A comparison of anesthesia induction by two different administration routes and doses of ketamine and medetomidine in red-eared sliders (*Trachemys scripta elegans*)

Ismar LUTVIKADIC[1,a,✉], Alan MAKSIMOVIC[1,b]

[1]University of Sarajevo, Veterinary Faculty, Department of Clinical Science, Sarajevo, Bosnia and Herzegovina

[a]ORCID: 0000-0002-3768-3007; [b]ORCID: 0000-0002-4127-9445

ARTICLE INFO

Article History
Received : 18.07.2022
Accepted : 25.07.2023
DOI: 10.33988/auvfd.1145264

Keywords
Intravenous
Ketamine
Medetomidine
Red-eared slider

✉Corresponding author
ismar.lutvikadic@vfs.unsa.ba

How to cite this article: Lutvikadic I, Maksimovic A (2024): A comparison of anesthesia induction by two different administration routes and doses of ketamine and medetomidine in red-eared sliders (*Trachemys scripta elegans*). Ankara Univ Vet Fak Derg, 71 (2), 231-237. DOI: 10.33988/auvfd.1145264.

ABSTRACT

This study aimed to assess the efficiency of ketamine and medetomidine by two different doses and routes on anesthesia depth and cardiac stability in red-eared sliders. Each turtle was anesthetized two times, with seven days wash-out period. Induction of anesthesia consisted of a bolus combination of ketamine (10 mg/kg) and medetomidine (0.2 mg/kg) administered in the left brachial biceps in the intramuscular protocol, or a bolus combination of ketamine (20 mg/kg) and medetomidine (0.2 mg/kg) administered in subcarapacial sinus after clear blood presence confirmation in the intravenous protocol. Vital signs, reaction on the skin palpation, manual mouth opening for endotracheal intubation, palpebral and cloacal reflex, and the withdrawal reflex of the front and hind limbs were measured and recorded every 5 minutes for 60 minutes after anesthesia injection. Atipamezole (1 mg/kg) was administered in the right brachial biceps one hour after ketamine and medetomidine administration. Needle insertion and possible painful reactions to drug administration were also evaluated and recorded. Obtained data were analyzed for normality and paired t-tests, Wilcoxon, or McNamar tests were performed where appropriate. The values of $P \leq 0.05$ were considered significant. A significantly less pronounced decrease in heart rate was observed with intravenous anesthesia protocol. Both protocols recorded complete anesthesia recovery 60 minutes after intramuscular atipamezole administration. A ketamine-medetomidine dose combination administered intravenously provides a more stable and consistent anesthetic plane in red-eared sliders than ketamine-medetomidine administered intramuscularly.

Introduction

The growing popularity of turtles as pets has led to increased demand for veterinary care for these animals. Sedation and/or anesthesia are often required for clinical examination, diagnostic procedures, and surgery. The evaluation of anesthetic protocols in turtles is scarce and anesthetic regimens are primarily based on those commonly used in mammals. General anesthesia in chelonians can be challenging due to their unique anatomical and physiological characteristics. Most previous studies have focused on anesthesia-related mortality in chelonians, whereas only a few have investigated the physiological effects of anesthetics (6). Therefore, ketamine has been used at various doses, combinations, and routes of administration (3, 5, 17). However, the results of the studies are variable.

Ketamine (5-10 mg/kg) in combination with medetomidine (0.1-0.2 mg/kg) administered intramuscularly or intravenously can lead to superficial anesthesia (26). Greer et al. (11) demonstrated that intramuscular administration of a lower dose of ketamine (5 mg/kg) in combination with medetomidine (0.1 mg/kg) induces anesthesia deep enough for endotracheal intubation of all turtles. Moreover, the same study results demonstrated that the higher dose (10 mg/kg ketamine and 0.2 mg/kg of medetomidine) could produce sufficient anesthesia even

for surgical incisions and suturing of the skin. It was suggested previously that intravenous ketamine at a dose of 5-10 mg/kg, either alone or in combination with other anesthetics, can provide endotracheal intubation of turtles (21, 27). However, this method of administration of ketamine has not been thoroughly evaluated in chelonians (25). Dennis and Heard (6) reported that the intravenous combination of ketamine (5 mg/kg) and medetomidine (0.1 mg/kg) could provide short-term surgical immobilization in gopher tortoises (*Gopherus polyphemus*). Information on the cardiopulmonary effects of these drugs in reptiles is limited (6). Due to the aforementioned limited data, propofol has been favorable in reptile anesthesia because of the fast induction and fast recovery (7) but in some countries, propofol is almost unreachable. Dosenovic et al. (7) concluded that ketamine and medetomidine combination is more suitable than propofol in red-eared sliders' anesthesia regarding the degree of oxidative stress.

This study aimed to assess the efficiency of ketamine and medetomidine by two different doses and routes of their administration (intravenously and intramuscularly) on anesthesia depth and cardiac stability in red-eared sliders.

Materials and Methods

The study was conducted at the Veterinary Faculty – University of Sarajevo, following approval by the Institutional Ethics Committee under approval number 01-02-153-2/21.

Animals: Twenty clinically healthy red-eared sliders (*Trachemys scripta elegans*), with equally distributed sex, were included in the study. Observation of all animals started 10 days before the anesthesia protocol and finished 10 days after the last protocol testing. They were kept indoors in a turtle terrarium with a water temperature of 25.5 °C. The daytime air temperature was between 26.0 and 28.0 °C with a basking spot heating up to 35.0 °C over the land area, and a UVB light source was provided for 12 hours each day. Dark photoperiod with lower temperatures (18.0 – 21.0 °C) was allowed. Red-eared sliders were fed with commercial food (Tetra®, Germany) and water was regularly cleaned using terrarium filters (Aqua-Tech 30-60 Aquarium Power Filter, USA).

Pilot study: Given the lack of existing literature on the intravenous ketamine and medetomidine combinations in red-eared sliders, it was deemed necessary to conduct a pilot study. A pilot study was conducted to assess the safety of the intravenous protocol and provide estimates for sample size calculation. Three healthy red-eared sliders underwent two different anesthesia protocols (intramuscular and intravenous protocol), with 7 days wash-out period in between. The intravenous protocol (IVP) was based on intravenous bolus administration of ketamine hydrochloride (*Ketaminol10*, MSD, Netherland) (10 mg/kg) in combination with medetomidine hydrochloride (*Sedastart*, Dechra, UK) (0.2 mg/kg), at doses recommended for intramuscular application (11). The effects of administered drugs were evaluated over 30 minutes. If adequate induction of anesthesia was not achieved, the lower ketamine doses were added incrementally in the subcarapacial sinus until the surgical plane of anesthesia was obtained as described by Bennett (3). The ketamine dose of 20 mg/kg and medetomidine dose of 0.2 mg/kg provided stable and effective induction of anesthesia, allowing endotracheal intubation of all animals, and it was defined as the final protocol dose. To determine the minimum required sample size for the final study, at which the significant difference between the anesthesia protocols' impact on vital stability would be found if such difference did exist, we performed an apriori power analysis using G power software (8). The desired significance level was set at 0.05, the desired effect size was set at medium, and the desired power level was set at 0.08.

Study design and procedures: Based on the physical examination and complete blood cell count results, 20 clinically healthy animals were selected for the study. They underwent two anesthesia protocols in a randomized crossover design, with 7 days wash-out period. Anesthesia procedures were performed in a surgical room with a controlled air temperature of 25.0 °C. In intramuscular protocol (IMP), a combination of ketamine (10 mg/kg) and medetomidine (0.2 mg/kg) was administered in the left brachial biceps following aseptic skin preparation. After the muscle relaxation, all animals were placed on the electrical heat pad to maintain body temperature. Three ECG leads were placed on the carapace at the front left, front right, and left hind quarter level. The body temperature probe was placed into the cloaca after lubrication. The SpO_2 sensor was placed on the foot and a side-stream capnography line was attached to the intravenous cannula and placed intranasally. A room air thermometer probe was placed between the patient's plastron and a heating pad. Blood pressure measurement was not possible. In intravenous protocol, a combination of ketamine (20 mg/kg) and medetomidine (0.2 mg/kg) was administered in the subcarapacial sinus. A newly prepared drug combination was repeated during the blood confirmation procedure in case of lymph contamination. A side-stream capnography line was reattached to the endotracheal tube after intubation. The rest of the procedure was identical to the intramuscular protocol. Adequate muscle relaxation and absence of limb retraction

were defined as time to induction after anesthesia injection.

Heart rate, body temperature, and thoracic impedance pneumography were measured and recorded every 5 minutes over the anesthesia monitor (Mindray iMEC8Vet, China) for 60 minutes following the administration of the anesthesia. Like in mammals, decreasing the heart rate by 30% of the basal values was considered as bradycardia (14). A masticatory muscle relaxation, palpebral and cloacal reflex, skin touch reaction, and withdrawal reflex using mosquito forceps on the front and hind limb were also evaluated and recorded every 5 minutes by an investigator blinded to the treatment. Relaxation and sensitivity were scored as described by Alves-Júnior et al. (1). Basal heart rate for each animal was obtained by an allometric scaling system ($bpm=33.4 \times (BWkg)^{-0.25}$) before induction (34). Relaxation of the masticatory muscles was an indication for endotracheal intubation which was performed using intravenous cannulas (Mediplus Limited, India), and the presence or the absence of the gag reflex during the procedure was recorded. Endotracheal intubation was attempted each time the masticatory muscles were relaxed.

An hour after anesthesia injection, atipamezole hydrochloride (*Sedastop*, Dechra, UK), a dose 5 times greater than medetomidine (26), was administered in the right brachial biceps and the presence of painful reaction on the needle insertion and drug application was observed. During the recovery phase, monitoring the physiological parameters was continued until the patient's voluntary locomotion was observed.

Statistical analysis: Experimental data were analyzed using PAST statistical analysis software (12). Recorded data were averaged across 13 measurements for each of the 20 turtles. Averaged data were tested for normality using the Shapiro-Wilk test. All data except the basal heart rate were found to be normally distributed. Box-plot inspection and IQR method were used to inspect for the presence of any outliers. Two outliers were identified in the IMP and IVP heart rate measurements, respectively, and thus excluded from the data analysis. Paired t-tests were conducted to assess differences in means of basal heart rate and heart rates obtained during monitoring in both groups. A mean percentage of heart rate reduction difference between intramuscular and intravenous anesthesia was also conducted. In light of the above, the t-test was chosen on the grounds of its sophistication in discerning between which conditions precisely the difference exists and how substantial is it.

Given that basal heart rate deviated from a normal distribution, to ensure results were not confounded by violation of parametric assumptions of the t-test, a non-parametric Wilcoxon test was also performed. In addition, log transformation was applied and transformed data were analyzed. There was no difference in the significance of results obtained using a non-parametric test, nor in comparison with those obtained on the transformed data. Therefore we will report the results obtained with a paired t-test, as the appropriate analysis method was determined. The mean percentage of heart rate reduction in intramuscular and intravenous anesthesia was also assessed. The difference in breathing frequency was tested using the McNamara test. Values of $P \leq 0.05$ were considered significant.

Results

Twenty clinically healthy red-eared sliders (*Trachemys scripta elegans*) had a mean age of 5 (SD=4) years and a mean body weight of 0.43 (SD=0.29) kg. All animals' complete blood cell count was within referent values given by Heatley and Russell (15). The mean time of muscle relaxation in IMP was 9 (SD=2.6) minutes and the mean time of possible manipulation with the animal was 11 (SD=2.9) minutes. In IVP, the mean time of muscle relaxation and animal manipulation was 12 (SD=3.9) minutes.

A significant difference was observed between basal heart rate (M=46.4, SD=3.0) and heart rate during IVP (M=34.7, SD=1.2), (t (19)=4.302, P=0.0004). Similarly, a significant difference was observed between basal heart rate (M=46.4, SD=3.0) and heart rate during IMP (M=30.6, SD=1.0), (t (19)=4.775, P=0.0001). A significant difference was also observed in a direct comparison of the heart rate values recorded during IMP and IVP (t (19)=-3.155, P=0.005). In contrast, an IVP pulse is significantly higher (M=34.7, SD=1.2) than in IMP (M=30.6, SD=1.0) (Figure 1).

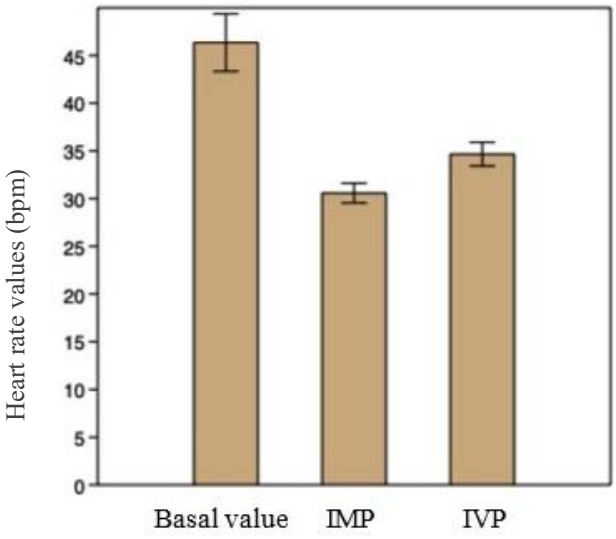

Figure 1. Difference between heart rate basal values and heart rate values in IMP and IVP groups.

A significant difference was observed in predetermined bradycardia values (decrease of the heart rate for 30% of basal values) between IMP and IVP (t (19)=-3.227, P=0.004). An average decrease of the heart rate for 31% (M=31.2; SD=3.8) of basal values was recorded during IMP, while during IVP was for 23% (M=23.4; SD=3.3) of preanesthetic values (Figure 2). In IMP, 95% of animals showed a decrease of the heart rate below 30% with a mean time of 29 (SD=19.8) minutes and only 55% of animals in IVP showed a heart rate decreasing below 30% with a mean time of 29 (SD=21.6) minutes.

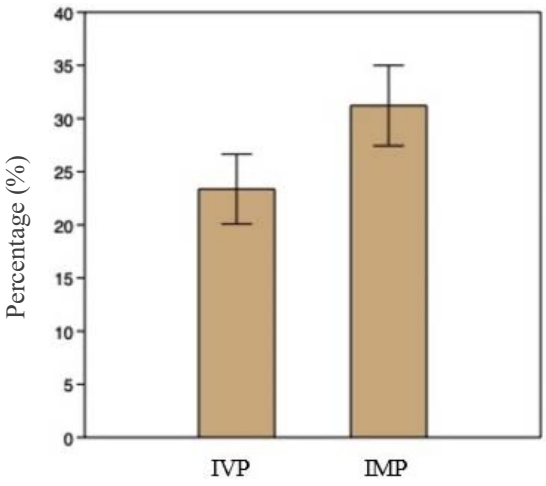

Figure 2. Heart rate reduction in IVP and IMP group during anesthesia expressed as a percentage.

No significant difference (P=0.451) was observed in respiratory rate between groups and arrhythmic breathing pattern was present in all animals. Pulse oximetry did not provide any saturation reading but indirect heart rate measurement was correlated with ECG values. An $EtCO_2$ by side-stream capnography line could not be obtained intranasally nor after endotracheal intubation, even after a probe by manual ventilation. Body temperature in all animals was successfully maintained between 24.0 and 26.0 °C.

In IMP, one animal achieved a surgical plane of anesthesia and endotracheal intubation was possible after 46 minutes. All animals in IVP reached the surgical plane of anesthesia and endotracheal intubation with a mean time of 47 (SD=15) minutes.

Reaction on the skin touch and withdrawal reflex was observed in all animals but the degree of response was pronounced in IMP. Spontaneous locomotion was not observed in IVP protocol during the anesthesia while it was recorded in 15% of animals in IMP, and it was recorded 30 (SD=2) and 55 (SD=3) minutes after ketamine and medetomidine administration.

Reaction on the needle insertion for atipamezole administration was increased in IMP. The mean time for spontaneous head movement was 6 (SD=2.9) minutes in both groups after atipamezole administration. The mean time for voluntary locomotion was 8 (SD=4.4) minutes in IMP and 9 (SD=4.2) minutes in IVP. Vomiting was observed only in the same turtle for both treatments 125 minutes after atipamezole administration in IMP and 30 minutes in the IVP protocol. An hour after atipamezole administration, all animals completely regained dorsal reflex which was a criterion for sufficient recovery to be returned to their enclosure. The difference in the recovery phase was not noticed between genders.

Discussion and Conclusion

Ketamine hydrochloride is a common anesthetic drug used in reptiles with a wide safety margin (22). It can be administered in various routes and its anesthetic doses, as a sole agent, usually cause prolonged recovery time in reptiles (17, 22, 35, 42). Medetomidine has a high affinity toward α2 adrenergic receptors with a sedative and analgesic effect. It is commonly used for anesthesia induction in combination with anesthetic agents (22, 29). In chelonians, medetomidine alone usually causes severe cardiopulmonary depression with heart rate and respiratory rate reduction and hypotension (36). According to some studies, these drugs in combination can provide effective intramuscular anesthesia in red-eared sliders (11, 38). Bouts and Gasthuys (4), suggest that medetomidine (0.1-0.3 mg/kg) in combination with ketamine (10 mg/kg) administered intramuscularly in most reptiles may induce general anesthesia. Contrary to the decreasing effect of the sympathetic tone by α2-adrenoreceptor activation with medetomidine, dissociative anesthetics increase sympathetic activity resulting in cardiac output, blood pressure, and heart rate increases (39, 43). Ketamine shouldn't significantly affect ventilatory drive due to hypercapnia in mammals (16, 37). Furthermore, Dennis and Heard (6) suggest that ketamine in combination with medetomidine cause hypoxemia that is not severe enough to trigger a respiratory response in chelonians.

Propofol is the anesthetic of choice in reptile anesthesia, but a recent study suggests that propofol has a more significant oxidative stress effect on red-eared sliders than the ketamine-medetomidine combination (7). In their combination, a counterbalance is enabled (40). The quality of anesthesia induction by intravenous administration of ketamine has not been tested in chelonians (25). Dennis and Heard (6) investigated the hemodynamic stability of the gopher tortoises after intravenous bolus administration of ketamine and medetomidine. In the same study, the quality of anesthesia was not evaluated. In our research, intramuscular administration provided satisfactory muscle relaxation and enabled endotracheal intubation only in 5% of animals. Furthermore, 15% of animals showed

DOI: 10.33988/auvfd.1145264

spontaneous locomotion during anesthesia monitoring in the same protocol, and all had significant heart rate reduction. On the contrary, intravenous administration in all animals allowed endotracheal intubation, and heart rate reduction was much closer to basal values. These differences among protocols could be due to increased sympathetic activity caused by the higher dose and administration route of ketamine in the intravenous protocol. The same animal was used in both protocols, so individual differences were prevented. Furthermore, the carry-over effect was prevented considering the pharmacokinetic properties of these drugs (30, 31).

It is essential to understand that aquatic chelonians can develop anaerobic breathing by tolerating an anoxic environment very well (9, 18). Considering all chelonians' physiological characteristics, compensatory mechanisms, and monitoring parameters (9, 13, 19, 20, 22, 23, 41), none of our animals showed compromise. Furthermore, the inability to detail respiratory evaluation during the investigation was not concerning for patient safety. Besides, in reptiles pulse oximetry, arterial blood gas analysis, and capnography are not validated yet (24). Heart rate and ECG readings were considered essential parameters in our study to assess induced anesthesia quality. In mammals, a heart rate decrease of 20-30% below the low normal is regarded as bradycardia which requires treatment (14). To the best of the authors' knowledge, a decrease in heart rate considered bradycardia in reptiles is not defined. Our study suggests that induction of anesthesia results in a significant drop in heart rate, while heart rate frequency was less affected by intravenous protocol. This signifies that the average degree of reduction in heart rate was significantly lower in IVP, so much anesthesia can be deemed safer. The ECG interpretation is critical during anesthesia as it can be helpful for the assessment of anesthesia depth (2). This is especially useful in the absence of other methods such as Doppler or esophageal stethoscopes. An ECG interpretation is similar to a mammalian, but diagnostic reference parameters data is limited in reptiles (24). It showed valuable methods in our study, especially when other monitoring methods during anesthesia have failed. However, ECG as the only method for anesthesia monitoring in reptiles is not recommended (24).

All animals in our study showed a reaction in different degrees of withdrawal reflex testing induced reaction of a certain degree in all animals regardless of anesthesia protocol and more pronounced on the hind limbs. A similar observation was reported by Greer et al. (11). More pronounced withdrawal reflex of hind limbs could be explained by the constatation that the anesthesia effect in reptiles goes from the cranial to the caudal direction (3, 10). Touch reaction and withdrawal reflex were notably different between the two anesthetic protocols. All animals showed a reaction but it was significantly intense in IMP evaluating the time and intensity of limb retraction. This observation correlates with detected differences in anesthesia depth induced between the two protocols. Furthermore, the palpebral reflex was absent in IVP, and the cloacal reflex was present in both protocols. We used reflexes evaluation in correlation with obtained heart rate values to decide does the patient requires urgent treatment. None of the patients required treatment and all animals recovered well. The presence of painful reaction on the needle insertion and atipamezole application was increased in IMP compared to IVP also indicating that intramuscular protocol causes superficial anesthesia.

Intravenous anesthesia agent application is preferable because of predictability and animal recovery. Unfortunately, intravenous standard methods can cause technical difficulties (33). Using subcarapacial sinus is an effortless technique, but some studies warn of the possibility of accidental intrathecal drug application. In that case, permanent paralysis or even death is expected mainly if high volumes or irritant drugs are used (28, 32). In our study, none of the red-eared sliders showed neurological issues 10 days after treatment.

In conclusion, the intravenously administered bolus mixture of ketamine and medetomidine in investigated doses provided superior induction of the general anesthesia and stability of the red-eared sliders compared to previously described and recommended doses of the same mixture administered intramuscularly. Therefore, intravenous administration of this combination should be the preferred route for inducing surgical plane anesthesia in this animal species.

Acknowledgments

The article is summarized from the first author's Ph.D. thesis. The authors are grateful to Majda Beslija, MSc Organizational, and Social Psychology for statistical analysis.

Financial Support

This research received no grant from any funding agency/sector.

Conflict of Interest

The authors declare that they have no conflict of interest.

Author Contributions

LI made a substantial contribution to the conception and design of the study and took the lead in writing the manuscript. Also, carried out anesthesia procedures and recordings of vital signs. MA recorded animals' reactions and reflex evaluation; contributed to drafting the article

and critical revision of the manuscript. All authors read and approved the final manuscript.

Ethical Statement

The study was conducted at the Veterinary Faculty – University of Sarajevo, following approval by the Institutional Ethics Committee under approval number 01-02-153-2/21, dated: 12.02.2021.

References

1. **Alves-Júnior JRF, Bosso ACS, Andrade BS, et al** (2012): *Association of midazolam with ketamine in giant Amazon River turtle* Podocnemis expansa *breed in captivity*. Acta Cirúrgica Brasileira, **27**, 144-147.
2. **Bailey JE, Pablo LS** (1998): *Anaesthetic monitoring and monitoring equipment in small exotic pet practice*. Seminars in Avian and Exotic Medicine, **7**, 53-60.
3. **Bennett RA** (1991): *A review of anesthesia and chemical restraint in reptiles*. J Zoo Wildl Med, **22**, 282-303.
4. **Bouts T, Gasthuys F** (2002): *Anesthesia in Reptiles. Part 1: Injection anesthesia*. Vlaams Diergeneeskundig Tijdschrift, **71**, 183-194.
5. **Cermakova E, Ceplecha V, Knotek Z** (2017): *Efficacy of two methods of intranasal administration of anesthetic drugs in red-eared terrapins (Trachemys scripta elegans)*. Veterinarni Medicina, **62**, 87-93.
6. **Dennis PM, Heard DJ** (2002): *Cardiopulmonary effects of a medetomidine-ketamine combination administered intravenously in gopher tortoises*. JAVMA, **220**, 1516-1519.
7. **Došenović M, Radaković M, Vučićević M, et al** (2020): *Evaluation of the effects of two anesthetic protocols on oxidative status and DNA damage in red-eared sliders (Trachemys scripta elegans) undergoing endoscopic colectomy*. Acta Veterinaria Hungarica, **4**, 337-344.
8. **Faul F, Erdfelder E, Lang AG, et al** (2007): *G*power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences*. Behavior Research Methods, **39**, 175-191.
9. **Frische S, Fago A, Altimiras J** (2000): *Respiratory response to short-term hypoxia in the snapping turtle, Chelydra serpentine*. Comp Biochem Phys A, **126**, 223-231.
10. **Frye FL** (1981): Anesthesia. 241-246. In: FL Frye (Ed), Biomedicine and surgical aspects of captive reptile husbandry. Veterinary Medicine Publishing.
11. **Greer LL, Jenne KJ, Diggs HE** (2001): *Medetomidine-Ketamine Anesthesia in Red-Eared Slider Turtles (Trachemys scripta elegans)*. American Association for Laboratory Animal Science, **40**, 9-11.
12. **Hammer Ø, Harper DAT, Ryan PD** (2001): *PAST: Paleontological statistics software package for education and data analysis*. Palaeontologia Electronica, **4**, 9.
13. **Harcourt-Brown F** (2002): Anaesthesia and analgesia. 121-139. In: F Harcourt-Brown (Ed), Textbook of Rabbit Medicine. Oxford.
14. **Haskins SC** (2015): Monitoring Anesthetized Patients. 86-113. In: KA Grimm, LA Lamont, WJ Tranquilli, et al (Eds), Veterinary Anesthesia and Analgesia: the fifth edition of Lumb and Jones. John Wiley and Sons, Inc.
15. **Heatley J, Russell KE** (2019): Hematology. 301-318. In: SJ Divers, SJ Stahl (Eds), Mader's Reptile and Amphibian Medicine and Surgery, 3rd edition. Elsevier.
16. **Hirshman CA, McCullough RE, Cohen PJ, et al** (1975): *Hypoxic ventilatory drive in dogs during thiopental, ketamine, or pentobarbital anesthesia*. Anesthesiology, **43**, 628-34.
17. **Holz P, Holz RM** (1994): *Evaluation of ketamine, ketamine/xylazine and ketamine/midazolam anesthesia in red-eared sliders (Trachemys scripta elegans)*. J Zoo Wildl Med, **25**, 531-537.
18. **Jackson DC** (2000): *Living without oxygen: lessons from the fresh-water turtle*. Comp Biochem Phys A, **125**, 299-315.
19. **Jackson DC, Crocker CE, Ultsch GR** (2001): *Mechanisms of homeostasis during long-term diving and anoxia in turtles*. Zoology, **103**, 150-156.
20. **Knotek Z, Divers SJ** (2019): Pulmonology. 786-804. In: SJ Divers, SJ Stahl (Eds), Mader's Reptile and Amphibian Medicine and Surgery, 3rd edition. Elsevier.
21. **Lock BA, Heard DJ, Dennis P** (1998): *Preliminary evaluation of medetomidine/ketamine combinations for immobilization and reversal with atipamezole in three tortoise species*. Bulletin of the Association of Reptilian and Amphibian Veterinarians, **8**, 6-9.
22. **Longley L** (2008): Reptile anesthesia. 185-210. In: Anaesthesia of exotic pets. Saunders.
23. **Maginnis LA, Ekelund SA, Ultsch GR** (2004): *Blood Oxygen Transport in Common Map Turtles during Simulated Hibernation*. Physiological and Biochemical Zoology, **77**, 232-241.
24. **Mans C, Sladky KK, Schumacher J** (2019): General Anesthesia. 447-464. In: SJ Divers, SJ Stahl (Eds), Mader's Reptile and Amphibian Medicine and Surgery, 3rd edition. Elsevier.
25. **McArthur S** (2004): Anaesthesia, analgesia and Euthanasia. 379-401. In: S McArthur, R Wilkinson, J Meyer (Eds), Medicine and Surgery of Tortoises and Turtles. Blackwell Publishing.
26. **Meredith A** (2015): Appendix II: Protocols. 315-322. In: BSAVA Small Animal Formulary, 9th ed.
27. **Norton TM** (2005): *Chelonian Emergency and Critical Care*. Topics in Medicine and Surgery. Seminars in Avian and Exotic Pet Medicine, **14**, 106-130.
28. **Perry SM, Mitchell MA** (2019): Routes of Administration. 1130-1138. In: SJ Divers, SJ Stahl (Eds), Mader's Reptile and Amphibian Medicine and Surgery, 3rd edition. Elsevier.
29. **Plumb DC** (2011): Medetomidine HCL. 3823-3834. In: Plumb's Veterinary Drug Handbook, 7th ed. PharmaVet, Inc.
30. **Posner LP, Burns P** (2009a): Injectable anesthetic agents. 729-834. In: JE Riviere, MG Papich (Eds), Veterinary Pharmacology, 9th edition. Wiley-Blackwell.
31. **Posner LP, Burns P** (2009b): Sedative agents: Tranquilizers, alpha-2 agonists, and related agents. 933-1067. In: JE Riviere, MG Papich (Eds), Veterinary Pharmacology, 9th edition. Wiley-Blackwell.
32. **Quesada RJ, Aitken-Palmer C, Conley K, et al** (2010): *Accidental submeningeal injection of propofol in gopher tortoises (Gopherus polyphemus)*. The Veterinary Record, **167**, 494.

33. **Schenllbacher RW, Shepard M** (2019): Sedation. 441-446. In: SJ Divers, SJ Stahl (Eds), Mader's Reptile and Amphibian Medicine and Surgery, 3rd edition. Elsevier.
34. **Schilliger L, Girling S** (2019): Cardiology. 669-698. In: SJ Divers, SJ Stahl (Eds), Mader's Reptile and Amphibian Medicine and Surgery, 3rd edition. Elsevier.
35. **Schumacher J, Yelen T** (2006): Anesthesia and analgesia. 442-452. In: DR Mader (Ed), Reptile Medicine and Surgery, 2nd ed. Saunders Elsevier, Missouri.
36. **Sleeman JM, Gaynor J** (2000): *Sedative and cardiopulmonary effects of medetomidine and reversal with atipamezole in desert tortoises (Gopherus agassizii)*. J Zoo Wildl Med, **31**, 28-35.
37. **Soliman MG, Brindle GF, Kuster G** (1975): *Response to hypercapnia under ketamine anesthesia*. Canad Anaesth Soc J, **22**, 486-494.
38. **Tatli ZB, Sen ZB, Gulaydin A** (2016): *Aural abscess in a red-eared slider turtle (Trachemys scripta elegans).* Harran Üniversitesti Veteriner Fakültesi Dergisi, **5**, 170-172.
39. **Thurmon JC, Tranquilli WJ, Benson GJ** (1996): Preanesthetics and anesthetics adjuvants. 183-209. In: JC Thurmon, WJ Tranquilli, GJ Benson (Eds), Lumb and Jones' veterinary anesthesia, 3rd ed. Baltimore.
40. **Verstegen J, Fargetton X, Donnay I, et al** (1991): *An evaluation of medetomidine/ketamine and other drug combinations for anesthesia in cats*. Vet Rec, **128**, 32-35.
41. **Vigani A** (2015): Cardiac Output Measurement. 473-482. In: KA Grimm, LA Lamont, WJ Tranquilli, et al (Eds), Veterinary Anesthesia and Analgesia: the fifth edition of Lumb and Jones. John Wiley and Sons, Inc.
42. **White PF, Way WL, Trevor AJ** (1982): *Ketamine – its pharmacology and therapeutic uses.* Anesthesiology, **56**, 119-136.
43. **Wright M** (1982): *Pharmacologic effects of ketamine and its use in veterinary medicine.* J Am Vet Med Assoc, **180**, 1462-1471.