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SHEEP GENERAL ANESTHESIA FOR EXPERIMENTAL RESEARCH PROCEDURES

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Abstract

We evaluated an anesthesia protocol for sheep as an experimental animal for surgical procedures. The entire group (10 sheep, breed Tsigai, 4 years old, 60.91 kg mean body weight) underwent experimental dentistry surgery and received a complete anesthetic protocol: intramuscular premedication (midazolam 0.18 mg/kg, ketamine 4.6 mg/kg, butorphanol 0.1 mg/kg), intravenous induction (propofol 4.45 mg/kg), intubation (endo tracheal cuffed tubes, size 7.5 mm and 8 mm), gas maintenance (isoflurane minimum alveolar concentration of 1.5-2%, a standard small animal circle circuit and spontaneous respirations during the procedures). The protocol was completed with analgesia (meloxicam 1 mg/kg) and clinical monitoring during the entire surgical procedure and in the recovery phase. Sheep were infused at a rate of 5 ml/kg/h with Ringer's Lactate solution, during anesthesia. The anesthesia duration varied between 32 -215 minutes, with a mean of 83.7 minutes. No incidents or complications were recorded during anesthesia. One sheep presented myopathy and lameness (right forelimb) in the first 72 hours after anesthesia, possibly associated with the positioning and the length of the procedure (135 minutes).

Key words*: anesthesia, intubation, isoflurane, protocol, sheep.*

INTRODUCTION

The scientific progress made in the recent years have enabled the research of new medical technologies, bringing together human and animal specialists in complex projects. In this context, it is important to design and conduct experimental studies with reliable animal models. While sheep (*Ovis aries*) are widely used as large animal models, current literature generally focuses on anesthesia and analgesia protocols used for the farm level (Stillman & Whittaker, 2019). The sheep is an excellent animal model for the evaluation of materials for bone regeneration and osteointegration of dental implant system in dentistry (Sartoretto et al., 2016). Adult sheep offer the advantage of a body weight, bone mineral composition and metabolic and remodelling rates similar to human patients (Pearce et al., 2007).
Due to the lack of literature comprehensive data regarding anesthesia for sheep animal models, it is necessary to contribute to the standardisation of the techniques and protocols, according to different types of experimental procedures, covering.
The choice of medication and the administration route used for anesthesia and analgesia during the procedures, need to be appropriate for the animal as well as compatible with the project.

MATERIALS AND METHODS

The researches described in this study were favourably approved by the ethics commission Cantacuzino National Military-Medical Institute for Research and Development, Bucharest, Romania, decision 43/13.04.2021, project authorization 625/17.05.2021.
All researches were performed in accordance with the rules for the care and use of animals used for scientific purposes established by national and European regulations and the application of the 3R principles of replacement, reduction and refinement.
The studies were performed in Băneasa location, in the Preclinical Testing Unit, part of Cantacuzino National Military-Medical Institute

for Research and Development and authorized as a user unit by the competent authority.

Ten adult sheep (*Ovis aries*), breed Tsigai, 4 years old, weighing between 53-69 kg (60.91 kg mean body weight) underwent general anesthesia for experimental dentistry surgery. The complete anesthetic protocol consisted in premedication with midazolam (0.18 mg/kg, i.m., Midazolam®, Baxter Holding B.V., Olanda), ketamine (4.6 mg/kg, i.m., Ketamidor®, Richter Pharma, Austria) and butorphanol (0.1 mg/kg, i.m., Butomidor® Richter Pharma, Austria), induction with propofol (4.45 mg/kg, i.v., Proposure® Richter Pharma, Austria), followed by endotracheal intubation and maintenance with isoflurane (Vetflurane®, France), started with 3-4% for 5 minutes and maintained at 1.5-2 % during the procedure.

In order to ensure a multimodal approach and analgesia, mandatory during long and complex procedures (Valverde & Doherty, 2009), the protocol was completed with ketamine infusion (0.5 mg/kg/h, i.v., Ketamidor®, Richter Pharma, Austria) during maintenance phase and one dose of meloxicam (1 mg/kg, i.m., Meloxidolor®, Le Vet Beheer B.V., Olanda) in the early recovery.

During anesthesia sheep were infused at a rate of 5 ml/kg/h with Ringer's Lactate solution (Soluție Ringer Lactat®, B Braun, Germany), thus ensuring the maintenance of homeostasis.

RESULTS AND DISCUSSIONS

The entire group was prepared for the surgical dentistry procedure, under general anesthesia. The choice of anesthesia and analgesia protocols was decided based on species particularities, type of surgery, possible pain and equipment avaible.

In order to minimize regurgitation and the development of ruminal tympany in the recumbent sheep, food was restricted for 24 h before anesthesia (Drake et al., 2021).

Free access to water was allowed until the premedication protocol was administered. Food and water withholding before anesthesia and endotracheal intubation with cuffed tubes after induction are both decreasing the risk for regurgitation and were included in our protocol for safety reasons.

Preanesthetic evaluation (physical condition, physical examination), was performed under no stress conditions, avoiding brutal contention methods or loud noises, in order to reduce stress factors (Costea, 2017). After the evaluation the entire group was assigned to ASA 1 risk group classification adapted for veterinary medicine, that allowed us to design an anesthetic protocol for healthy animals, with a low anesthesia risk compared to other patient's risk categories (Table 1).

Table 1. American Society of Anesthesiologists-ASA classification (Costea, 2017)

ASA 1	A normal healthy patient, with no organic disease
ASA 2	A patient with mild systemic disease
ASA 3	A patient with severe systemic disease that limits activity but it's not incapacitating
ASA 4	A patient with sever systemic disease that is a constant threat to life
ASA 5	A moribund patient who is not expected to survive 24 hours without intervention

Weight was measured with an electronic scale weighing between (60.91 kg mean body weight). For an accurate estimation of weight, in order to improve the safety of anesthesia, we decided to exclude the wool weight (57.89 kg mean body weight without wool).

Taking in to consideration the breed particularities, regarding wool production and data form the literature (Hutu et al., 2020; Pascal et al., 2014), we decided to use for the entire group a "wool free weight", by reducing the total body weight measured, with 5%. The results were recorded on individual anesthesia sheet.

The anesthetic protocol began with the administration of premedication by intramuscular injection in the triceps muscle. Sheep were premedicated with a combination of midazolam (0.18 mg/kg, i.m., Midazolam®, Baxter Holding B.V., Olanda), ketamine (4.6 mg/kg, i.m., Ketamidor®, Richter Pharma, Austria) and butorphanol (0.1 mg/kg, i.m., Butomidor® Richter Pharma, Austria).

Ketamine in combination with a benzodiazepine (midazolam) and an opioid (butorphanol) provides moderate relaxation, good analgesia and minimal cardiovascular depression (White & Taylor, 2000).

An intravenous catheter (20 gauge) was placed in the cephalic vein after premedication, prior to induction. The catheters were easily placed, the animals premedicated and under sedation.

Mean time between premedication and prior to the induction of anesthesia was 17 minutes (11–20 min).
Anesthesia was induced with propofol (4.45 mg/kg, i.v., Proposure® Richter Pharma, Austria) by slow intravenous injection until effect and after the intubation procedure was started.
With sheep in sternal recumbency and the neck in hyperextension, the trachea was intubated. A long laryngoscope blade was necessary to visualize the laryngeal opening. The thick of the base of the tongue increased the difficulty of intubation.
The intubation was done carefully, under direct visual control, without forcing the insertion of the tube into the trachea. During the intubation, especially if the procedure lasted longer, we tried to additionally provide oxygen to the patient through a flow by technique.
Tracheal palpation and direct visualisation during intubation, determined us to select the appropriate tube size. Sheep required an endotracheal tube with an internal diameter of 7.5 or 8 mm. After intubation the endotracheal tube was cuffed and secured to the mandible with textile ropes, leaving the tongue free.
Eyes were lubricated with a mild ophthalmic ointment (OptixCare® Plus Eye Lube; CLC MEDICA), in order to reduce the risk of corneal lesions during anesthesia (Riebold, 2007).
A support was placed in the submandibular region (textile roll), to raise the cephalic extremity and to avoid saliva regurgitation (Figure 1).

Figure 1. Sheep in sternal position, intubated and connected to the anesthesia machine, with a support placed in the cervical region

Particular care was taken to ensure adequate padding in order to prevent excessive pressure against nerves and major muscle groups.
Anesthesia was maintained with isoflurane 1.5-2% (Vetflurane®, France),) in 100% oxygen delivered through a standard small animal circle circuit and supplemented with ketamine by continuous rate infusion (0.5 mg/kg/h, i.v., Ketamidor®, Richter Pharma, Austria).
Sheep were positioned in sternal recumbency allowing a good surgical access for the oral cavity and increasing the chances to maintain a stable ventilation, cardiac output and arterial blood pressure during anesthesia (Desmecht et al, 1995).
The anesthesia machine with ventilator was prepared with settings for 10 breaths per minute, inspiratory: expiratory ratio to 1:2, positive end expiratory pressure to 10 cm H_20, tidal volume 10 mL/kg, PIP 15 cm H_2O.
The mean duration of the maintenance phase (from induction to extubating time, in the recovery phase), was 66.7 minutes, times varying from 32 minutes to 215 minutes.
During the procedures, the entire group, all 10 sheep were breathing spontaneously, none requiring assisted ventilation, even during deep anesthesia.
Intravenous fluid therapy during anesthesia was considered necessary to prevent hypotension and hypoperfusion, with a maintenance rate of 5 ml/kg/h with Ringer's Lactate solution (Soluție Ringer Lactat®, B Braun, Germany).
Monitoring the anesthetic plane was continuous throughout the procedures (absence of the palpebral reflex, mandibular tonus, cardiopulmonary stability, any movements or swallowing).
Unlike for other species, rotation of the eye cannot be considered a reliable indicator of the depth on anesthesia (White & Taylor, 2000).
Advanced clinical monitoring was used while maintaining anesthesia: pulse-oximetry (probe positioned on tongue or ear pinna), capnography, rectal temperature measuring (rectal probe), blood pressure measurement (indirectly by using a pressure cuff) and continuous electrocardiography (Figure 2).
A mean arterial blood pressure of at least 75 mmHg (systolic 100 mmHg, diastolic 60 mmHg) was maintained. Values were recorded in the anesthesia sheet, every 5 minutes.
Saliva production during anesthesia was not problematic, since the patient was positioned with the cervical area elevated, allowing secretions to drain.

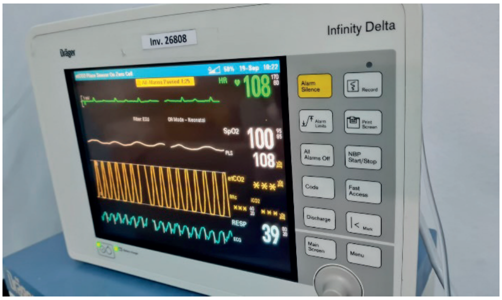

Figure 2. Monitoring anesthesia, during maintenance phase

We did not used anticholinergic drugs, since salivation is not markedly reduced by anticholinergics and may become more viscid and therefore more liable to produce airway obstruction (White & Taylor, 2000), we preferred to assure an optimal positioning.
In order to prevent excessive gas production during anesthesia a stomach tube was placed during the entire procedure.
Given the need for continuous monitoring as well as ensuring the possibility of the gastric tube maneuver to eliminate gas at any time, the animal was positioned with its head elevated, in a lateral position, thus facilitating direct and unrestricted access to the surgical procedure (Figure 3).

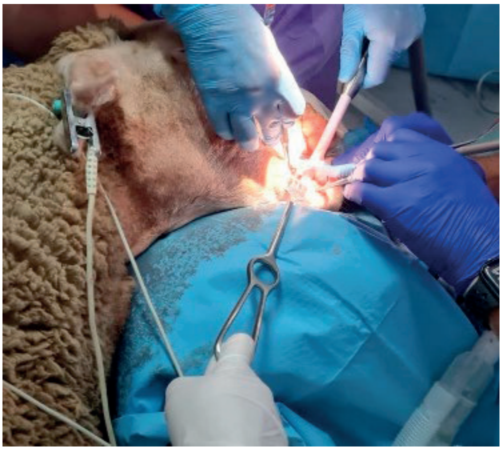

Figure 3. Surgical field access

Cuff deflation and extubating were delayed until the patient was clinically light enough and palpebral reflex, coughing, swallowing and movements of the limbs or head were regained. The mentioned reflexes reappeared extremely quickly after the cessation of the administration of isoflurane, at about 2-3 minutes the animals can be extubated.
Certainly, the fact that the animals were spontaneous throughout the maintenance of anesthesia represented an advantage for the recovery phase.
After extubating, the careful monitoring of the vital functions was continued, as well as the administration of the infusion solution, for another 10 minutes. During this phase animals were calm and cooperative. Efforts have been made to reduce any additional stressors, such as loud noises, excessive light or sudden movements.
After this interval, the animals were moved from the operating room to the wake-up area where their clinical monitoring continued. Animals were placed in a warm, sheltered space, to avoid hypothermia that often results in a prolonged recovery (Galato, 2011). No incidents were recorded in the early recovering phase.
Recovery from anesthesia was uneventful, sheep recovering completely after extubating in 15-22 minutes (mean 19 minutes), with no corelation regarding the duration of anesthesia.
Despite the duration of the procedures and the anesthesia protocols used, which involved a deep anesthesia with a high degree of analgesia, all the animals recovered very quickly.
When animals were able to adopt the four-legged position and to move without hesitation, they were transferred to their accommodation where they had access to food and water.
Animals resumed their routine of free movement, drinking and feeding at about 40- 45 minutes (mean 44 minutes) after completion of anesthesia (Figure 4).

Figure 4. Feeding after the recovery phase

One incident was noted in the late recovery phase. It was noticed 1 hour after anesthesia and it lasted for 72 hours. A sheep presented acute lameness (right forelimb). Myopathy was suspected. Muscle perfusion may be affected during recumbency due to arterial hypotension, pressure on muscles due poor positioning, noncompliant surfaces or prolonged periods of recumbency, especially for large animals (Seddighi & Doherty, 2016). In this case myopathy was possibly associated with the positioning and the length of the procedure (135 minutes for this case), despite padding, the careful positioning and the attention for maintaining adequate arterial blood pressure.

This sheep received anti-inflammatory therapy (meloxicam- Meloxidolor®, 1 mg/kg, i.m., for 3 consecutive days), during which time lameness gradually subsided.

CONCLUSIONS

The anesthetic protocols developed for experimental research procedures, should provide quick and deep anesthesia and analgesia to suit the type and duration of the surgical procedure and the individual characteristics of the patient.

Choosing the dosages for the protocol and the right equipment for anesthesia should take into account the weight of the patient, ideally without the wool weight, which can vary according to age, breed or raising system and requires experience for a correct estimation.

The premedication protocol used provided sedation and analgesia enabling the subsequent procedures, calming the patients and assuring a smooth recovery.

Orotracheal intubation can be performed quickly and safely, after positioning the sheep in sternal recumbency with the cervical region in hyperextension and using a long blade laryngoscope.

Gas maintenance should be considered mandatory for all prolonged surgical procedures and included, if necessary, in multimodal protocols.

Spontaneous breathing during maintenance phase proved to fulfil a good state of anesthesia and analgesia, while no respiratory depression was noted.

Recovery should be rapid and uneventful, while the patient must be exposed to a minimum number of stress factors.

Monitoring vital functions as well as animal behaviour assessment have to continue throughout the entire recovery phase in order to assure a good evolution and to correct any possible problems that may occur during this phase.

Assuring adequate arterial blood pressure and providing a comfortable padding during anesthesia are important factors in order to decrease the risk of myopathy.

Sheep tolerate general anesthesia with isoflurane very well, the absence of any anesthetic incidents as well as their rapid and complete recovery are important benefits for their successful use in various research procedures.

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