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THE USE OF TWO DIFFERENT ANESTHETIC PROTOCOLS FOR OVARIECTOMY IN *TRACHEMYS SCRIPTA ELEGANS*

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Abstract

This study was performed in order to evaluate two anesthetic protocols used for ovariectomy in turtles belonging to Trachemys scripta elegans species, between June and July 2019 at the Faculty of Veterinary Medicine of Bucharest. Patients aged one year old were divided in two study groups depending on the anesthetic protocol used. Group A was premedicated with Midazolam (2 mg/kg), Dexmedetomidine (0.1 mg/kg) and Ketamine (5 mg/kg) administered intramuscularly (IM) while group B had Midazolam (2 mg/kg), Buprenorphine (0.2 mg/kg) and Ketamine (2 mg/kg) IM. Induction was made with Isoflurane 5% in the induction chamber followed by Ketamine (10 mg/kg) given intravenously, in the supravertebral sinus (IV). All patients were intubated with an 18G IV catheter and maintained with Isoflurane 3% and 100% Oxygen. Patients in group B required two boluses of Ketamine (10 mg/kg/bolus) compared with patients in group A that didn't have any additional doses. Therefore the recovery was smoother and faster in patients from group A.

Key words*: anesthesia, ovariectomy, Trachemys scripta elegans.*

INTRODUCTION

The study analyzes and compares the effectiveness of two anesthetic protocols utilised in ovariectomy in *Trachemys scripta elegans* (red-eared slider turtle). The anesthesia of turtles have some dificulties, compared to the anesthesia of small animals. First of all, turtles are considered exotic animals, with anatomical and physiological particularities. Working with turtles for the first time can be very difficult. The doses need to be carefully calculated because they are different from the doses used in small animals.

The female presents a pair of ovaries that are saccular in shape and are covered with follicles. The ovaries are continued with the oviducts. The oviduct is continued with infundibulum, uterine tubes, isthium, uterus and vagina that open in the exterior in urodeum. The egg has three membranes inside and the eggshell, that is water resistant, allows gas exchange. All of these play a very important role in the development of the embryo (O'Malley, 2005).

Because of all this anatomical particularities, the turtles are prone to some conditions such as oviductal rupture, ectopic eggs in the coelum, chornic oviductal impaction or obstructive dystocia (Mans & Sladky, 2012). To avoid these conditions, as a prophylactic measure, the ovariectomy is recommended.

Turtles have anatomical and physiological particularities different from other small animals so the anesthetic protocols have to be modified according to those. One of these particularities is thermoreglation. Turtles are poikilothermic animals, which means that the body temperature is influenced by the outside temperature. Decrease in the body temperature affects the metabolic rate, which will increase the recovery period. There are also differences of the cardiovascular system. A unique feature of the cardiovascular system is the renal portal system. This cranial and the caudal portal vein form a ring of blood vessels around the kidney. The clinical importance of this particularity is that if the medication is administered in the bottom half of the body, the metabolism and excretion will be faster. Therefore, it is important to underline that the site for the intramuscular injection is in the forelimb muscles. As for the respiratory system, the turtles have a pair of saccular shaped lungs. The respiratory rate and the oxygen demand are

physiologically lower compared to the mammals. The hypoxia is well tolerated because turtles can change from an aerobic to an anaerobic metabolism (Sladky & Mans, 2012).

MATERIALS AND METHODS

The study took place at the Faculty of Veterinary Medicine Bucharest, between June and July 2019. A number of 10 turtles were used, all females, of age one year old and that were divided in two study groups, A and B, based on the premedication.

The first step in choosing anesthesia protocol was a thorough history and clinical examination. All the turtles were fed with turtle pellets and dried shrimps and kept in a glass aquarium filled with water. The physical examination revealed them to be bright, alert and responsive, with a heart rate and respiratory rate within normal limits (40 beats per minute and 5 breaths per 2 minutes).

Group A was premedicated with o combination of Midazolam, Dexmedetomidine and Ketamine, while group B received Midazolam, Buprenorphine and Ketamine. The main classes of substances that were used for the anesthesia were: benzodiazepines, α_2-adrenoreceptor agonist, opioids, dissociative agents and inhalant agents.

Midazolam is a benzodiazepine used for its sedation, muscle relaxation and anxiolytic effects. It is a water soluble compound, which means is not painful when administered intramuscularly. Midazolam is metabolised by the liver, with fast onset and a short action period. It doesn't have analgesic properties so it is used in different combinations with other classes of medication, like opioids and α_2-adrenoreceptor agonists. Flumazenil is competitive antagonist used to antagonize the effects of benzodiazepines (Costea, 2017).

Dexmedetomidine is the most potent and specific α_2-adrenoreceptor agonist available. This class of substances is known for its sedation, anxiolytic and analgesic properties. Because of its analgesic properties, dexmedetomidine reduces the need of opioids to almost 40% (Costea, 2017). Dexmedetomidine has some cardiovascular effects that include bradycardia, initial hypertension, followed by prolonged period of lower blood tension. As a result of the bradycardia, the cardiac output falls (Tranquilli et al., 2013). The respiratory depression is minimal. Other side effects of the dexmedetomidine administration include hyperglycaemia, decrease in the gastrointestinal motility and decrease in the intraocular and intracranial pressure (Clarke et al., 2013).

α_2 agonists actions can be antagonised by using Atipamezol. When used, Atipamezol antagonises both its sedation and analgesic effects.

Ketamine is a dissociative agent that produces a state called "dissociative anesthesia". This state is characterized by a dissociation of the thalamocortical and limbic system (Tranquilli et al., 2013). Ketamine acts as a non-competitive N-methyl-D-aspartate antagonist (Tranquilli et al., 2013). When administered alone, it has sympathomimetic effects, such as increase in the heart rate, cardiac output and blood pressure. (Costea, 2016).

Buprenorphine is a μ opioid receptor partial antagonist. It is more effective compared to morphine for chronic pain. The peak effect appears after 20 minutes of IV administration and the duration of the analgesic effects last 8 up to 12 hours. (Tranquilli et al., 2013)

RESULTS AND DISCUSSIONS

Before any medication was administrated, the turtles were measured so that the doses are according to the animals weight. The weight of the turtles varies from 1.2 to 1.7 kg (Figure 1).

The 10 turtles were randomized divided in 2 groups. Group A was premedicated with Midazolam (2 mg/kg), Dexmedetomidine (0.1 mg/kg) and Ketamine (5 mg/kg). Group B had Midazolam (2 mg/kg), Buprenorphine (0.2 mg/kg) and Ketamine (2 mg/kg). In both groups, the medication was given IM, in the forelimb muscle and then we kept them in a quiet place for 10 minutes so that the external sounds don't disturb them.

Figure 1. Weight measurement

For induction, all the turtles were kept for 10 minutes in the induction chamber where they received Isoflurane 5% and Oxygen 100%, followed by a bolus of Ketamine (10 mg/kg) IV, in the subcarpial sinus (Figure 2).

The next step after premedication and induction was intubation with a 18G IV cannula (Figure 3). Because so small endotracheal tubes were not available at the moment, we used an IV cannula, removing the insertion needle before intubation of the turtles.

Turtles are obligate nasal breathers (Kirchgessner & Mitchell, 2009). After opening the mouth, the glottis can easily be identified at the back of the tongue. The IV cannula is easily introduced inside the trachea. After the intubation, a breathing circuit is used to deliver 1 L of Oxygen 100% and Isoflurane 3% to the patient. The patient was maintained on Isoflurane 3% and 1 L of 100% Oxygen during the surgery (Figure 4).

Figure 2. Intravenous administration in the subcarpial sinus

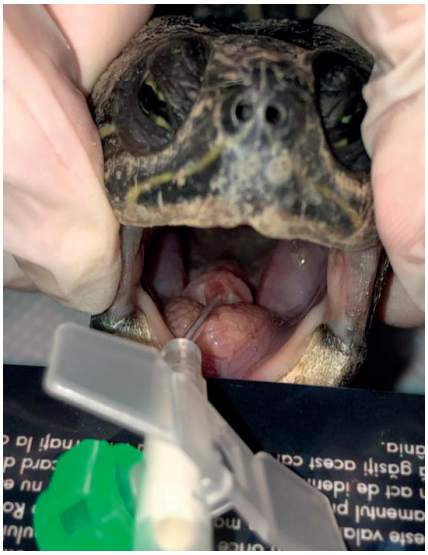

Figure 3. Intubation of the turtle using a 18G IV cannula

Figure 4. The turtle is connected to a breathing system to receive Isoflurane 3% and 100% Oxygen

Monitoring the pacient during anesthesia is a very important part of any surgery. The heart rate, respiratory rate and the end tidal CO_2 in the expired gas were recorded at regular intervals to facilitate early recognition of adverse trends. The normal vital signs of the turtles, before any medication was administrated were: heart rate 40 beats per minute and respiratory rate 5 breaths per 2 minutes.

This parameters change depending the substances that are used. For example, Table 1 presents the vital signs monitored during surgery: heart rate, respiratory rate and end tidal CO_2 in the expired gas of the turtles from group B after 5 minutes from receiving a Ketamine bolus (10 mg/kg, IV). Note the increase in the heart rate due to the positive inotropic action of the Ketamine.

Table 1. Vital signs of the turtles from group B after receiving a bolus of Ketamine (10 mg/kg, IV)

Parameter	T1	T 2	T 3	T 4	T 5
Heart rate (bpm)	75	73	65	70	63
Respiratory rate (rpm)	8	7	8	10	13
ET CO_2 (mmHg)	9	8	9	6	5

Table 2. Vital signs of the turtles from group A

Parameter	T 1	T 2	T 3	T 4	T 5
Heart rate (bpm)	35	30	41	38	36
Respiratory Rate (rpm)	8	7	8	9	8
ET CO_2 (mmHg)	9	9	8	8	8

Table 2 describes the same vital signs monitored during surgery of the turtles from group A. Note the decrease of the heart rate due to the use of α_2-adrenoreceptor agonist. In comparison to group B, the turtles from group A did not required additional boluses of Ketamine.

The mean duration of the surgery was 52.4 ± 7.4 minutes. During the surgery, an increase in the heart rate and the movement of the animal indicated that the plane of anesthesia was light so it required an extra bolus of Ketamine (10 mg/kg, IV). All the turtles from group B required one or two extra boluses of Ketamine (10 mg/kg, IV) during surgery. The time between each boluses was an average of 25 minutes.

After all the surgical procedures were completed, the turtles were extubated and kept under observation. During surgery and on the recovery period, the animals were kept on the heating pad. Cloacal temperatures were consistently the same as the ambient room temperature (28° ± 1°C) in all animals at each recorded time (at the beginning of the surgery and at the end of the surgery). All the turtles received Meloxicam (single dose, 0.5 mg/kg, IM) and Enrofloxacin (5 mg/kg/per day, 5 days). Every turtle from the two groups benefit from the administration of specific antagonists: group A receive Atipamezol (0.5 mg/kg, IM), while group B receive Flumazenil (0.05 mg/kg, SC). After 10 minutes from the administration of the antagonist, all the turtles in both groups presented an increase in the mandibular reflex. Also, the heart rate of the turtles from group A began to increase. After 20 minutes from the injection of the antagonists, the turtles from group A began to move (mean time of 15 ± 2.5 minutes), while the turtles from group B were alert and responsive (mean time of 21.8 ± 2.1 minutes).

A previous study by Greer, Jenne ans Digs evaluate the effects of a low and high dose combination of Medetomidine and Ketamine. The parameters that were evaluated were heart rate, palpebral reflex, limb and neck reflexion

and cloacal temperature and also the response to minor procedures. Using an α_2-adrenoreceptor agonist assures an adequate level of sedation and anesthesia and also allows endotracheal intubation (Greer et al., 2001).

Turtles from group B that didn't receive a α_2-adrenoreceptor agonist required one or two additional Ketamine boluses (10 mg/kg). Because of the additional bolus that the turtles in group B received, the recovery was prolonged, compared with the turtles in group A, in which the recovery was faster and smoother.

Figure 5 presents in comparasion the time that each turtle required to recover after the antagonist administration. Note that for group A, all the turtles required less than 20 minutes to recover (mean time of 15 ± 2.5 minutes), while the turtles in group B require more than 20 minutes (mean time of 21.8 ± 2.1 minutes).

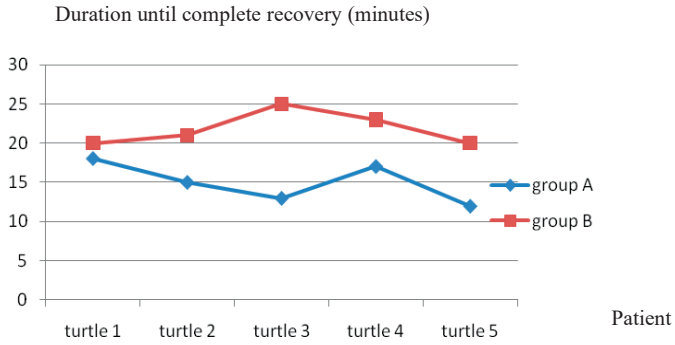

Figure 5. The recovery period (minutes) for the two groups

CONCLUSIONS

When using Midazolom - Dexmedetomidine - Ketamine in captive red eared slider turtles the recovery is smoother and the return to the normal physiologic parameters is faster.

To have a smooth and fast recovery, always consider using substances that have an antagonist to treat and prevent the emergency situations.

The monitoring of the vital parameters is very important during the anesthesia as it is in the recovery period.

For the anesthesia of the red eared slider turtle including an α_2-adrenoreceptor agonist increases the level of sedation and analgesia.

In combination with other drugs (ex. Benzodiazepines or Ketamine), the use of α_2-adrenoreceptor agonist produce a sufficient anesthesia for short to medium time duration procedures, without any addition boluses of substances.

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