelines for animal welfare, with all necessary permits and approvals obtained.

Thirteen adult sheep (*Ovis aries*) of different breed, aged 2-4 years, weighing 43-67 kg (with a mean body weight of 54.11 kg) were anesthetized at the Faculty of Veterinary Medicine of Bucharest, for different experimental surgical procedures.

The premedication intramuscularly (i.m.) protocol consisted of midazolam (0.2 mg/kg, i.m.), ketamine (5 mg/kg, i.m.), and butorphanol (0.1 mg/kg, i.m.) and fallowed by intravenous (i.v.) induction with propofol (3-5 mg/kg, i.v.) or alfaxalone (1-3 mg/kg, i.v.), intubation and maintenance with isoflurane (1.0-3.0 vol %) in oxygen 100%.

RESULTS AND DISCUSSIONS

Before anesthesia, it is essential to acknowledge that sheep are sensitive to stress, making the implementation of appropriate handling techniques crucial for their welfare. Inadequate or inappropriate physiological, immunological, metabolic, and behavioral responses can result in stress, leading to distress characterized by poor health or compromised well-being (Ignătescu et al., 2018), which may ultimately affect the outcomes of the procedure or the research.

Sheep (*Ovis aries*) are ruminants with a complex stomach system consisting of four compartments: the rumen, reticulum, omasum, and abomasum. While this system aids in digesting fibrous plant material, it also poses specific risks during general anesthesia. The primary concerns are regurgitation and aspiration of ruminal contents. Also, sheep can produce an increased saliva volume when under anesthesia, leading to airway obstruction. Recognizing these risks and applying preventive measures is crucial for safeguarding the animals' well-being and ensuring the success of the experimental procedures (Costea et al., 2022).

The choice of anesthesia and analgesia protocols was based on species-specific characteristics, the type of surgery, anticipated pain, and the available equipment. After analyzing these parameters, we decided to implement a 7-step preanesthetic protocol, including fasting management, preanesthetic evaluation, selection of preanesthetic medication, preanesthetic monitoring, administration of premedication, sheep stabilization before anesthesia induction, and post-preanesthetic monitoring.

The first step of our study was fasting management. Fasting in sheep it is necessary to reduce the risk of regurgitation during general anesthesia. Fasting has a limited effect on the volume of contents in the rumen but may reduce the incidence of ruminal tympany, which is gas accumulation in the rumen caused by bacterial fermentation (Wang et al., 2023). To avoid complications the sheep in our study were not given food 24 hours before general anesthesia and had unrestricted access to water until the premedication protocol was administered.

After the fasting period, the second step was to conduct a preanesthetic evaluation of the sheep. This was done in a low-stress environment, avoiding harsh restraint methods and loud noises to minimize stress. The preanesthetic evaluation included a clinical physical examination and the measurement of the sheep's weight.

We conducted for all the cases a comprehensive physical examination to evaluate the overall health of the sheep before administering anesthesia. This examination focused on the respiratory, cardiovascular, and digestive systems. We monitored the respiratory rate to identify any pre-existing respiratory conditions that could complicate the anesthesia process. Evaluating heart rate and rhythm was equally important, as any irregularities could affect the animal's response to anesthetic agents. Additionally, we checked the gastrointestinal tract of

the sheep for signs of distension or discomfort, which could indicate digestive issues requiring special consideration during surgery.

Hematological and biochemical tests were conducted to evaluate the clinical and metabolic status of the sheep (Mihai et al., 2023).

After the evaluation, the entire group of 13 sheep was classified as the ASA 1-2 risk group, according to the American Society of Anesthesiologists veterinary medicine standards, indicating a normal, healthy patient without any organic disease.

Sheep's body weight was accurately measured, which is crucial for determining the correct dosages of anesthetic agents. Since anesthetic drugs are typically administered based on weight, any inaccuracies could lead to underdosing or overdosing, both of which pose significant risks. An electronic scale was used to obtain precise weight readings, and excluded the weight of the wool to obtain precise weight estimation. A "wool-free weight" formula (Costea et al., 2022) was applied for the entire group by reducing the measured total body weight by 5%.

In the third step of our research, we focused on selecting preanesthetic medication. Preanesthetic drugs have several important functions, including reducing the amount of anesthetic needed for induction and maintenance, calming the animal for a smoother induction, and contributing to a more stable anesthesia. The choice of premedication is based on the results according to the preanesthetic examination and on the expected maintenance time of anesthesia.

Sedatives, sedative-opioid combinations, and anesthetic agents like ketamine can be used to achieve the desired sedation effects (Hall et al., 2001).

The preferred routes of administration, known for providing more predictable outcomes, are intramuscular or intravenous.

The selected cases received premedication protocols using benzodiazepines as the sedative of choice for sheep, typically in combination with other agents like opioids, ketamine.

The intravenous administration of a benzodiazepine, such as midazolam at a dose of 0.10-0.25 mg/kg, can produce sedation and ataxia, typically lasting for about 30 minutes. When administered intramuscularly at the same dose range (0.10-0.25 mg/kg), midazolam significantly increases the depth of sedation, especially when combined with another sedative or opioid. For instance, butorphanol at 0.1 mg/kg has a rapid onset of action and, when given 5-10 minutes before midazolam or diazepam, and ketamine, helps facilitate a smooth and relaxed induction of anesthesia. The effect typically lasts for 1-2 hours (Hall et al., 2001). The combination of ketamine, a benzodiazepine (midazolam), and an opioid (butorphanol) provides moderate sedation, and effective analgesia, and has minimal impact on cardiovascular function (White & Taylor, 2000).

α2-agonists may be less effective for pain relief in sheep compared to other animals, and at higher doses, they can cause excitement or changes in behavior (Kästner, 2006). Xylazine is a popular choice, but it can reduce ruminal motility and lead to bloat and is likely to develop pulmonary edema (Bacon et al., 1998). Ketamine can be administered alongside xylazine and butorphanol to achieve sedation and pain relief.

All these premedication combinations allow for administering smaller doses of each drug, thereby reducing potential side effects and facilitating a multimodal approach.

Based on these considerations, we analyzed the best combination of sedatives and pain relievers to keep the sheep calm during surgery and manage their pain effectively. By carefully selecting our preanesthetic medication, we aimed to optimize both sedation and pain relief, thereby improving the overall safety and effectiveness of the surgical procedures.

We decided to administer the premedication via intramuscular injection into the triceps muscle. After choosing the preanesthetic medication, we conducted the preanesthetic monitoring to ensure the safety and stability of the sheep. Before administering any anesthetic drugs, we recorded the baseline heart and respiratory rates for each sheep. This was crucial to establish a reference point, allowing us to identify any potential abnormalities or changes in the sheep's condition after sedation and during the anesthetic process.

Body temperature and pulse oximetry were monitored to ensure the sheep were in optimal

condition for anesthesia and surgery. It is crucial to maintain stable body temperature, as hypothermia can complicate the anesthetic process. Continuous monitoring of pulse oximetry was vital for assessing oxygenation and ventilation after administering the preanesthetic medications (Figure 1).

Figure 1. Monitoring during anesthesia (original)

To address the significant saliva production of approximately 6-16 liters over 24 hours and to prevent regurgitation, the sheep were positioned in sternal recumbency with their heads elevated (Somers, 1957).

Tear production decreases during anesthesia in sheep, making it essential to implement protective measures to prevent eye dryness and potential lesions. Proper eye care, such as closing and taping the eyes or applying appropriate lubricants, is crucial for maintaining ocular health during anesthesia. Ideally, protective eye care measures should be implemented starting from the premedication phase (Tudor et al., 2018). Early intervention ensures optimal eye protection throughout the entire anesthetic process, reducing the risk of ocular complications in sheep (Pavel et al., 2024). After administering the premedication, a 20-gauge intravenous catheter was easily inserted into the cephalic vein to prepare for the induction of anesthesia (Figure 2).

The venous catheter helps administer intravenous fluids, additional doses of parenteral anesthetic and analgesia, and emergency medication if needed. Before inducing general anesthesia, it was mandatory to stabilize each animal to ensure the best possible conditions for the procedure. This step was the sixth phase of our preanesthetic protocol.

Figure 2. Intravenous catheter into the cephalic vein (original)

The main aim was to ensure that the sheep were calm and stable before anesthesia. To achieve this, we used various techniques to minimize stress, such as gentle handling and maintaining a quiet environment. By creating a low-stress atmosphere, we aimed to help the sheep feel secure, which is crucial for their safety and a smooth transition into anesthesia.

Another important goal was to support the cardiovascular function and to maintain the blood pressure of the sheep. We achieved this by administering intravenous fluids based on the specific condition, hydration status, and cardiovascular health of each animal (Musk, 2024).

During anesthesia sheep received a maintenance rate of fluids of 5 ml/kg/h Ringer's Lactate solution. This step was crucial for ensuring adequate hydration and promoting hemodynamic stability, thereby enhancing the overall safety of the surgical procedures.

After administering preanesthetic medications a thorough monitoring protocol was implemented to ensure safety and stability during the transition into anesthesia. We were prepared to reverse sedation or address any potential side effects that could arise during this phase. If excessive sedation or respiratory depression occurred, we were ready to modify our approach and provide necessary supportive care. This allowed us to manage any potential complications promptly and effectively. Through this anesthetic monitoring, we prioritized the well-being of each sheep, ensuring their stability throughout the induction process, which ultimately contributed to the success of the surgery procedures.

CONCLUSIONS

Adherence to comprehensive preanesthetic protocols is crucial to minimizing the risks of regurgitation and aspiration of ruminal contents in sheep (*Ovis aries*). Under conditions where a rigorous preanesthetic evaluation protocol is followed and preparations are made for potential risks and complications, sheep can serve as a valuable resource for research, yielding predictable results.

This includes careful management of fasting, the administration of appropriate medications, the use of specific preanesthetic techniques, and continuous monitoring. By following these seven steps, we can greatly reduce potential risks and enhance the safety and well-being of sheep undergoing general anesthesia.

This approach is vital for ensuring successful outcomes in experimental surgical procedures involving sheep. For researchers and veterinarians involved in such interventions, this article provides a comprehensive guide to the essential steps of the preanesthetic process, offering guidelines that can enhance success rates and reduce potential complications.

ACKNOWLEDGEMENTS

This research on preanesthetic guidelines in sheep was made possible through the support of our colleagues from the University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Veterinary Medicine, who assisted us in completing the cases that formed the study group.

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