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Intramuscular vs Intravenous Administration of Alfaxalon for Induction of General Anaesthesia in *Geochelone Platynota* and *Astrochelys Radiata*

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Table of Content

Part 1

1 INTRODUCTION4

2 GENERAL FACTS ABOUT ALFAXALONE5

2.1 HISTORY5
2.2 EFFECTS AND SIDE EFFECTS6
2.2.1 Pharmacodynamic6
2.2.2 Pharmacokinetics7
2.2.3 Side Effects8
2.3 COMBINATION WITH OTHER ANAESTHETIC DRUGS8
2.4 OTHER INDUCTION AGENTS**FEHLER! TEXTMARKE NICHT DEFINIERT.**
*2.4.1 Propofol**Fehler! Textmarke nicht definiert.***
*2.4.2 Ketamine**Fehler! Textmarke nicht definiert.***
*2.4.3 Comparison to Alfaxalone**Fehler! Textmarke nicht definiert.***

3 ANATOMY AND PHYSIOLOGY IN CHELONIANS9

3.1 RESPIRATORY SYSTEM9
*3.1.1 The Effects of Hypoxia on Breathing Patterns**Fehler! Textmarke nicht definiert.***
3.2 GAS EXCHANGE AND TRANSPORT13
3.3 CARDIOVASCULAR SYSTEM15
3.4 CARDIOPULMONARY CIRCULATION16
3.4.1 Cardiac Shunts17

4 ALFAXALONE IN REPTILES20

4.1 OTHER ANAESTHETIC DRUGS USED IN REPTILES22
4.1.1 Volatile Agents22
4.1.2 Ketamine22
4.1.3 Alpha-2-adrenergic Antagonists23
4.1.4 Benzodiazepines23
4.1.5 Propofol23
4.1.6 Comparison to Alfaxalone24
4.2 COMBINATION WITH OTHER ANAESTHETIC DRUGS IN REPTILES26
4.3 ALFAXALONE USE IN CHELONIANS**FEHLER! TEXTMARKE NICHT DEFINIERT.**

Part 2

1 MATERIAL AND METHODS27

2 RESULTS29

3 DISCUSSION35

Part 1

1 Introduction

As the popularity of exotic pets, especially reptiles, has grown in recent years, the need for safer evidence-based anaesthesia and sedation has increased. Many of those species are extremely sensitive when handled and even some non-invasive or minimally invasive procedures, such as blood collection and radiographs or a thorough clinical examination, may cause extensive stress levels, which can harm the animal. Current veterinary practice of anaesthesia in exotic animals often relies on anecdotal drug dosing recommendations or even extrapolation of non-related species, such as cats and dogs (Balko and Chinnadurai 2017). As anatomy, physiology, as well as pharmacokinetic behaviour vary between species, new evidence is needed to help us understand the effects of anaesthetic drugs better in different species. Findings should be documented and published, as this information can help gain a better understanding of the animals and differences between species. The goal of this study was to share the information gained from reviewing the collected data and to find differences in-between an intramuscular vs. intravenous induction with Alfaxalone in the two species *Geochelone Platynota* and *Astrocheylis radiata.*

2 Alfaxalone

Alfaxalone is a steroid derived anaesthetic agent used in veterinary medicine for producing short term general anaesthesia or as induction agent for a long-term inhalation anaesthesia. Since there is little danger of accumulation, it can also be used for TIVA (total intravenous anaesthesia) (Eberspächer 2016).

2.1 History

Neurosteroids, such as Alfaxalone, were first found to have anaesthetic properties in the 1940s. Hans Selye showed that a reversible unconsciousness could be produced in rats by injecting intraperitoneally large quantities of steroid hormones. One of the most potent steroids injected - and devoid of hormonal activity - was pregnenedione. In 1955, an even more potent analogue of pregnaendione, called hydroxydione was found. It was safer than the anaesthetic drugs used at the time, thiobarbiturate and thiopentone. Unfortunately, it was not ideal, as induction was slow (up to three minutes) and the drug needed to be solubilized in an alkaline pH solution, which lead to venous thrombosis. Eventually, by altering certain carbon positions of hydroxydione, the Glaxo UK Pharmacology Department discovered the active molecule 3α-hydroxy-5α-pregnane-11,20-dione, alias Alfaxalone. Its main mechanism of action is through the GABAa receptor, similarly to barbiturates, benzodiazepines, propofol and isoflurane (Jurox Pty. Limited 2021).

After thorough investigations and molecular modifications, Alfaxalone was first launched on the marked in the 1970s as part of the drugs Saffan®, an intramuscular anaesthetic for veterinary use, and Althesin®, an intravenous induction agent for humans (Jones 2012, Jurox Pty Limited 2021). Already then, neurosteroid anaesthesia was found to possess excellent anaesthetic properties in combination with minimal effects on the cardiovascular system(Jurox Pty. Limited 2021). The two components, Alfaxalone and alfadolone (921-acetoxy-3α-hydroxy-5-α-pregnane-11,20-dione), were combined with a 20% polyoxyethylated caster oil-based surfactant (Cremophor EL) (Jones 2012). Alfadolone improved the solubility of Alfaxalone and was included for this purpose. The finished formulation used in Saffan® and Althesin® was called CT1341, which showed significant advantages over other injectable anaesthetic agents. The margin of safety was higher, and it was non-irritating to tissues including veins. Furthermore, it was proved to be compatible with the adjuvant and preanesthetic drugs, no accumulation was

documented, and it produced a pleasant anaesthetic experience for the patient as well (Jurox Pty Limited 2021). But the cremophor EL component included in this formular was associated with severe histamine release, especially in dogs, which could result in anaphylactic shock and death. Overall, Saffan®' s common adverse effects in dogs of were salivation, oedema of paws and eyelids, pulmonary oedema, rhinorrhoea and hypotension. In cats, it caused oedema of distal thoracic limbs, pinnae and lungs, hyperaemia, partial laryngeal spasm and cyanosis. In humans as well severe histamine release was found in combination with Althesin® use. Therefore, these drugs were quickly taken off the market (Jones 2012, Barletta 2019).

In the 1990s, Alfaxalone was solubilised in aqueous solution for the first time, eliminating the reaction observed with the previous formulation in cremophor EL. This process was made possible by complexing Alfaxalone to cyclodextrins (large sugar molecules) first and solubilizing it afterwards (Jurox Pty. Limited 2021). In 2000 the brand name Alfaxan® brought a new formulation of Alfaxalone as an intravenous injection onto the market. It was formulated in 2-hydroxypropyl-beta-cyclodextrin (2-HPCD,) which does not cause histamine release and is excreted through the kidneys. Finally, in 2018 the formulation as being further improved by adding preservatives. As a result, AlfaxanMultidose®, can be used multiple times for 28 days after initial vial broaching (Jurox Pty Limited 2021).

2.2 Effects and Side Effects

2.2.1 Pharmacodynamic

Generally, neurosteroid agents decrease cerebral metabolic demands, as well as blood flow and the intracranial pressure. Therefore, in human medicine they are considered to have a positive neurologic profile (Jones 2012).

Alfaxalone is a synthetic steroid derived drug, which reaches its effect, unconsciousness of the patient, through interaction with the $GABA_A$ (gamma-aminobutyric acid sub-type) receptor complex where it enhances the GABAergic neurotransmission (Löscher and Richter 2016). $GABA_A$ – receptors are inotropic ligand-gated channels with GABA as the endogenous ligand, which is the main inhibitory neurotransmitter in the Central Nervous System (CNS). These receptors are widely distributed throughout the CNS and in fact are present on almost half of all neurons (Barletta 2019, Jurox Pty Limited 2021). Low concentrations of Alfaxalone positively modulate chloride current through its channels. Through opening a pore in the receptor,

higher concentrations of Alfaxalone increase the transmembrane chloride ion transport leading to hyperpolarization of the postsynaptic neuron cell membrane thus inhibiting the propagation of action potential and stopping impulse transmission. This process decreases the activation of pathways related to arousal and awareness (Jones 2012, Barletta 2019, Jurox Pty Limited 2021). Alfaxalone does not bind to the receptors of sex hormones, glucocorticoids or mineralocorticoids, and it is therefore void of any hormonal effect (Jurox Pty Limited 2021). In short, the main effects of Alfaxalone are hypnosis and muscle relaxation (Jones 2012).

2.2.2 Pharmacokinetics

Due to the high lipophilicity, Alfaxalone reaches effective concentration in the brain very fast, and therefore has a short onset time until an effect is seen in the patient. It is quickly transformed into inactive metabolites in the liver and discharged through the bile and intestine with the drug being cleared from the body within a few hours (with certain variability between different species) (Löscher and Richter 2016, Jurox Pty Limited 2021). Pharmacokinetics of Alfaxalone are nonlinear, meaning that absorption, distribution, metabolism or elimination of the drug may vary in time (West 2017). The mentioned time ranges mostly depend on the health status of a patient, concurrent medications and, of course, the dose of Alfaxalone given. According to the producer of AlfaxaloneMultidose®, Jurox Pty Limited, a healthy un-premedicated cat administered a 5 mg/kg dose i.v., with no noxious stimuli, will stay in an anesthetised state for approximately 25 minutes and a healthy un-premedicated dog administered 2 mg/kg i.v. will remain anaesthetised for approximately 10 minutes. The mean terminal plasma elimination half-life of Alfaxalone at recommended dosage (cats 5 mg/kg and dogs 2 mg/kg) was observed by the manufacturer to be 34 minutes in dogs and 43 minutes in cats. To prolong the duration of anaesthesia, Alfaxalone can be combined with other drugs, which also reduces the dose requirements for Alfaxalone itself (Jurox Pty Limited 2021). At standard doses, there was no clinically relevant accumulation detected (West 2017).

In one study in felines, there was an unexplained rebound effect of plasma concentration at supraclinical dosage of 25 mg/kg observed, suggesting that prediction of onset of duration and effect might be difficult in cats (West 2017). Even though this was observed, the Australian producer Jurox states that no adverse effects to overdoses up to five times the recommended

dosage in cats and dogs has been observed, besides requiring ventilation (Jurox Pty Limited 2021).

Alfaxalone is missing analgesic effect, and furthermore it has been shown not to enhance the nociceptive effects of opioids and unlike other neurosteroids (West 2017).

2.2.3 Side Effects

The primary known side effects consist of respiratory depression and cardiovascular derangements (Jurox Pty Limited 2021). Respiratory side effects include a brief period of apnoea during or after intravenous administration (Jones 2012). Alfaxalone causes a vasodilatation leading to temporary mild decrease of blood pressure, which results further in the possibility of decreased cardiac output leading to a reflex tachycardia (Jones 2012, Löscher and Richter 2016). These side effects of Alfaxalone c can be reduced to a minimum by slow intravenous administration to effect. However, if it is administered intramuscularly there is no possibility to control it to effect (Jones 2012).

The quality of recovery after use of Alfaxalone is species dependent and is affected by the concomitant administration of other drugs. It may include minor twitches, paddling or even violent motions, if Alfaxalone is used as the sole anaesthetic agent or in sole combination with benzodiazepines. Use of the right premedication can help reduce the risk of a rough recovery (Jones 2012). Further side effects, which may need prevention, are hypothermia and emesis (Jurox Pty Limited 2021).

Lastly, it should be mentioned that Alfaxalone does have the potential for abuse in the same equivalent of midazolam and diazepam. The US Drug Enforcement Administration classifies it as a Controlled Schedule IV substance, meaning that its abuse may lead to physical or psychological dependence (Barletta 2019).

2.3 Combination with other anaesthetic Drugs

Alfaxalone can be safely used with opioids, alpha-2-agonists, benzodiazepines, anticholinergics, phenothiazines and NSAIDs (nonsteroidal anti-inflammatories). It is advised to combine it with the right premedication, especially because of the lack of analgesic effect (Jones 2012, Eberspächer 2016). The combination of Alfaxalone with other intravenous anaesthetic agents

is contraindicated. Also, if possible, one should refrain from using it in patients with a compromised liver function, as it is metabolized mainly through the liver. When used for intermittent bolus or TIVA the dosage is lower than when used for induction (Jones 2012). For induction a bolus dose of 2-3mg/kg in dogs or 5mg/kg in cats is recommended by the producer Jurox. These doses will provide anaesthesia for 8-12 minutes in dogs and 20-25 minutes in cats. When used for TIVA in dogs 0.10-0.15mg/kg/min and in cats 0.11-0.18mg/kg/min are recommended. For intermittent boluses (every 10 minutes) the recommended dosages for dogs are 1-1.5mg/kg and for cats 1.1-1.8mg/kg (Jurox Pty. Limited 2021).

3 Anatomy and Physiology in Chelonians

3.1 Respiratory System

One of the special characteristics in Chelonians is the bony shell, which also influences the respiratory function. Due to it being a stiff structure surrounding the lungs, there is less room for lung expansion while breathing. These animals are obligate nasal breathers (Bennett 2011).

The airways start at the glottis at the base of the tongue, which is supported by the hyoid apparatus and then opening into the trachea, which is lying ventral to the oesophagus (Wyneken et al. 2007). The glottis closes at rest and opens during expiration and inspiration (Longley et al. 2008) Craniodorsal to the heart, the trachea divides into two separate bronchi. Compared to other species, this bifurcation lies more cranially in chelonians leading to difficulty with correct intubation, i.e. a higher risk of accidental one-lung intubation (Wyneken et al. 2007).

Instead of a diaphragmatic muscle, some species have a membranous ligament which separates pulmonary and coelomic compartments (Wyneken et al. 2007). The lungs are embedded in the dorsal carapace, extending caudal from the nuchal region up to 78% of the carapace. A pulmonary ligament attaches the lungs to the vertebral column (Wyneken et al. 2007). Chelonian's lungs have no bronchial tree, only one interpulmonary bronchus stretching through each lung and separating it into medial and lateral chambers. Furthermore, there are no alveoli, as the parenchyma is parted into small niches divided by thin trabeculae. These then part into shallow ediculi and faveoli, where the gas exchange takes place. The shape differs in the various types of chelonians (Wyneken et al. 2007).

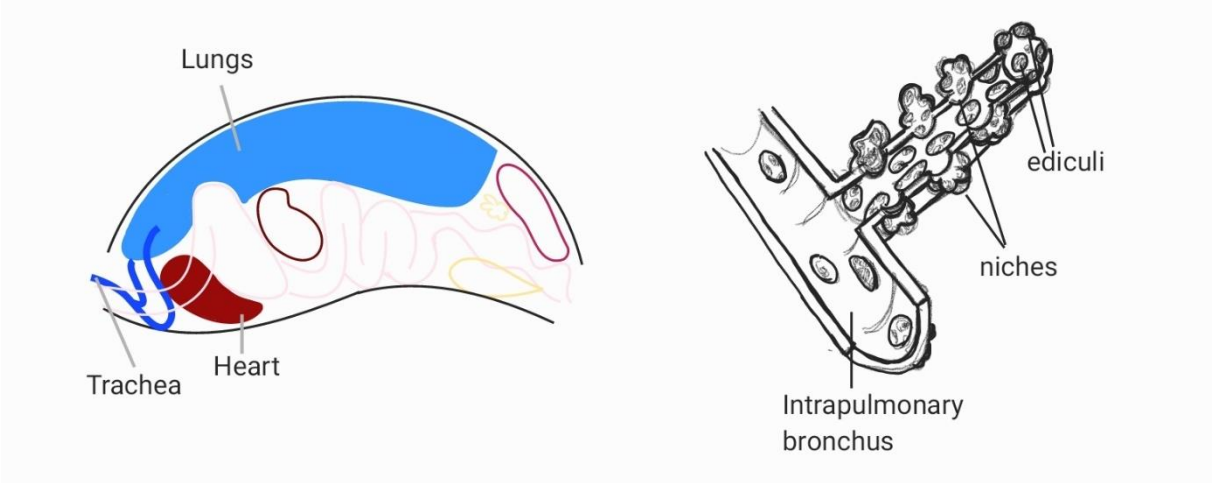

Figure 1: A sketch of the position of lungs, the heart and trachea in the chelonians shell and of the pulmonal parenchyma.

Due to their anatomical peculiarity, chelonians are bound to accomplish ventilation by changing the intracoelomic pressure rather than using their thoracic cavity. Because the thoracic wall is a rigid structure and chelonians are lacking a true diaphragm, there are limited possibilities to generate negative intracavitary pressure to accomplish pulmonary expansion. Hence, they accomplish the task by moving their shoulders and their inguinal muscles. Expiration, as well as inspiration are processes which are separated from locomotion. Therefore, a slight movement of front feet and head can be notable in physiological breathing in chelonians (Balko and Chinnadurai 2017).

Reptile respiratory physiology includes a unique process for exchanging respiratory gases. Aquatic chelonians can cope with extensive periods of apnoea between breaths and terrestrial species do not breath continuously. In contrast to mammals, were merely one to five minutes are needed to identify breathing rhythm, breathing sequence in chelonians can only be revealed if the animals are observed over extensive time periods. The normal breathing pattern of chelonians is episodic breathing, and it is hypothesised to be related to an overall respiratory drive (the sum of all inputs to regulate breathing). An increase in this drive generally causes this episodic breathing pattern to become continuous. This hypothesis is supported by experimental data, but the theory goes even further suggesting that terrestrial chelonians breathe mostly with a singlet pattern (one breath separated by a period of apnoea less than a minute long from the next breath) and aquatic species breath more often episodically (apnoea lasting for several minutes punctuated by a breathing series of 5-10 breaths). Furthermore, hypoxia also alters the

breathing pattern, especially the number of breaths per episode. As an example, in unidirectionally-ventilated semi-aquatic turtles this number decreased by 50% when oxygen supply was cut from 30% to 0 (Johnson et al. 2015). In 2015 Johnson et al. published a study about the influence of hypoxia on the breathing pattern of red-eared slider turtles, a semi-aquatic species, which is naturally experiencing hypoxia and therefore are extremely hypoxia-resistant. The animals were put into a respiratory tank to obtain baseline breathing for three hours and then the gas was switched to 100% nitrogen gas for the next four hours, to create hypoxia. In this study it is mentioned that past studies often are difficult to compare, as experimental protocols might have had an impact on breathing. Some hypoxia-induced changes in breathing patterns might have occurred due to involved anaesthesia and the necessary instruments, such as an endotracheal tube, chronic artery catheter placement and such(Johnson et al. 2015). Still, this is only a theory as terrestrial chelonians breathing patterns have not yet been analysed in detail (Burggren and Shelton 1979, Johnson et al. 2015). Other studies in aquatic and semi-aquatic turtles showed no or little increase in tidal volume during hypoxia. Thus, it can be assumed, that there are cofounding factors and conflicting reports due to different experiment approaches (Johnson et al. 2015). Johnson et al. suggest that because of all the issues mentioned above hypoxic ventilation response in ectothermic vertebrates and its underlying physiological mechanisms are not well characterised so far and the goal of their study is to change that and shine lighter on hypoxia and its physiology in turtles(Johnson et al. 2015).

In mammals, fish or birds intermittent breathing is not possible, as the perfusion of the lung is closely matched with its ventilation where gas transport occurs in a narrow, strictly defined range of internal conditions. In mammals and birds, the processes controlling the maintenance of this homeostasis is well researched and understood (Burggren and Shelton 1979). Especially aquatic species possess the significant ability to use anaerobic metabolism for an extensive period. The build-up of lactic acid and hydrogen ions are compensated by special buffer systems within the blood, pericardial fluid and, dependent on species(Wyneken et al. 2007). The intermittent ventilation of chelonians lungs leads to fluctuations in respiratory gas tensions, acid-base-balance and metabolic end-product concentrations which no mammal or bird can tolerate (Burggren and Shelton 1979). Unfortunately, this also leads to a good ability in coping with respiratory illnesses and masking their clinical signs until pathology is severe (Longley et al. 2008). In other species, the main factors driving respirations are the pH, oxygen and carbon

dioxide partial pressure in arterial blood, and body temperature. In chelonians the importance of the individual factors mentioned varies depending on the species. Not only being a terrestrial or aquatic specie plays a role, but also weather conditions in their natural habitat, such as temperature and humidity, have an extensive influence on breathing patterns. Normally one could assume that, since a higher temperature promotes a higher metabolic rate, this would lead to more frequent breathing. Surprisingly it was found that temperature induced rise of metabolic rate does not have the same stimulating effect on respiration as a rise in metabolism at a constant temperature. This can be explained by an arterial pH, which changes in an inverse manner with temperature (Harless and Morlock 1979). As there are so many different types of chelonians (terrestrial, semi-aquatic, aquatic, soft shelled, etc.) it is hard to put up one rule set concerning respiration for all of them. Furthermore, past studies are difficult to compare, as experimental protocols might have had an impact on breathing. Some hypoxia-induced changes in breathing patterns might have happened due to involved anaesthesia and the necessary instruments, such as an endotracheal tube. Therefore, it is possible that these turtles were not acting freely during the hypoxic exposure and cofounding factors may have caused the reactions documented(Johnson et al. 2015). This should always be kept in mind, when working with chelonians, but in general one can use the following rules concerning respiration in chelonians: most importantly, in chelonians the main stimulus to breath are low oxygen levels in blood, leading to an increase in respiratory rate and volume (Longley et al. 2008), whereas the main stimulus to breath in mammals, including humans, are high carbon dioxide levels, which will lead to increase of alveolar ventilation, meaning higher frequency of breathing and increase of tidal volume. The alveolar CO_2 concentration is inversely proportional to the alveolar ventilation (von Engelhardt and Aurich 2010). In chelonians high levels of carbon dioxide lead to increase ventilation mainly by an increase in tidal volume rather than frequency. An elevated temperature or long dive in aquatic species leads to a higher oxygen demand, which also results in an increase of tidal volume, while the respiratory rate stays the same. High levels of environmental oxygen result in a reduction of ventilation, as the respiratory rate and tidal volume decrease (Longley et al. 2008). The positioning changes in lung volume, due to gravity, were documented and it was found that the optimal breathing position for lung expansion in chelonians is sternal recumbency with the limbs extended (Balko and Chinnadurai 2017).

3.2 Gas Exchange and Transport

To improve gas exchange efficacy during the periods of ventilation in-between apnoea, a temporal matching of perfusion and ventilation of the lungs is provided by an increase in pulmonary blood flow and heart rate. In mammals, birds and fish lung perfusion and ventilation is kept in close margins at all times. A rise in pulmonary blood flow is associated with a left-right shunt in the heart, leading to a pulmonary recirculation of already oxygenated blood back into the lungs (Wang 2011). The intermittent ventilation of the chelonian lungs therefore creates an environment of fluctuations in respiratory gas tensions, acid-base balance and metabolic end-product concentrations which are unimaginable in any mammal. Thus, the regulating mechanisms controlling the limits of these fluctuations are not as well understood as control mechanisms in birds or mammals. Overall, there are two phases in any intermittent breathing pattern – apnoea (however long) and a period of breaths in-between. In most chelonian species breath-holding effectively stops the gas exchange with the surrounding environment which obviously leads to large variations in the respiratory gas levels in the lungs but also in the blood during intermittent breathing and diving. To cope with this, chelonians tissues have the outstanding ability to metabolise at low levels during ongoing oxygen depletion, therefore surviving long periods of total anoxia. Furthermore, they are able to control blood flow through the heart by shunting it, so that the arterial blood from the substantially reduced cardiac output, preferably enters the systemic circulation. In unrestrained chelonians the lungs perfusion is most profoundly influenced by the change from active ventilation to apnoea, which results in a dramatic fall in blood flow (to the unventilated lung) caused by vasoconstriction and, more so, shunting of the heart (Shelton and Burggren 1976, Burggren and Shelton 1979). Similar to blood flow, fluctuations in the respiratory gas tensions are very closely correlated to ventilatory events. During ventilation of the lung, proportionally more CO_2 than O_2 is exchanged in the lung, creating a pulmonary gas exchange quotient greater than 1, while during apnoea this quotient falls progressively to values smaller than 1 (Burggren and Shelton 1979, Johnson et al. 2015). This may be achieved by a considerable redistribution of the two gases between different compartments in the chelonians body, meaning the lungs, the blood and the tissues. As CO_2 has a considerably higher solubility in tissue fluid and blood than oxygen. Only 2% of the required oxygen during apnoea comes from dissolved O_2 stores in the tissue. All 48% of the produced CO_2 will remain stored in tissues until the return of ventilation when the large gas tension gradients

favour the rapid removal of CO_2 to the blood and lungs. Additional CO_2 is stored in the blood in a dissolved form, such as carbamino compounds and bicarbonate. Interestingly, this leads to only about 10-15% of the produced CO_2 being transported back to the lungs during the time of apnoea, leading to the high fluctuation in the gas exchange quotient in the lungs. So, for CO_2 the blood does not only function as transport towards the lungs but can also store it. On the other hand O_2 is rather transported than stored, and only 10% of the oxygen consumed during apnoea comes directly from the O_2 initially contained in the erythrocytes before apnoea. A 90% of the oxygen metabolized during non-ventilated periods is constituted of O_2 transported from the lungs into the tissues. This is aided by a gas tension gradient for oxygen that favours a continuous transfer of pulmonary oxygen into the blood. Pulmonary oxygen can continue at any time during ventilation and in vitro data of blood affinities showed that the oxygen partial pressure remained high enough to afford almost full haemoglobin saturation with oxygen during all but the longest dive measured (Shelton and Burggren 1976, Burggren and Shelton 1979). The lung therefore, functions as an oxygen store, like an oxygen tank a diver would wear, while not letting CO_2 take up space in it, but rather within the body tissues. Different solubilities of the gases in the different body compartments result in highly disproportionate quantities of each gas being removed from and added to these compartments during apnoea, with an overall O_2 gas tension gradient from the lungs towards the tissues and an CO_2 gas tension gradient from the tissues to the lungs. This CO_2 gradient is strongly increased at the onset of lung ventilation, while the oxygen stores are replenished. As, during non-ventilatory periods, there was only a little amount of CO_2 excreted into the lungs, about 85% must be rapidly transported out of the tissues into the blood to the lungs during a brief period of ventilation. This period can occupy as little as 15% of the total activity of a turtle, depending on the species. Pulmonary CO_2 exchange occurs rather cyclical than pulmonary exchange of oxygen and happens only when large gas tension gradients during the breathing intervals favour the rapid movement of CO_2 towards the lungs (Burggren and Shelton 1979). During intermittent ventilation there are substantial differences between the respiratory gas tensions in the lung gas and arterial blood. This is supposedly a result of arterial-venous shunting in the chelonian ventricle and from the failure of pulmonary venous blood to reach an equilibrium with the pulmonary gas. The latter could be caused by barriers to gas diffusion within the lung or inappropriate alveolar ventilation/perfusion ratios, where some alveoli are hypo perfused and physiological shunting, meaning venous

admixture, representing hyper perfusion of other alveoli. These conditions produce a different venous oxygen partial pressure from the ideal value achieved in a perfect ventilation/perfusion ratio. This pulmonary venous admixture was measured in a study conducted by Burggren and Shelton in 1979 with two species, *Testudo graeca* and *Pseudemys scripta* where it amounted to approximately 2% in the former and 6% in the latter during the periods of lung ventilation. But as apnoea proceeded this percentage increased three- to four-fold and it is hypothesised that the distributional or alveolar dead space also increased during non-ventilation, since the oxygen partial pressure gradient from the alveolar gas towards the pulmonary venous blood increased as well. Therefore, it appears that the lung is less efficient as a gas exchanger mainly due to changes of vascular nature, during periods of apnoea (Shelton and Burggren 1976, Burggren and Shelton 1979, Johnson et al. 2015).

3.3 Cardiovascular System

In the cranial part of the coelomic cavity the heart is to be found midline cranially to the liver, ventral to the lungs, and just dorsal of the plastron (Longley et al. 2008). The heart is surrounded by a pericardial sac, which physiologically contains a low amount of clear pericardial fluid. Pericardium and ventricle are - in their posterior aspect - attached to the peritoneum through the gubernaculum cordis, a collagenous cord. This anchor holds the heart in place when it contracts (Wyneken et al. 2007).

The heart consists of a large thin-walled sinus venosus, two atria and one ventricle, which is parted into three communicating compartments – the cavum arteriosum, cavum venosum and the third, cavum pulmonale (Wyneken et al. 2007, Longley et al. 2008). The sinus is attached dorsally to the atria, sitting slightly more on the right side. It has a muscular but very thin wall. There are four major vessels who enter the heart through the *sinus venosus*: the right and left precaval vein, the postcaval vein and the left hepatic vein. The sinus and the right atrium are connected by a sinuauricular aperture guarded by a valve. Left and right atrium are separated by the interauricular septum and both contain musculi pectinate. The right atrium is bigger than the left and the walls of both are rather thin (Harless and Morlock 1979, Longley et al. 2008). The right atrium leads into the cavum venosum and the left into the cavum arteriosum. Overall, the ventricle is the most muscular part of the heart, but holds relatively little volume (Harless and Morlock 1979). The cavum arteriosum and venosum are a continuous chamber, separated

by an intraventricular canal. A right and left single-cusped atrio-ventricular valve prevents regurgitation from this canal back into the right and left atrium and during the diastole they prevent the exchange between the two cava. Furthermore, these first two chambers of the ventricle are, to some extent, separated from the third (cavum pulmonale) by a muscular ridge called the interventricular septum. In the cavum venosum the left and right aortic arches originate. Out of the cavum pulmonale the pulmonary trunk leads through the pulmonary arteries into the lungs (Harless and Morlock 1979). Figure two shows the anatomy of the chelonian heart. Blood enters the heart through the sinus venosus continuing into to right atrium, which leads the blood into the cavum venosum. From here it either directly leaves the heart through the left and right aorta, with no oxygenation of the blood taking place. From the cavum venosum the blood can also enter the cavum pulmonale and continuing through into the pulmonary artery into the lungs. Afterwards the blood re-enters the heart through the pulmonary vein into the left atrium, then flows into the cavum arteriosum and entering the cavum venosum where oxygenated blood and non-oxygenated blood mix (Harless and Morlock 1979, Wyneken et al. 2007).

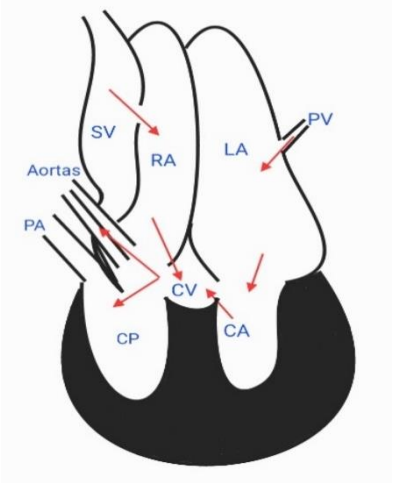

Figure 2: SV - sinus venosus, RA-right atrium, LA-left atrium, CA-cavum arteriosum, CV-cavum venosum, CP-cavum pulmonale, PV-pulmonary vein, PA-pulmonary artery, Aortas-right and left aortic arch, the arrows symbolize the possible blood flow routes.

3.4 Cardiopulmonary Circulation

The deoxygenated blood comes in through the major vessels, as described above, into the sinus venosus and through the right atrium into the cavum venosum. From here, the blood takes one of two separate paths. Either it leaves the heart into the left and right aorta (the systematic pathway) or it flows into the cavum pulmonale and exiting the heart from there into the pulmonary trunk (the pulmonary pathway) (Harless and Morlock 1979). Through the lungs and the

pulmonary veins, the now oxygenated blood re-enters the heart into the left atrium. From there it passes into the cavum arteriosum and then through the interventricular canal into the cavum venosum again, where the same options as before are given to the blood coming into the cavum venosum (Harless and Morlock 1979, Wyneken et al. 2007). The interventricular septum can, to some extent, control how much blood will take which pathway. It was found in the species Pseudemys scripta, that when breathing, 60% of blood volume will be directed towards the pulmonary circuit leaving the rest to take the systemic pathway. In the same study it was demonstrated that this species can shunt their heart either right-to-left or left-to-right, depending on the breathing situation of the animal (Harless and Morlock 1979).

3.4.1 Cardiac Shunts

The cardiovascular system of chelonians presents a certain flexibility in blood flow, which cannot be achieved in mammals or birds, as ventilation and blood flow need to be closely matched to stay within certain parameters in the CO_2 and O_2 levels as well as the acid-base balance. As chelonians lack complete anatomical separation of the cardiac ventricle, blood can be actively shunted towards the pulmonary or systemic circuits. These shunts are defined based on the overall direction of the blood flow (Greunz et al. 2018). In the absence of separate ventricular pumps, mixing of blood from the systemic and pulmonary circulation can and does occur during ventilation and apnoea. Shunts occur in both directions and can be identified through flow measurement. Still, shunts cannot eliminate the differences of the blood from left and right auricle completely as the ventricle directs blood both into the pulmonary and systemic circulation. These differences are thought to be maintained by laminar blood flow in the ventricle, aided by muscular ridges, the trabeculate nature of the ventricle walls and other internal structures(Shelton and Burggren 1976). A right-to-left (L-R) shunt occurs, when systemic deoxygenated blood passes from the right side of the ventricle across the incomplete septum, the muscular ridge, towards the systemic circulation, bypassing the lung and recirculating the systemic venous blood. As there is no chance of reoxygenation, this shunt decreases the oxygen saturation of systemic arterial blood (Hicks 2002). A left-to-right (L-R) shunt, on the other hand, facilitates the recirculation of oxygen rich blood from the pulmonary circulation back into the lungs (Greunz et al. 2018). The extend, of the ability to shunt the heart is different in every species of tortoise. For example, in the leatherback turtle the abilities of this muscular ridge is exceptionally well developed, even for marine species (Harless und Morlock 1979,

Wyneken et al. 2007). Leaving species-specific differences aside, the magnitude and direction of the shunt is primarily controlled by the balances of vascular resistance in the systemic and pulmonary vasculature. If, in response to increased parasympathetic tone, the pulmonary vasculature resistance is high, the lung can be completely bypassed with a R-L-shunt (Shelton and Burggren 1976, Greunz et al. 2018). Regulation of the vasculature resistances not only results from changes in autonomic vagal tone, but also from the realise of neurohumoral factors. These also influence heart rate, contractility and blood volumes of the ventricle, all off which can contribute to the size and direction of the shunt (Hicks 2002). Shunts are closely connected to ventilation and therefore, dependent on the breathing pattern. R-L shunting dominates during apnoea and L-R shunting prevails during ventilation (Greunz et al. 2018). During apnoea a bradycardia is developed by testudines and the pulmonary vascular resistance increases leading to a reduction in the pulmonary blood flow. These changes promote the development of a R-L shunt, which can be as large as 90% of systemic blood flow being recirculated into the systemic circulation. This shunt is believed to facilitate oxygen uptake while sequestering carbon dioxide away from the lungs and enhancing the tissue stores of this gas to prevent acidification of the blood in the pulmonary circulation. It ensures a high blood oxygen affinity in the pulmonary circulation while blood pressure levels of oxygen decline throughout apnoea. This shifts the oxygen equilibrium curve right in the systemic circulation, augmenting oxygen delivery. As apnoea proceeds the oxygen removal from the lungs greatly exceeds the CO_2 excretion into the lungs, decreasing the respiratory exchange ratio at the blood gas barrier (Shelton and Burggren 1976, Burggren and Shelton 1979, Hicks and Wang 1996). During ventilation the heartrate rises and the pulmonary vasculature resistance decreases. In Pseudemys scripta the decrease can be more than 50% which leads to a threefold increase in pulmonary blood flow, which is made up by about 60-65% of the cardiac output. These numbers were measured through analysing O_2 blood pressure in the different atria and arteries and comparing them (Shelton and Burggren 1976). Each vessel leaving the ventricle contains a different mixture of blood and has an unique relationship to the changing patterns of blood distribution in the ventricle. If more than half of the cardiac output goes back into the lungs, part of the blood coming from the lung has to re-enter it, which is facilitated through a L-R shunt. The cardiovascular changes are controlled, as mentioned before through cholinergics and can be abolished by vagotomy or atropine (Shelton and Burggren 1976, Hicks and Wang 1996b).

4 Alfaxalone in Reptiles

Alfaxalone has become a widely used drugs in reptiles, but studies about its effect and dosing in different species are scarce. The new and current formulation of Alfaxalone has been shown to provide successful anaesthesia in chelonians, crocodiles, lizards and snakes. In small lizards, intravenous administration provided a safe and fast route and, in comparison to intramuscular or subcutaneous route, reduced the induction and recovery periods (Morici et al. 2018). In leopard geckos, 5 mg/kg Alfaxalone was successfully used intravenously to achieve deep anaesthesia which lasted for 11-15 minutes. Recovery was smooth and took approximately 20 minutes, with the fastest recovery taking 10 minutes and the longest about 53 minutes. They administered the drug through the jugular vein, which is a feasible option in leopard geckos. In this and other studies tracheal intubation was or could have been possible. As there was apnoea observed in four out of twenty geckos, it has been suggested that dosage should not be raised much higher than 5 mg/kg. Yet, combinations with other anaesthetic drugs still need evaluation in leopard geckos (Morici et al. 2018).

A study in ball pythons investigated the effect of intramuscular Alfaxalone use dependent on dose and injection site. They used low, medium and high dosage (10, 20, and 30 mg/kg) and administered them in the caudal body part. Two weeks later, a dosage of 20 mg/kg was given in a cranial location (cranial of the heart). The use of the two injection locations was meant to assess the first-pass effect through the renal portal system in reptiles. As a result, cranially injected Alfaxalone anaesthetic duration was signifiable longer than when injected caudally. Furthermore, intubation was only possible after a dosage of 30 mg/kg, when injected caudally. Cranially administered, intubation was already possible with a dosage of 20 mg/kg used. Regardless of injection site, maximum effect was reached within a mean of 10 minutes and both, 30 mg/kg injected caudally and 20 mg/kg injected at the cranial site, led to apnoea after about 10 minutes post-injection (which can be managed by intubation and mechanical ventilation). Overall, Alfaxalone provided a rapid and non-eventful induction when administered intramuscularly in pythons and leads to a deeper anaesthesia when injected cranially to the heart, (James et al. 2018)

However, it is always important to bear in mind, that when it comes to reptiles there may be great differences between species and a study concerning one species, might not provide enough

or the right information for a different species. For example, in some snakes Alfaxalone only resulted in a light sedation and in all species sometimes responses to pain stimuli were maintained. In some species one might need a higher or lower dose, than studies in other reptilian species have suggested (Jones 2012).

4.1 Alfaxalone Use in different Chelonian Species

Alfaxalone has been used intramuscular or intravenous. It has been shown that at 5 mg/kg intravenously Alfaxalone leads to rapid induction and fast recovery in different terrapins and tortoises (red-eared terrapins, Hermann's tortoises, spur-thighed tortoises, marginated tortoises and Russian tortoises were included in the cited study). Minimum induction time was 15 seconds and the maximum 40 seconds. Time until reaching surgical plane of anaesthesia differed from 26 to 35 minutes, while full recovery was only reached shortly after, between 29-45 minutes post induction (Knotek 2014).

Another study focussed on temperature induced change of the effects of Alfaxalone administered intramuscularly in red-eared sliders. Cooler temperatures lead to a longer recovery period and deepen the anaesthetic effect, showing that body temperature should be monitored and taken into account when planning the anaesthesia protocol for a procedure. In general, higher doses of Alfaxalone led to a significantly longer recovery time, independent of the temperature. Turtles with shortest recovery time were kept in warm temperatures and were given a lower dose of Alfaxalone. The longest recovery times in this study took up to four hours. Neither dose nor temperature changed the reflexes tested (palpebral reflex, corneal reflex) or the reaction towards toe pinch stimulus. This is important to know, as most practitioners use these parameters to define anaesthetic depth (Shepard et al. 2013). Furthermore, in a study in Horsfield's tortoises and another study in red-eared sliders showed transient fluctuation in muscle tone and reflexes at the end or during the plateau phase of anaesthesia (Hansen and Bertelsen 2013, Kischinovsky et al. 2013). When the temperature-dependent effect of intramuscular Alfaxalone injection was researched in another study including red-eared sliders (Trachyemis scripta elegans), it was found that in general the anaesthetic leads to a rapid and easy induction and a smooth recovery. The high dose used was 20mg/kg and low 10mg/kg. As for the temperature, 35°C were defined to be high and 20°C to be low. If the higher dose was used in combination with cooler temperatures the induction time was longer, but the anaesthesia itself was deeper

and longer. Intubation was successful in 80-100% of animals induced during a lower temperature, but only in 0-30% who had the lower dose, but higher temperature (Jones 2012). Nonetheless, these studies proved a dose-dependent sedation after intramuscular Alfaxalone administration in red-eared sliders and alerts practitioners to the likelihood of enhanced anaesthetic effects through hypothermia. Regarding temperature changes, one should take into account circadian rhythms within the animals. Circadian and biannual changes affect metabolic rate, especially ventilation rate and oxygen consumption, in red-eared sliders. Depending on when the peaks of a species are this metabolic rate in- or decreases. Therefore, effects of Alfaxalone might also differ with time of the year (Shepard et al. 2013).

4.2 Other anaesthetic Drugs used in Reptiles

4.2.1 Volatile Agents

The use of inhalation anaesthetics is difficult in chelonians, because of their ability to hold their breath for a very long time and use anaerobic metabolism quite well (Knotek 2014). Furthermore, their ability to shunt the heart towards the systemic circulation route and leaving out the lungs helps them to avoid the intake of inhalation gas further. This can lead to a prolonged recovery time, as the inhalation drug used will reach it full effect later or in small doses over a longer period of time, depending on the species and the extent to which the animal can shunt their heart (Wyneken et al. 2007, Balko and Chinnadurai 2017). Furthermore, induction through inhalation gas requires high doses of the drug used which will also contaminate the environment putting the veterinary personnel at unnecessary health risk.

In absence of other alternatives, gas induction can be used in snakes and lizards, even though medium and large snakes can be intubated while conscious and therefore do not need any injected induction drugs at all (Sladky and Mans 2012).

4.2.2 Ketamine

Ketamine is not a narcotic drug in the classical sense, as it leads to dissociative anaesthesia and has mild hallucinogen effects. As a non-competitive agonist for the NDMA (N-Nitrosodimethylamin) receptor, it efficiently blocks flows of natrium, kalium and calcium ions. This provides good somatic analgesia, mild sedation, but also catalepsy. The hallucinogen effect can last long and lead to a rough recovery (Löscher and Richter 2016). Furthermore, the induced

catalepsy disables the patient's ability to react to pain stimuli, which indicates the risk of performing surgery on an animal with subanaesthetic dose of this drug. The analgesic properties are dose and specie dependent and are in generally not sufficient for visceral pain, so that for most surgical interventions further analgesic drugs are required. Additionally, it leads to a rise of blood pressure, and has a positive inotropic and chronotropic effect on the heart (Eberspächer 2016). The negative effects of ketamine can, however, mostly be compensated by the right premedication or combination with other drugs for induction (Löscher and Richter 2016). Ketamin modulates the pronociceptive sensitization process and therefore effectively counteracts the postoperative hyperalgesic response (Choi et al. 2015). This drug is best used in combination with alpha-2adrenergic agonists or benzodiazepines. In combinations, the dosage of ketamine can be lowered considerably, while it still provides additional sedation and analgesia (Sladky and Mans 2012).

4.2.3 Alpha-2-adrenergic Agonists

Alpha-2-adrenergic agonists, as for example medetomidine or dexmedetomidine, provide sedation and muscle relaxation. It is unclear, if there is an analgesic effect with this group of drugs in reptiles. A disadvantage is the documented dose-dependent cardiovascular depression after administration. On the other hand, their reversibility will lead to a fast and more predictable recovery. As mentioned before, alpha-2-adrenergic agonists are often used in combination with ketamine to achieve a safe, reliable and reversible anaesthesia (Sladky and Mans 2012).

4.2.4 Benzodiazepines

Benzodiazepines have a sedative, anxiolytic and muscle relaxant effect. Midazolam can be administered subcutaneously, intramuscularly or intravenously but provides highly variable sedation. This can be sufficient for minor procedures. More common than using it alone, is the combination with ketamine or alpha-2-adrenergic agonists, where it reduces the dosages of all drugs and attenuates the cardiovascular depressant effects and prolonged recovery periods observed in the other two drugs. Furthermore, this drug can be antagonized to shorten recovery periods even further (Sladky and Mans 2012).

4.2.5 Propofol

Propofol is an induction agent used in human and veterinary medicine. Through its interaction with the presynaptic cells of the GABAergic synapses, it enhances inhibitory neurotransmission. The main effects of propofol are sedative, anxiolytic and hypnotic. Additionally, it binds

radicals and can therefore have dose-dependent neuro- and cardioprotective effects. Furthermore, it reduces the cerebral oxygen uptake, its consumption and cerebral metabolic rate (Löscher and Richter 2016). Fast induction and uneventful recovery characterize propofol and lead to its popularity. Even though it cannot be antagonized, duration of its effects is rather short, as it is metabolized quickly in the liver and eliminated through the kidneys (Eberspächer 2016). Fast metabolization of this drug also minimizes the risk of accumulation. Side effects of propofol include post injection respiratory depression or even apnoea, transient decrease of blood pressure and a mild negative inotrope effect on the heart. Propofol should be injected strictly intravenous, which makes its use in reptiles not always practical. Even upon i.v. injection, it can lead to irritation of the vascular walls and to pain reactions (Löscher and Richter 2016).

Propofol can be used in all species of reptiles and can be administered only intravenous or intraosseous. Compared with mammals however, induction time can be prolonged and intravascular access is required. In Chelonians there have been documented complications secondary to accidental extravascular and/or intrathecal injections. These complications included paralysis of fore- and hindlimbs, coma and spinal necroses. Therefore, attempting propofol administration into the subcarapacial sinus is not recommended.

Depth and duration of anaesthesia and cardiopulmonary depressant effects are dose dependent. Because of low risk of accumulation and fast metabolization, chances of recovery after an overdose are rather high, if assisted ventilation is possible. Due to the short actio, propofol is most often used to facilitate intubation and the maintenance of anaesthesia will be provided by an inhalation anaesthetic agent. As sole agent it can be used for short surgical procedures – oesophageal feeding tube placement, abscess treatment or hemipenile amputation. This drug, however, does not contain any analgesic properties and therefore, appropriate analgesic administration needs to be included for painful procedures (Sladky and Mans 2012).

4.2.6 Comparison to Alfaxalone

Even though Alfaxalone and propofol seem to be similar in certain ways, they do have their differences. The first advantage of Alfaxalone is the possibility of intramuscular use, as with

some animals and even some species a strict intravenous administration is not feasible. Especially in its use in reptiles, it is a great advantage to be able to administer the drug intramuscularly. This is also very valuable in the wildlife sector considering free ranging animals, as well as stressed or fractious animals. Also, Alfaxalone does not appear to have increased adverse effects after continued or repeated use, such as the slow recovery or Heinz Body formation witnessed in cats after repeated use of propofol (Jones 2012). Concerning the side effects, they both have the possibility of short apnoea after intravenous injection. This appears to be less severe in Alfaxalone compared to propofol. Studies comparing both drugs have not found statistical differences, as they are dependent on the scoring system and statistical methods used (Maney et al. 2013, Bigby et al. 2017). Ketamine has quite different effects compared to Alfaxalone. Its biggest disadvantages are the possible hallucinations, catalepsy and the poor muscular relaxation, up to muscular rigidity when administered alone. Furthermore, it is not as quickly metabolized as Alfaxalone, leading to an undesirable protracted recovery phase, especially in reptiles (Eberspächer 2016, Löscher and Richter 2016).

Concerning reptiles, Alfaxalone has the advantage of faster induction times and fewer equipment requirements in comparison to inhalation anaesthesia. Although it is common practice to induce anaesthesia in reptiles by administered inhalation anaesthetics, this practice is considered more stressful than an injection (Jones 2012). For short procedures, such as a thorough examination of the animal or treatment of wounds can be managed without the requirements needed for inhalation anaesthesia. The advantages over Ketamine include that Alfaxalone has reportedly a very short induction time, provides sufficient muscle relaxation and recovery time in reptiles is, dependent on the species, fast and uneventful (Balko and Chinnadurai 2017). The advantage of ketamine over Alfaxalone, is the analgesic effect. Otherwise Alfaxalone has been documented to provide more reliable anaesthesia, a smoother induction and shorter recovery time. As for benzodiazepines, they can help as premedication when Alfaxalone is used, but do not provide the same depth or reliability of anaesthesia. Their advantage would be the possibility of antagonization, but the elevated cost of flumazenil, the compound used to antagonise benzodiazepines, may preclude the use of benzodiazepines in larger animals (Sladky and Mans 2012). The anaesthetic drug with the clinical effect most similar to Alfaxalone is propofol. Both are not reversible and both lead to a rather smooth induction. Propofol, however, does have known dose-dependent cardiopulmonary depressant effects in reptiles, which can cause apnoea

and are more frequently if the drug is administered fast. As known in mammals, Alfaxalone does have these effects as well, but only minimally. Furthermore, Alfaxalone has the clear advantage of the possibility to administer it intramuscularly or subcutaneously. This is by far the biggest shortcoming of propofol concerning reptiles, as intravascular access cannot always be provided (Sladky and Mans 2012).

4.3 Combination with other Anaesthetic Drugs in Reptiles

As mentioned before, it has been recommended not to combine Alfaxalone with any other intravenous anaesthetic drugs. Intramuscular premedication, however, can be provided before induction to reduce the necessary dose and smooth some of the side effects. A study including nine adult male Horsfield's tortoises has shown the differences between Alfaxalone used alone and in combination with medetomidine. An intramuscular dose of 10 mg/kg Alfaxalone intramuscular alone led to moderate sedation with no analgesia and endotracheal intubation was not possible. Also, 20 mg/kg Alfaxalone intramuscular alone in resulted in variations of moderate to deep anaesthesia with still no possibility of intubation. The analgesic effect was minimal. When medetomidine was added, deep sedation or anaesthesia was the result, as well as a significant increase in total anaesthetic time. Additionally, peripheral nociceptive sensation was significantly reduced. Anyway, this combination of Alfaxalone and medetomidine was observed to have significant respiratory depression and bradycardia as side effects (Hansen and Bertelsen 2013).

Part 2

1 Material and Methods

For this retrospective study, anaesthesia data was collected in chelonians belonging to two species (*Astrochelys radiata* and *Geochelone platynota*). The animals were undergoing general anaesthesia in the context of another study and submitted to MRI imaging, CT imaging, ultrasound examination and finally intracelomatic endoscopy. The study was approved by the ethics commission of the Veterinary University in Vienna (ETK-61/04/2019). All the animals came from the same breeder and were kept in the same standard husbandry condition. Animals were excluded if deemed not healthy on clinical examination or if any abnormal finding was reported after the diagnostic imaging procedures performed. The collection of date took place on various days from Mai-July 2019. The author of the study was collecting the data for anaesthtic reports and afterwards it was decided to use the data in this diploma thesis.

All tortoises where premedicated with 0.15 mg/kg Medetomidine (Narco Start® 1mg/ml, Richter Pharma), 0.1 mg/kg Midazolam (midazolam® 2 mg/ml, öog GmbH, Salzkammergut-kl. Bad Ischl Apotheke) and 0.2 mg/kg Hydromorphon (hydal®) or 0.5 mg/kgmorphine-(Vendal® 10mg Ampullen, G.L. Pharma GmbH), mixed in the same syringe and administered intramuscularly into the pectoral muscles. Meloxicam (Metacam 5mg/ml®, Boehringer Ingelheim) in a dose of 0.2 mg/kg was separately injected in the same muscle. After premedication, general anaesthesia was induced in all animals by injecting Alfaxalone. Seventeen animals (9 *Astrocheylis radiata*, 8 *Geochelone platynota*) received Alfaxalone through intramuscular administration (group IM) and seventeen subjects (9 *Astrocheylis radiata*, 8 *Geochelone platynota*) received Alfaxalone through intravenous administration (group IV). The tortoises were brought to the clinic in the groups they live in at the breeder's facility. Subjects in each group were alternatingly induced either i.m. or i.v..

Group IM received 10 mg/kg Alfaxalone (Alfaxan Multidose® 10mg/ml, Jurox, Dechra) 10 minutes after premedication in the pectoral muscles. Group IV received 7 mg/kg Alfaxalone (Alfaxan Multidose® 10mg/ml, Jurox, Dechra) into the subcarapacial sinus thirty minutes after premedication and subsequentially they received orotracheal intubation with an IV catheter

(Vasofix® Safety Braun). Tracheal intubation was performed only if the jaw muscles were relaxed enough to open the jaw without force. If intubation was not possible at the first attempt, the animal received a second dose of Alfaxalone intravenously into the subcarapacial sinus and the event was noted. The second dose was half of the original dosage, meaning 5 mg/kg for the IM group and 3.5 mg/kg in the IV group. The time from induction through Alfaxalone either i.m. or i.v. until intubation was noted. During MRI, the animals received 2% Sevoflurane in 100% oxygen at a flow rate of 1 l/min through a rebreathing circle circuit and were let to breath spontaneously. At the end of the procedure, all turtles received 0.5 mg/kg atipamezole and 0.008 mg/kg flumazenil i.m. and were extubated as soon as it was confirmed that the patient was consistently breathing spontaneously.

Data collected during anaesthesia included respiratory rate, checked clinically through front limbs movement and through capnography during MRI, and any reaction to stimulations through movement. During the MRI, heart rate was also measured through ECG with MRI compatible ECG pads placed on the legs and recorded every 5 minutes. Body temperature was measured cloacally with a temperature probe at least once before and after MRI. Temperature loss per minute was calculated as (Rectal temperature before MRI – Rectal temperature after MRI)/time in minutes. After MRI, animals who did not breath spontaneously were ventilated with an AMBU bag. Two breaths were administered every 5 minutes. Heart rate was measured with a doppler probe placed at the base of the neck, once before MRI and then, after MRI, every five minutes until extubation. If it was not possible to record heartrate with ECG, the change in heartrate during anaesthesia was calculated (heartrate after MRI – heartrate before MRI). Pinch reflexes were measured with a Pean clamp on all four extremities. The test result was defined positive when it evoked a motoric response. For the different procedures the leg of the animals were fixed with twill tape above the knees. The animals were pinched before MRI and after MRI every five minutes until full recovery. The palpebral reflex was measured by touching the medial corner of the eye with the closed Pean clamp. If the reflex was inducible in one or both eyes, it was counted as positive. This reflex was measured right before and after the MRI and every five minutes until full recovery. Time from induction until intubation, then from intubation until extubation and from antagonization until extubation was recorded. Data collected from both groups was compared and analysed through a t-test for independent samples ($\alpha = 0.05$).

2 Results

A total of 40 animals were initially enrolled in the study, 22 *Astrocheylis radiata* and 18 *Geochelone platynota*. Six animals were excluded (four *Astrocheylis radiata* and two *Geochelone platynota*). Of the *Astrocheylis radiata*, one was excluded because of arrhythmia, the second because of an accidental bronchial intubation, which was identified through MRI, the third one due to suspected cardiac pathology, and the fourth due to a mistake in dosing the anaesthetic. From the species *Geochelone platynota,* one was excluded because in the MRI fluid could be seen in the lung, and in another one it was not possible to achieve a successful intravenous injection and was then injected i.m.. Age varied from 2 years to 5 years (3,53 ± 1,11 years) and weight varied from 121g to 1017g (455,94 ± 243,03).

I.M. Group				I.V. Group			
Species	**Age(years)**	**weight (g)**	**sex**	**Species**	**Age(years)**	**weight (g)**	**sex**
AR	3	507	w	AR	3	457	w
AR	3	429	w	AR	3	250	w
AR	3	250	w	AR	2	258	w
AR	2	232	w	AR	5	781	w
AR	5	1001	w	AR	5	998	w
AR	5	726	w	AR	1	169	w
AR	1	136	m	AR	1	121	m
AR	3	1017	w	AR	3	560	w
GP	4	261	m	GP	4	333	m
GP	4	424	w	GP	4	332	m
GP	4	430	m	GP	4	504	m
GP	4	467	m	GP	4	430	m
GP	4	490	m	GP	4	695	m
GP	4	401	m	GP	4	417	m
GP	4	352	m	GP	4	298	m
GP	4	310	m	GP	4	306	m
AR	3	322	w	AR	5	838	w

Figure 3:Demographic data of all subjects, I.M. – intramuscular, I.V. – intravenous, GP-Geochelone Platynota, AR – Astrocheylis Radiata, w – female, m – male,

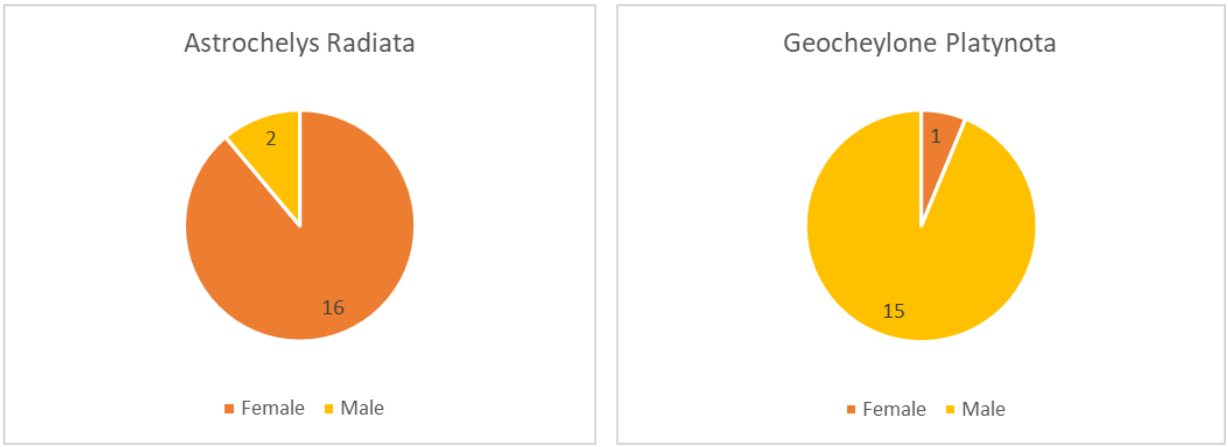

Figure 4: Distribution of gender in both species

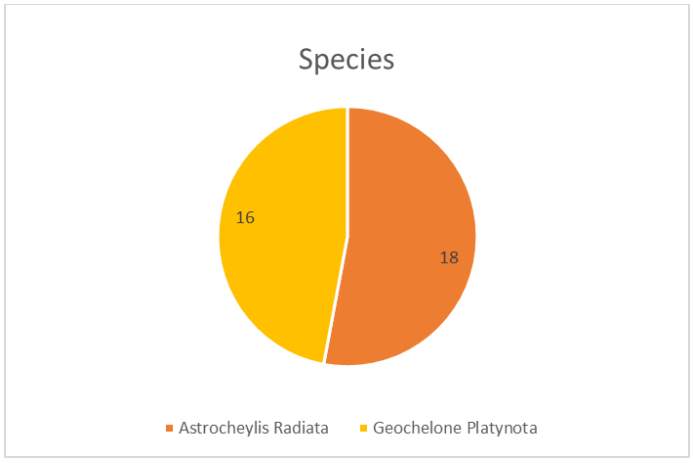

Figure 5: Distribution of species

At the end, eighteen animals received Alfaxalone as an induction agent intravenously and seventeen animals received the induction agent intramuscular. In total 4 subjects, 3 in the IM group and 1 in the IV group, needed a second dose of Alfaxalone to achieve orotracheal intubation.

The average time from administration of Alfaxalone to intubation was significantly different in the 2 groups, with 25.36 ± 6.62 minutes in the IM group, and 4.88 ± 3.07 minutes in the IV group ($p < 0.01$). These numbers were calculated after excluding the subjects which required a second dose. The subject in IV group which required a second dose for intubation took 9 minutes from the second dose (half of the original dose) of Alfaxalone until intubation was possible, while in the IM group an average of 1.33 ± 0.58 minutes after second dose was required.

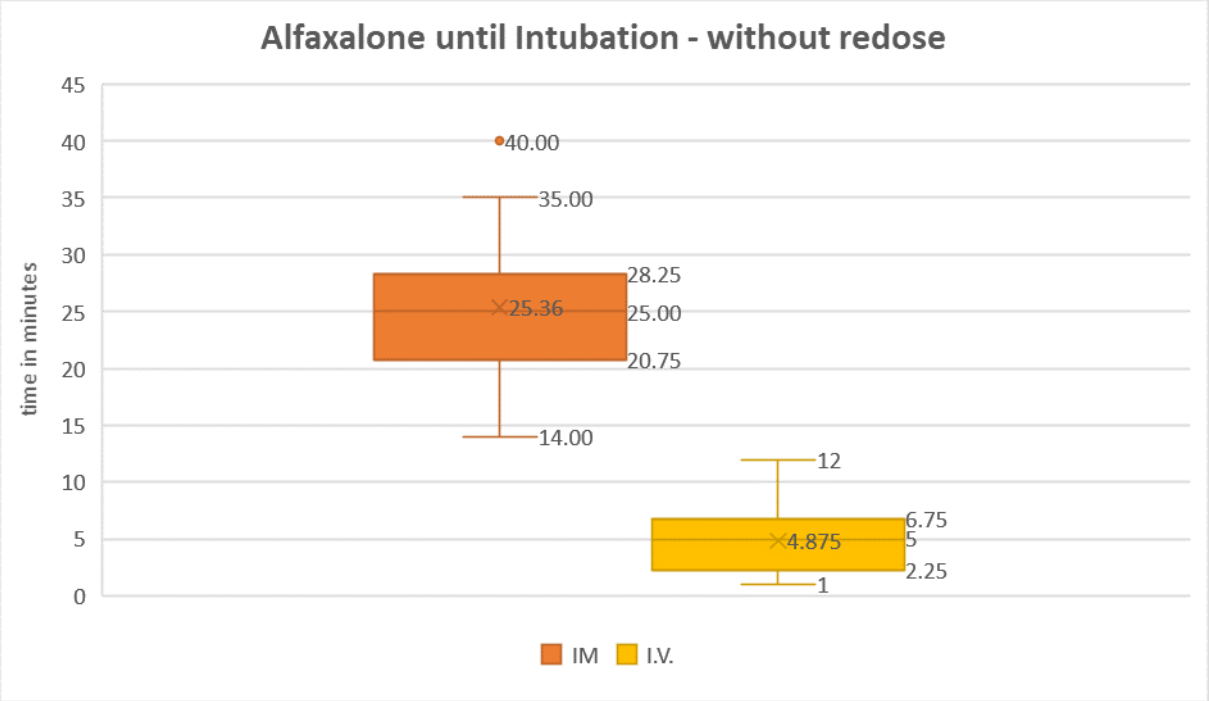

Figure 6: Time from administration of Alfaxalone until intubation excluding subjects which needed a redose

Time from intubation until extubation in the IM group was 57.65 (± 15.41) minutes, while in the IV group was 57.71 (± 18.89) minutes, showing no significant difference between the two groups (P= 0.42).

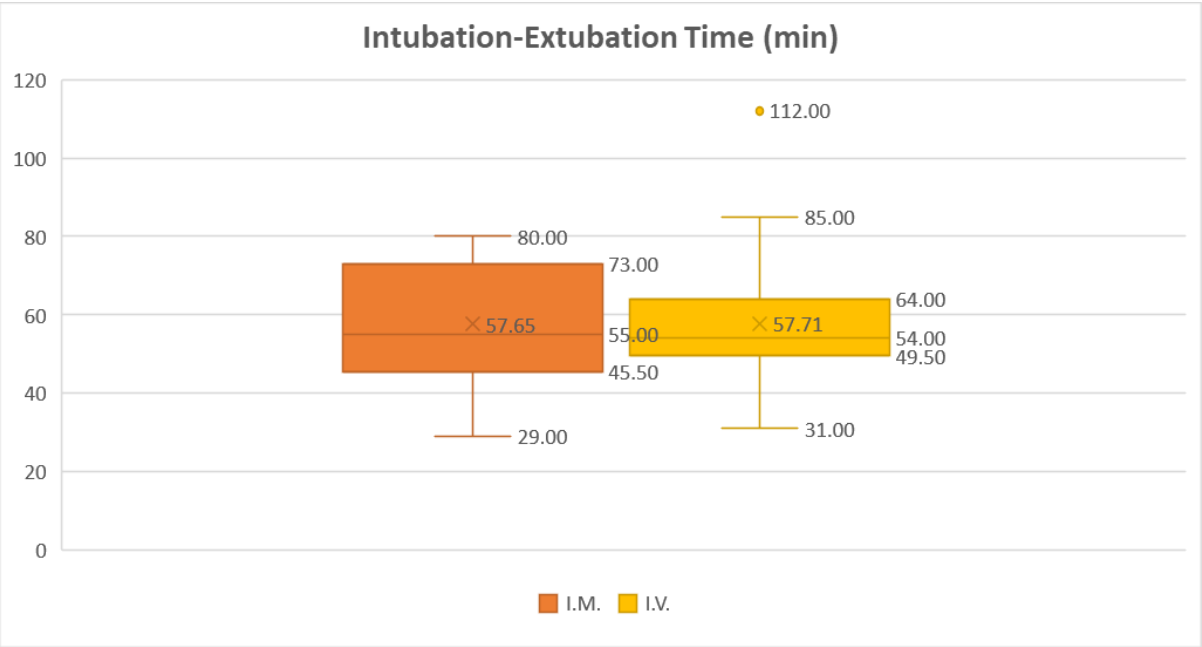

Figure 7: Time noted from intubation until extubation of the patients from the IM and IV group.

On average, the animals were in the MRI for a duration of 17 minutes (17.22 ± 4.38 min). Difference in time were only related to the size of the animal and the required number of slides.

Body temperature loss per minute was similar in the 2 groups (group IM 0.06 ± 0.05; group IV 0.07 ± 0.08; p=0.47). The loss was calculated during the time animals were not heated externally with a heating mat, which was during the MRI. Dependent on animal size, the MRI took different amounts of time for each animal, which is why the temperature loss has to be calculated per minute, so it can be compared. The total temperature loss averagely was 1.05°C ± 1.11°C in the IV group and 0.89 ± 0.77°C in the IM group.

Temperature Loss over time (°C)					
I.M.			I.V.		
Total Temp Loss	*Time*	*Temp Loss/Time*	*Total Temp Loss*	*Time*	*Temp Loss/Time*
-1.2	15	-0.08	-1.3	20	-0.07
-1.8	20	-0.09	-1.8	10	-0.18
-2.4	15	-0.16	-0.9	10	-0.09
-1.7	10	-0.17	0	15	0.00
-0.4	20	-0.02	0.1	20	0.01
-0.2	15	-0.01	-2.5	25	-0.10
-2.4	15	-0.16	-4.6	15	-0.31
-0.9	25	-0.04	-0.7	15	-0.05
-1	15	-0.07	-0.8	15	-0.05
-0.4	20	-0.02	-0.5	20	-0.03
-0.6	15	-0.04	-1.1	15	-0.07
0	10	0.00	-0.1	15	-0.01
-1.2	20	-0.06	-1.2	15	-0.08
-0.8	15	-0.05	-0.9	20	-0.05
-0.4	20	-0.02	-0.5	15	-0.03
-0.3	15	-0.02	-0.5	15	-0.03
-1.8	20	-0.09	-0.6	25	-0.02

Figure 8: Numbers concerning temperature loss over time.: each line is representing the temperature loss for one individual during the time they were not heated externally.

The starting temperature – which was measured after intubation – ranged from 26.9 to 36.3°C (mean 29.95 ± 2.17°C) in the IV on average. In the IM group it ranged from 27.6 to 32.6°C (mean 29.74 ± 1.59°C). There was no significant difference found between the two groups.

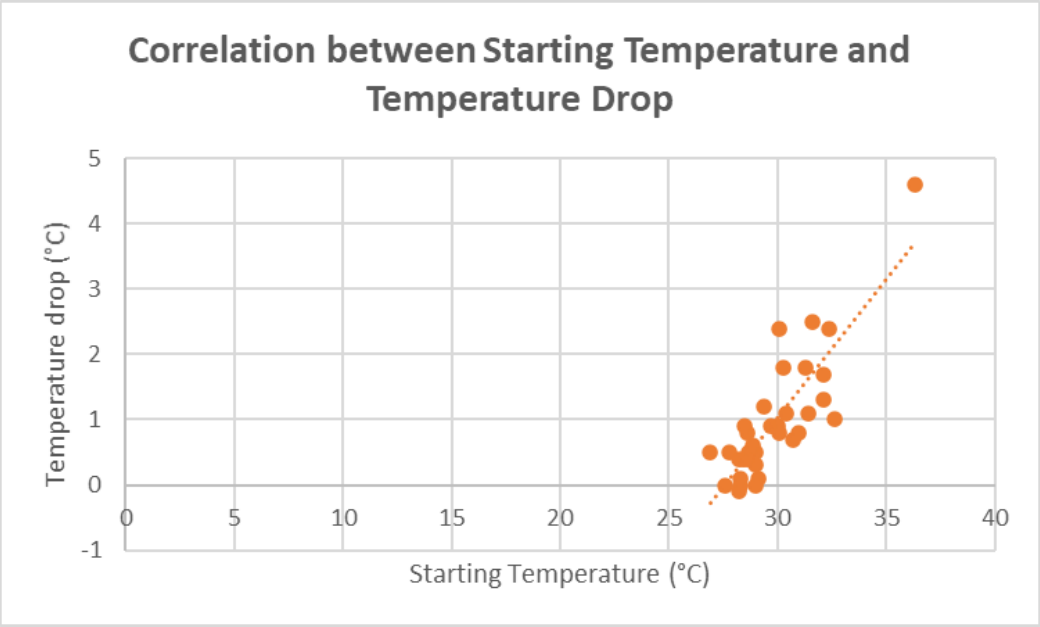

Figure 9: Correlation between the starting temperature of the individual and the temperature drop during the MRI. One individual gained 1°C of temperature, which is shown here as -1°C.

In 17 animals it was not possible to record heart rate during MRI (10 subjects from the IM group and 7 from the IV group). For the subjects, which were successfully monitored with ECG during the MRI (in 8 subjects from IV group and 7 subjects from the IM group), heart rates were recorded up to the moment of extubation. This is put together in the figure below, showing a rather dramatic drop in heartrate of 6.80 bpm in the IV group in the first 35 minutes. After that the lowest point of averagely 22 bpm is reached and heart rate rises again. In the IM group the drop is less prominent (2.67 bpm), and heart rate is going up again after 25 minutes already. In both groups, however there is a short rise of heart rate in the first five minutes of averagely 0.70bpm in the IV group and 2.00bpm in the IM group. As heart rate measurements were stopped after extubation, the date thins out, which is why the diagram below stops at the point in time where most animals did not require heart rate monitoring anymore.

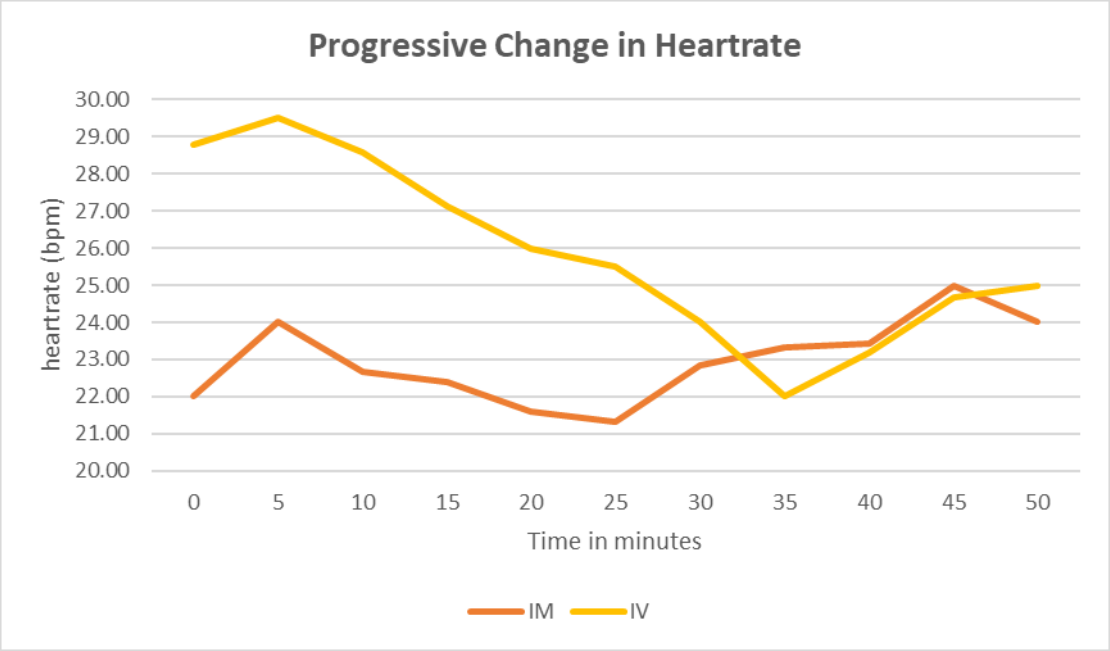

Figure 10: Progressive changes in heart rate in both groups over time

Reflex response to pinching test and corneal reflex were inconsistent and changing in each subject of both groups along time and did not appeared to relate to the depth of anaesthesia. Any further comparison was therefore impossible.

3 Discussion

In this study, we had the rare opportunity to evaluate data from many individuals from rare chelonian species as *Astrocheylis radiata* and *Geochelone platynota*. Furthermore, data concerning anaesthesia of these species are scarce. Information gained includes length of anaesthesia, changes in heartrate and temperature and, most importantly, the differences between administration routes (i.m. and i.v.), which is especially interesting for clinicians working with terrestrial chelonians.

As shown in the results, mean time from induction until intubation took significantly longer in the IM group than in the IV group (25.36 ± 6.62 minutes and 4.88 ± 3.07 minutes respectively). This is expected and already been assessed in cats, dogs, or humans. In general, it can be said that after intramuscular administration the effect of Alfaxalone commences after 5-10 minutes in cats and dogs, but as intravascular administration bypasses the step of resorption, it leads to an onset of effect within one or two minutes, about 5-folds less than in i.m. administration. Furthermore, considering the outcomes in this study, the time until effect commences is about 2.5-5 times longer in chelonians than in dogs or cats. This is something the clinician should consider carefully, when assessing the effect of this drug in chelonian and before considering a top-up dose (Eberspächer 2016, Löscher and Richter 2016).

Overall, only 4 out of 34 animals needed a top-up dose to achieve an adequate depth of anesthesia for intubation. Interestingly, from all the individual who received a top-up dose, the only individual from the IV group requiring a second dose, took significantly longer (9 minutes) to be ready for intubation, than the average time it took all re-dosed subjects of the IM group (1.33 ± 0.58 minutes). As mentioned, only animals that appeared clinically healthy and, where no abnormalities in the MRI or CT were found, were included in this study. Therefore, there were no apparent organic alterations or clear medical issues which could justified the significantly longer induction time in that specific individual. Anyway, there was no preoperative bloodwork available for any of the patients, so we cannot exclude the presence of a subclinical problem as cause for this unexpected delay. Variations in body temperature are expected to affect the circulation and perfusion of reptiles, and therefore can affect the on-set time of drugs (Williams et al. 2020). However, all subjects were in a similar range of environmental temperature and therefore is unlikely that a marked difference in body temperature could explain the long onset

time for the subject of the IV group. Accidental non-i.v. injection could also lead to a prolonged induction phase. Anyway, specifically in this subject, the MRI imaging did not show any presence of fluid in the surrounding tissue, a sign observed in other animal who received accidental perivascular injection. It is therefore also very unlikely that both injections were not correctly placed in the sub-carapacial sinus.

Concerning body temperature, no significant difference in temperature loss could be determined between groups. The mean starting temperature in the IV group was 29.95 ±2.17°C and in the IM group 29.75 ± 1.59°C. Starting temperature only differed 0.19°C on average between the two groups. The biggest temperature loss occurred in one animal of the IV group with 4.6°C lost over 15 minutes. This individual was also the subject with the highest starting temperature of 36.3°C. For both species it is rather hard, to define a regular body temperature (when they are active) as they are ectotherm. Furthermore, their average body temperature during their active phases seems to be seldomly recorded and published, due to the rarity of these species. Anyway, a body temperature of 36°C is in general on the higher end of the spectrum in terrestrial tortoises (Rasoma et al. 2013). A reason, why this subject from the IV group lost 12.67% of its starting body temperature in 15 minutes could be, that the gradient from body temperature to surrounding temperature was much bigger in this animal than in all the others, as the others had lower starting temperatures. A higher starting temperature will create a higher gradient to the surroundings and therefore initially lead to a faster loss of temperature at the beginning of anaesthesia, when temperature loss is mainly attributed to deregulation of peripheral vascular tone and core-to-superficial body temperature gradient. In the IM group the highest loss of temperature was 2.4°C in 15 minutes from a starting temperature of 32.4°C, which translates to 7.40% of temperature loss. In the IM group the individual with the highest temperature loss only had the second highest starting temperature. The highest starting temperature was 32.6°C and that individual only lost 1°C in 15 minutes. These two very high losses of temperature (4.6°C and 2.4°C in 15 minutes) where far from the average total loss of temperature during MRI, which were 1.05 ± 1.11°C (0.07 ± 0.08°C/min) in the IV group and 0.89 ± 0.77°C (0.06 ± 0.05°C/min) in the IM group.

Why the two mentioned subjects were showing higher body temperature and or losses, given that they were all kept in the same husbandry condition, is not easy to explain. As mentioned,

temperature loss is not a linear process, but rather an exponential regression over time, Therefore, even small differences in initial measuring time-point could have been the cause of marked discrepancies in the recorded body temperature. Since it was unfortunately not possible to measure temperature during the MRI, we have no way of knowing how the regression of temperature went and therefore can only speculate. When looking at the starting temperature of the individuals together with the drop of temperature over time, there does seem to be a correlation: the higher the starting temperature, the higher was the temperature drop during the MRI. Furthermore, in both groups the standard deviation of temperature loss is almost as high and even higher than the mean, which shows a great fluctuation in temperature loss between the individual subjects. This underlines the theory, that dependent on where in the temperature loss regression one starts to measure, the drop is higher or lower.

Heart rate was measured with a doppler probe and, during the MRI, through an ECG when possible. A doppler is normally applied at the base of the neck in chelonians to acoustically detect pulse along large vessels. Especially when the doppler is provided with a stylus probe, it can be applied to animal of practically any size. In contrast, some patients were unfortunately too small for the magnetic resonance compatible ECG pads and therefore heartrate could not be measured in all patients during the MRI. When an ECG trace was visible on the monitor, anyway, heart rate had to be counted manually, as the electric potentials were so small that the system did not recognise them as heartbeats. In all the patients monitored with ECG, the sequence of the electrical activities did not show any gross disturbance of rhythmicity, but the trace was too small to differentiate the morphology of the different waves. Therefore, for small turtles, a more sensitive ECG monitor than the ones normally provided in anaesthesia multiparameter monitors would be helpful when accurate cardiac monitoring is required. Heart rate was successfully recorded during MRI, and therefore throughout the whole time, in 8 subjects from IV group and 7 subjects from the IM group. For these subjects the ECG-data was collected. This enabled us to see, how heartrate changed over time (it was noted down every five minutes) from intubation until most patients were stable enough to discontinue counting the heartrate. In both groups a short rise of heart rate was observed during the first five minutes after intubation. This was more than twice as high in the IM group (2.00 bpm) than in the IV group (0.70 bpm). After that, a drop-in heart rate proceeded over the next 20 (IM group) to 25 minutes (IV group). This drop was more severe in the IV group with 6.80 bpm, while the drop in the IM

group amounted to only 2.67 bpm. After 20 and 25 minutes in the IM and IV groups respectively, the heart rate started to rise until the animals were awake enough to stop monitoring the heartrate as frequently. The drops in heartrate might originate from the peripheral vasodilation and the resulting hypotension Alfaxalone can cause. The initial rise in heart rate might also be caused by Alfaxalone. In pigs Alfaxalone caused a significant decrease in diameter of the common carotid artery and a significant increase in heart rate 15 seconds after injection. This was allotted either to a direct vasoconstrictoric effect of the drug or to the acute reduction of mean arterial pressure measured at that said time point. Contrary to what happened in the carotid artery, in said study involving pigs, a decrease in resistance in the peripheral vessels was shown, which was probably cause by a direct vasodilatory effect at this level. After these acute changes vascular diameter re-increased in the common carotid artery and after 1 minute the mean arterial pressure reached baseline values again. Heart rate decreased as well, which might be explained with a possible direct negative inotropic effect of Alfaxalone (Pfeiffer et al. 2013). Basically, what observed in the chelonian subjects included in our study seems to reflect what occurred in the pigs from studied by Pfeiffer, but in a much shorter time frame, which leads to the assumption that either this can be attributed to different metabolism of chelonians, or to inaccuracy of our measurements. The drop in heart rate was unrelated to the Alfaxalone and rather an effect of the premedication. in fact, we started measuring heart rate only after intubation. This was done to not bother the subjects with the doppler probe when they were still superficial, in case the stimulation would cause them to stay awake longer.

To the author's best knowledge, there are no published data about the duration of Alfaxalone effect in these two rare chelonian species. In our patients, time from intubation to extubation was 57.71 ± 18.89 minutes in the IV group and 57.65 ± 15. minutes in the IM group. The difference between group was not significant, and there is therefore no advantage of one administration route over the other when it comes to extubation time. Furthermore, these data confirms that the combination of the premedication and Alfaxalone used in this study could suffice for short procedures, when inhalation anaesthesia maintenance is not available or desirable.

As mentioned in the results, the evaluation of the reflexes was not conclusive. Not only were the reactions to reflex stimulation different between the individuals, even for each individual

the reactions changed during anaesthesia, and did not appear to correlate to depth of anaesthesia. Regarding the pinching response, it has to be taken in account that it could have been affected by the preparation necessary for the procedures, where the hind legs were bound to be straight, as well as by the ECG pads which covered the whole extremities in some patients and may therefore have stiffened the legs further. Besides that, reflexes have been found to be very unreliable markers for depth of anaesthesia in reptiles in general before (Longley et al. 2008), which is supported by the findings of this study. The only conclusion we can draw is that the evaluation of reflexes to judge depth of anaesthesia is not suitable when using the described anaesthetic protocol in these chelonian species.

Often breathing is thought to be unimportant in chelonian anaesthesia, because a lot of chelonians do not breathe as often as mammals would, as they are occasional breathers. But just because breathing frequency is lower and there are longer times of apnoea in-between breaths, this should not bring to underestimate the importance of monitoring and eventually correcting ventilation (Hicks and Wang 1996, Longley et al. 2008). In the case of this study, it was not possible to monitor breathing accurately and continuously, as the subjects were rather small, and their tidal volume was insufficient for being consistently detected by the MRI-compatible side-stream capnograph available. Therefore, it was impossible to record any respiratory frequency or even any presence of spontaneous breathing during the MRI sequencing. Nonetheless, especially in animals breathing so little, it should be a priority to monitor breathing. Therefore, for procedures involving longer anaesthesia time, it would be advisable to use more sensitive instrument to quantify not only the respiratory rate, but also the adequacy of ventilation through an accurate measurement of expiratory CO2. For this purpose, the use of a main-stream capnograph or a micro stream capnograph could be more indicated than a side-stream capnograph. Furthermore, Alfaxalone does have a negative impact on breathing frequency in cats and dogs (Eberspächer 2016) and this might also be the case in reptiles. Unfortunately, the data we collected cannot bring any light on the respiratory effect of this drug in chelonians, and especially if the route of administration has any relevance. Therefore, it would be interesting to further investigate the severity of respiratory depression caused by Alfaxalone chelonians as well as in other reptilian species, to differentiate between physiological periods of breathing pauses and drug induced breathing pauses.

Unfortunately, there was no blood pressure measured in this study. In the future, blood pressure measurements would be helpful to provide further information on the cardiovascular effects of Alfaxalone. Furthermore, information on the metabolization and elimination of Alfaxalone in the chelonian organ system would be interesting and, of course, blood work with solid referential numbers would complete the picture of all possible effects and side effects of this drug. Especially the renal system has already been shown to work differently in reptilians than in mammals. While in most domestic animal species numerous studies are conducted when a new drug is brought to the market, reptilian species are plenty and often vary a lot in their metabolism. Studies often only include the commonly held species, such as green iguanas or red eared sliders. But even in the family of terrestrial tortoises there are differences between soft-shelled and hard-shelled species, some living in a dry environment and others in a humid environment (Wyneken et al. 2007, Kölle and Blahak 2015). This lack of information could be filled with further studies in different reptilian species including blood work before and after anaesthesia.

In the personal experience of the medical personnel involved in this study, the IM group seemed to be drowsy longer after recovery, but slept less deep, while the IV group seemed to recover fully faster and slept deeper. While these subjective observations were not quantified, further studies concerning Alfaxalone might be able to prove these experiences wrong or right.

The limitations of this study include the fact that there was not the right equipment at hand during the collection of data, for example for the ECG monitoring during the MRI: The pads were too big for some of the subjects and therefore the results are not as wholesome as they could be. As already mentioned, many existing studies include more common species, whick makes comparison to existing data difficult. Furthermore, as these animals are cold-blooded, temperature plays an important role, and there is no data of the surrounding room temperatures, which might have been different for some turtles and might have affected reactions to medication.

In conclusion, as it is often either unpractical or impossible to administer medication intravenously in reptiles, and more specifically in small chelonians, Alfaxalone seems to offer as a rather promising alternative for other induction anaesthetics, such as propofol. Both propofol and Alfaxalone have a short induction time and a relatively short duration of action, providing at the same time variable but normally sufficient muscle relaxation. Propofol anyway present

the disadvantage to require a strictly intravenous administration. In contrast, ketamine, similarly to Alfaxalone, can be administered intramuscularly, but is unable to provide satisfactory muscle relaxation, and especially in chelonians is often associated with extremely prolonged recovery phase. Alfaxalone can also be a practical alternative to gas induction, that although popular, is associated with more stress for the patient due to the prolonged time required by the specific respiratory pattern of these species. Especially in reptiles breathing patterns depend a lot on the natural environment of a species. Semi-aquatic types of chelonians can hold their breath for extensive time periods. Because of this, when it is not possible to provide controlled ventilation, anaesthesia duration might be limited to the time frame injectable drugs can provide, as volatile agents cannot function without breathing (Shelton and Burggren 1976, Jackson 1985, Hicks and Wang 1996). The anaesthesia duration provided by the protocol used in this study would then offer a practical alternative for short procedures. The clinician should anyway keep in mind that the administration of Alfaxalone is associated with a reduction in heart rate and a progressive loss in body temperature. The data we collected do not show any significative difference associated with the way of administration for the body temperature. In contrast, i.m. administration appears associated with a more marked initial increase in heart rate, followed by a less marked a shorter drop in frequency. Unfortunately, is impossibly to affirm if the heart rate changes reflect a difference in hemodynamic stability as no blood pressure measurements were performed in our study. Similarly, our set-up did not allow to verify the respiratory effect of the drug and if a way of administration would have any advantage other the other in that sense. More studies should be granted to further investigate the full potential and limitations of Alfaxalone in chelonians as well as in other reptile species.

Abstract:

Alfaxalone is a long-known veterinary drug and has only recently been introduced into the field of veterinary medicine in reptiles. This diploma thesis is a retrospective study on Alfaxalone's effects in chelonians dependent on the route of administration (intravenously versus intramuscularly). The study took place at the University of Veterinary Medicine, Vienna, where 35 healthy chelonians of the rare species *Geochelone platynota* and *Astrochelys radiata* were anesthetized with Alfaxalone. Subjects were divided into one group receiving Alfaxalone intravenously (IV group) and one receiving it intramascular (IM group). Findings showed a significant difference in time from induction with Alfaxalone until intubation, where the IV group (4.88 ± 3.07 minutes) took significantly shorter than the IM group (25.36 ± 6.62 minutes) and a significant higher heartrate over the duration of anesthesia in the IV group (24.71 ± 2.27bpm) than in the IM group (22.57 ± 1.90 bpm). In total 4 subjects needed a second dose of Alfaxalone and there was no significant difference in the temperature and length of anesthesia between the two groups. This retrospective study serves to collect and show data on reptile anesthesia and give indications on where to look for filling in the knowledge gaps existing in this field of research.

Zusammenfassung:

Alfaxalone ist ein altbekanntes Medikament, welches aber erst seit kurzem in der Veterinärmedizin Einzug genommen hat. Diese Diplomarbeit ist eine Retrospektive Studie über die Effekte von Alfaxalone in Schildkröten je nach Administration. 35 gesunde Schildkröten der Arten *Geochelone Platynota* und *Astrocheylis Radiata* wurden mit Alfaxalone intravenös oder intramuskulär für eine Vollnarkose induziert. Dafür wurden die Schildkröten in zwei Gruppen unterteilt und diese wurden dann verglichen. Hier war signifikant, dass die Zeit von Induktion bis zur Intubation in der IM Gruppe (25.36 ± 6.62 Minuten) länger war als in der IV Gruppe (4.88 ± 3.07 Minuten). Weiters war der Puls der IV Gruppe (24.71 ± 2.27bpm) durchgehend signifikant höher als in der IM Gruppe (22.57 ± 1.90 bpm). 4 Tiere mussten nachdosiert werden, um die nötige Anästhesietiefe zu erreichen, um intubiert werden zu können. Es gab keine signifikanten Unterschiede in innerer Körpertemperatur oder der Länger der Anästhesie zwischen den beiden Gruppen. Diese retrospektive Studie soll dazu dienen Daten über Anästhesie in Reptilien zu sammeln und damit zu der Wissenserweiterung in diesem Bereich bei zu tragen.

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List of Figures

FIGURE 1: A SKETCH OF THE POSITION OF LUNGS, THE HEART AND TRACHEA IN THE CHELONIANS SHELL AND OF THE PULMONAL PARENCHYMA. 10
FIGURE 2: SV - SINUS VENOSUS, RA-RIGHT ATRIUM, LA-LEFT ATRIUM, CA-CAVUM ARTERIOSUM, CV-CAVUM VENOSUM, CP-CAVUM PULMONALE, PV-PULMONARY VEIN, PA-PULMONARY ARTERY, AORTAS-RIGHT AND LEFT AORTIC ARCH, THE ARROWS SYMBOLIZE THE POSSIBLE BLOOD FLOW ROUTES. 16
FIGURE 3:DEMOGRAPHIC DATA OF ALL SUBJECTS, I.M. – INTRAMUSCULAR, I.V. – INTRAVENOUS, GP-GEOCHELONE PLATYNOTA, AR – ASTROCHEYLIS RADIATA, W – FEMALE, M – MALE, ... 29
FIGURE 4: DISTRIBUTION OF GENDER IN BOTH SPECIES 30
FIGURE 5: DISTRIBUTION OF SPECIES 30
FIGURE 6: TIME FROM ADMINISTRATION OF ALFAXALONE UNTIL INTUBATION EXCLUDING SUBJECTS WHICH NEEDED A REDOSE 31
FIGURE 7: TIME NOTED FROM INTUBATION UNTIL EXTUBATION OF THE PATIENTS FROM THE IM AND IV GROUP. 31
FIGURE 8: NUMBERS CONCERNING TEMPERATURE LOSS OVER TIME.: EACH LINE IS REPRESENTING THE TEMPERATURE LOSS FOR ONE INDIVIDUAL DURING THE TIME THEY WERE NOT HEATED EXTERNALLY 32
FIGURE 10: CORRELATION BETWEEN THE STARTING TEMPERATURE OF THE INDIVIDUAL AND THE TEMPERATURE DROP DURING THE MRI. ONE INDIVIDUAL GAINED 1°C OF TEMPERATURE, WHICH IS SHOWN HERE AS -1°C. 33
FIGURE 11: PROGRESSIVE CHANGES IN HEART RATE IN BOTH GROUPS OVER TIME 34