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Electrocardiographic parameters of *Sapajus libidinosus* (SPIX, 1823) after chemical immobilization with tiletamine-zolazepam

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Abstract

Background: Tiletamine-zolazepam is a widely used as an alternative chemical immobilization method in non-human primates, with a safe application pathway and necessary relaxation. In order to determine the electrocardiographic parameters of *Sapajus libidinosus* after intramuscular tiletamine-zolazepam administration at the dose of 5 mg/kg, nine animals were submitted to anesthesia.

Methods: The interpretation of the electrocardiogram determined: heart rate in bpm and heart rhythm, P wave, P-R interval, R wave, QRS complex, T wave, Q-T interval, corrected QT interval and electrical axis. The mean HR was 206 ± 32 bpm.

Results: The majority of the monkeys showed normal sinus rhythm, but one animal showed sinus tachycardia. The most commonly observed electrical axis was between +30° and +90°. Two animals presented bigger alterations in the tracing such as low amplitude QRS and T wave bigger than 1/4 of the R wave.

Conclusions: The administration of tiletamine-zolazepam was safe and efficacious, with minimal cardiovascular effects.

KEYWORDS

anesthesia, electrocardiogram, primate

1 | INTRODUCTION

Black-striped capuchin monkeys (*Sapajus libidinosus*) are diurnal omnivores found on the American continent and have the widest geographic distribution of the neotropical primates.[1] Constant deforestation means that these animals migrate to urban centers, becoming targets of predatory hunting and leading to a significant number of specimens in captivity.

So that the veterinary physician can intervene efficaciously in the health of these animals, in addition to clinical knowledge, the choice of the chemical immobilization method of the animal, anesthesia and the anesthetic administration route is fundamental to the success of the procedures.[2]

The association of tiletamine-zolazepam (TZ) has been widely used in wild and exotic animals because it requires a small injection volume, is well absorbed intramuscularly, and has a wide safety margin and permits immobilization to anesthesia with minimum cardiopulmonary effect.[3,4] For most species, 2-5 mg/kg are sufficient to promote anesthesia for minimal procedures, but smaller species require a bigger dose.[2] However, the use of this association is still controversial in some species due to a lack of studies.

Monitoring of the cardiorespiratory function is fundamental as a base for anesthetic procedures, because the mortality index of small wild mammals is larger during anesthesia or sedation compared to domestic animals.[5]

In the face of this, the electrocardiogram (ECG) is a simple, essential examination to determine heart rate and rhythms that are important data during the course of anesthesia and it is the only way of establishing the diagnosis of arrhythmia that may occur as a result of anesthesia or surgical manipulations, thus providing therapeutic direction.[6]

For the genus, studies on ECG are restricted to evaluations of comparisons of anesthetic protocols, such as ketamine and midazolam + propofol,[7-9] tiletamine-zolazepam and ketamine-xylazine in *Sapajus apella*,[10] but descriptive studies determining the electrocardiographic standard in *S libidinosus* were not found.

With these considerations and due to the scarcity of literature on the subject, the objective of the present study was to determine the electrocardiographic standard for the black-striped capuchin (*S libidinosus*) after chemical immobilization with tiletamine-zolazepam.

2 | METHODS

2.1 | Humane care guidelines

All the procedures were carried out after approval of the experimental protocol by the Ministry of the Environment, by the Biodiversity Authorisation and Information System—SISBIO (no 40334-2) of the Chico Mendes Institute—ICMBio and by the Committee of Ethics in the Use of Animals (CEUA) of the Rural Health and Technology Center (CSTR) at the Federal University of Campina Grande (UFCG), PB, Brazil, protocol number 116-2013.

2.2 | Experimental design

Nine adult male black-striped capuchin (*S libidinosus*) specimens weighing 2.95 ± 0.61 kg were selected from the Wild Animal Screening Center (CETAS/IBAMA), located in the municipality of Natal/RN, Brazil. The animals, coming from clandestine captivity or illegal trafficking and already long-term residents of the facility, were kept in 9.5 m^2 enclosures and received a diet consisting of varied fruits and vegetables offered daily and extruded feed and protein source (boiled egg) offered two or three times a week. The monkeys were subjected to water and food fasting 12 hours before the anesthetic procedure.

2.3 | Immobilizing the animals

Thirty minutes before starting the procedures, all the animals intended for the experiment were netted and placed in squeeze cages to minimize the influence of capture stress on the study. The animals were sedated individually using tiletamine-zolazepam association (TZ) 10% at an estimated dose of 5 mg/kg, administered intramuscularly.[11] After the anesthesia, the animals were weighed on a hand hook scales and the body length, tail length, and thorax diameter were measured using a tape measure.

2.4 | Determining the parameters and performing the electrocardiogram

Five minutes after applying the drug, before performing the ECG exam, the physiologic parameters were checked a single time: heart rate (HR), peripheral oxygen saturation (SpO_2), systolic arterial pressure (PAS), diastolic arterial pressure (PAD), and mean arterial pressure (PAM)—by means of cuff no. 4, located in the femoral region— all using a multiparametric monitor (Model DL 1000. Deltalife, Brazil); respiratory rate (RR), assessed by direct inspection of the respiratory movements; and body temperature (TC), using a digital clinical thermometer inserted in the rectum. The ECG was carried out immediately after registering the parameters and lasted approximately three minutes, with a single recording.

To perform the ECG examination, the animals were placed in dorsal decubitus, on a rubber insulating surface to prevent interferences. A veterinary electrocardiograph was used (Eletrocardiógrafo TEB ECGPC, Brazil), and the cables were placed in the following manner: the red and yellow cables were placed on the right and left thoracic limbs, respectively, and the black and green cables were placed on the right and left pelvic limbs, respectively. The electrodes were fixed above the cubital joint (humerus-radial-ulna) and the knee joint (femur-tibia-patella) by clip-type metal connectors. The thoracic electrodes were placed as proposed for humans[12] in the following manner: V1, located at the fourth intercostal space, immediately to the right of the sternum; V2 located at the fourth intercostal space, immediately to the left of the sternum; V3 placed between V2 and V4; and V4 placed at the fourth intercostal space, on the left of the midclavicular line (Figure 1). The skin was moistened with alcohol prior to placing the electrodes.

The ECG was recorded at speed of 25 mm/s, sensitivity N (1 cm = 1 mV) in the DI, DII, DIII, aVR, aVL, and aVF derivations. The electrocardiographic tracing was then interpreted in the DII derivation, determining heart rate (HR) in bpm and heart rhythm, P wave (ms/mV), P-R interval (ms), R wave (mV), QRS complex (ms), T wave (mV), Q-T interval (ms), corrected QT interval (ms), and electrical axis (°) following the axis table (DI/DIII). The corrected QT interval was calculated using the formula[13]:

$$QTc = \frac{QT}{\sqrt{R-R}}$$

The following were also assessed: the latency period, as being the time elapsed from the end of the anesthetic injection until the animal fell (decubitus position) and the period of useful anesthesia, assessed as the time elapsed from the latency period to the start of limb movement.

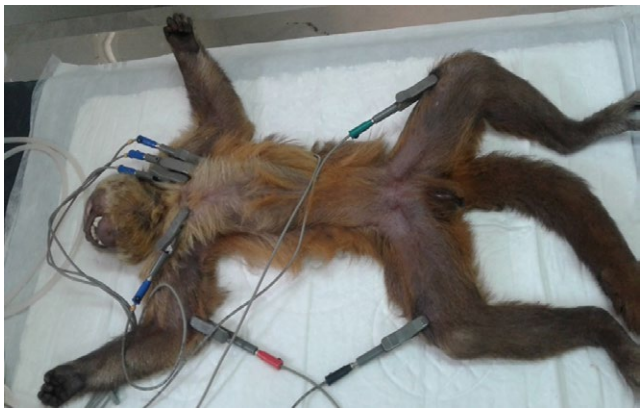

FIGURE 1 Disposition of the electrodes in black-striped capuchin monkeys (*Sapajus libidinosus*) for achievement electrocardiographic exam

2.5 | Statistical analysis

The data were analyzed by descriptive statistics, using the Microsoft Excel 2013 computer program, and are presented as mean and standard deviation.

3 | RESULTS

The animals showed no signs of malnutrition or obesity, and weighed 2.1-3.8 kg (mean 2.95 ± 0.61 kg), with 38-44 cm body length (mean 41.33 ± 2.24 cm) and the tail length, 43-47 cm (mean 44.39 ± 1.36 cm), was longer than the body length, which is in line with the literature.[14] The thorax circumference varied from 24 to 33 cm (mean 27.78 ± 3.15 cm).

The physiologic parameters assessed prior to the ECG are shown in Table 1. The data were expressed in individual values, mean, and standard deviation.

The individual electrocardiographic parameters of the nine animals are shown in Table 2.

The HR during the ECG ranged from 150 to 258 beats per minute (bpm), showing sinus rhythm (Figure 2) in eight animals (88.8%) and sinus tachycardia in one animal (11.1%).

The P wave was increased in time in animals 1, 5, and 6 (33.3%) and in amplitude in one animal (11.1%), when compared to the others.

The PR and QT intervals were inversely proportional to the heart rate.

The QRS complexes exhibited low amplitude in two animals, with positive R wave in the DI, DII, and aVF derivations in nine animals (100%) that was also positive in aVL in six animals (66.6%). In the aVR derivation, the R wave was negative in all the tracings. Two animals (22.2%) showed negative R wave in the DIII derivation.

The T wave showed rounded, slightly symmetric morphology, and was smaller than 1/4 R wave in seven tracings and bigger than 1/4 R wave in two tracings. T wave polarity was positive for all the animals.

The electrical axis varied among the individuals, and in three animals it showed deviation to the left. Two of the total of nine animals presented axis between −30 and 0°, four were between +30° and +60°, and three were between +60° and +90°.

The tiletamine-zolazepam latency period in the present study was 1.8 ± 0.6 minutes and the useful anesthetic period was 33 ± 10 minutes.

The following figure demonstrates an electrocardiographic tracing recorded in the augmented bipolar and unipolar derivations, N sensitivity and speed 25 mm/s in an animal with normal sinus rhythm, 197 bpm heart rate, 0.24 mV × 43 ms P wave, 68 ms PR interval, 42 ms QRS complex, 182 ms QT interval and positive T wave smaller than 1/4 R wave.

4 | DISCUSSION

The data obtained in the present study demonstrated animals with relatively larger weight and size than those described in the literature

TABLE 1 Assessment of the physiologic parameters of the nine black-striped capuchin monkeys (*Sapajus libidinosus*) sedated with tiletamine-zolazepam (5 mg/kg/IM), verified immediately before the ECG

Animals	RR (mpm)	HR (bpm)	RT (°C)	SAP (mm Hg)	DAP (mm Hg)	MAP (mm Hg)	SpO_2 (%)
01	112	227	38,9	180	80	110	97
02	96	184	38	120	40	67	99
03	100	162	37.9	160	50	97	99
04	48	156	37.5	150	40	99	99
05	100	177	38.2	123	114	119	98
06	52	150	36.3	143	83	109	98
07	48	197	37.7	109	51	89	99
08	48	217	37.6	186	176	179	98
09	80	230	37.6	164	150	157	99
$\bar{X}$	76	189	37.7	148	87	114	98
s (±)	27	31	0.7	27	50	34	1

bpm, beats per minute; DAP, diastolic arterial pressure; MAP, mean arterial pressure; mpm, movements per minute; mm Hg, millimeters of mercury; HR, heart rate; RR, respiratory rate; RT, rectal temperature; SAP, systolic arterial pressure; SpO_2, peripheral oxygen saturation.

TABLE 2 Variables of the individual ECG of the nine black-striped capuchin monkeys (*Sapajus libidinosus*), sedated with tiletamina-zolazepam (5 mg/kg/IM). Parameters recorded in the DII derivation, 25 mm/s, speed and N mode

Animals	FC (bpm)	P (ms)	P (mV)	P-R (ms)	QRS (ms)	R (mV)	T (mV)	T (pol)	Q-T (ms)	QTc (ms)	Axis DI/DIII(°)
01	225	53	0.19	90	47	0.84	0.18	+	143	276.9	83
02	197	43	0.24	68	42	0.59	0.09	+	182	329.8	79
03	208	47	0.20	87	43	0.73	0.13	+	170	316.5	55
04	174	43	0.33	75	38	0.86	0.07	+	187	318.4	73
05	200	57	0.20	92	47	0.27	0.28	+	170	310.4	0
06	150	57	0.13	102	33	0.18	0.09	+	193	304.1	−11
07	219	37	0.13	83	40	0.76	0.12	+	147	280.8	30
08	227	40	0.19	78	38	0.47	0.07	+	142	276.2	49
09	258	40	0.18	60	33	0.66	0.09	+	140	290.3	55
$\bar{X}$	206	46	0.20	82	40	0.60	0.12		164	300.4	46
s (±)	32	7.6	0.06	13	5.2	0.24	0.07		21	20	34

bpm, beats per minute; HR, heart rate; mV, millivolts; ms, milliseconds; pol, polarity; P, P wave; P-R, PR interval; QRS, QRS complex; R, R wave; T, T wave; Q-T, QT interval; $\bar{X}$, (mean); σ (±), standard deviation.

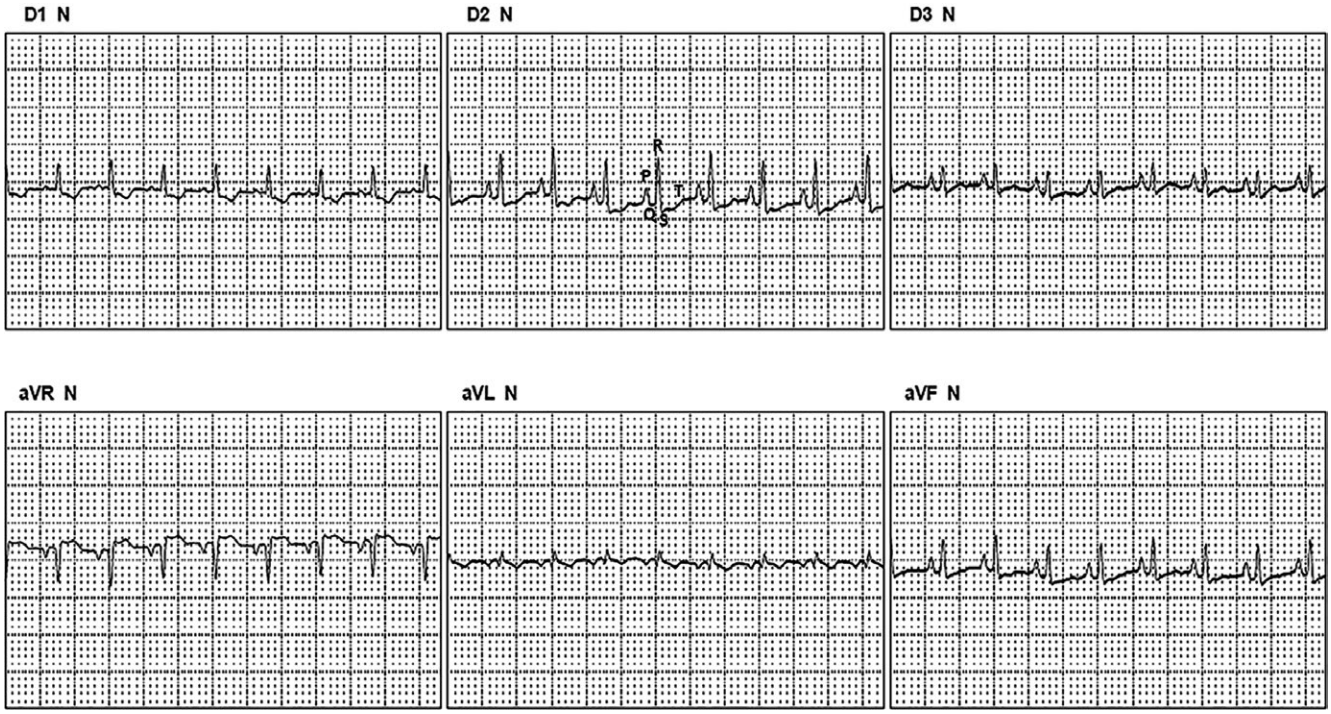

FIGURE 2 Electrocardiographic trace in leads DI, DII, DIII, aVR, aVL e aVF, sensitivity N and velocity 25 mm/s of *Sapajus libidinosus*. P wave (ms/mV), P-R interval (ms), R wave (mV), QRS complex (ms), T wave (mV), Q-T interval (ms), corrected QT interval (ms) and electrical axis (°)

for captive animals of the species *S libidinosus*.[15] However, based on the weight: length ratio, the animals showed good nutritional state since, in spite of increased weight compared to the standard for the species, the morphometric variables were also bigger, that indicated that these animals did not present factors of obesity or malnutrition that might influence their electrocardiographic parameters, bearing in mind that, according to the literature, obesity, characterized by excess adipose tissue, can causes subepicardial fat deposits and small degrees of fat infiltration in the mycardium, affecting mainly the right ventricle.[16]

The variables body temperature, respiratory rate, and arterial pressure[17] did not show large alterations, according to the literature and in relation to research using other pharmacological classes.[10,18,19] Thus, tiletamine-zolazepam was shown to be safe when administered at the dose studied and that small alterations are very often responses to the individual temperament

of the animal, in addition to the stress caused by capture and immobilization.

Several electrocardiographic studies have been carried out in various non-human primate species[7,10,20-26] but nevertheless there are no electrocardiographic studies on *S libidinosus*.

The appearance of high amplitude P waves is not an uncommon fact in non-human primates[23] and was reported in three animals of the present study (33.3%), in 40% rhesus monkeys,[26] 37% Japanese monkeys,[25] and commonly in cynomolgus monkeys.[27] The P wave represents atrium activation and is generated by the start of the electric impulse in the sinoatrial node, followed by its rapid transmission through the atrium.[28] Measurements of time and amplitude are indicative of left atrial overload (duration in ms) and right atrium (voltage in mV),[15,22] but for the correct assertion that overloads would be necessary to perform chest X-rays and/or echocardiogram, taking into account in view of the fact that tiletamine-zolazepam, due to sympathomimetic action, would determine a reduction in the time of atrial depolarization by intensifying the electrical conductance in the cardiac tissue and not causing a delay. In this case, these animals may be carriers of some cardiomyopathy or simply these values may be considered as borderline for the species.

The mean value found in this study (82 ms in *S libidinosus*) for the PR interval was very close to that described in other research on primates, that ranges from 70 ms for rhesus monkeys,[26] to 80 ms for cynomolgus monkeys,[20] *Macaca arctoides*[24] and Japanese monkeys,[25] with a bigger variation for interval duration of 120-200 ms in humans,[29] and 56 ms in the black-tufted marmoset.[22] The time that the electrical impulse takes to cross the atria and the atrioventricular node is represented by this interval, that is measured from the start of the P wave to the first deflection of the QR S complex.[28]

The mean QT interval was 164 ms in the present study. Variation has been reported for other primates of 200 ms in cynomolgus monkeys[20] and Japanese monkeys,[25] 270 ms in *M arctoides*,[24] 130 ms in black-tufted marmosets,[22] and 170 ms in *S apella*,[7] that belong to the same genus under study. The period between the start of ventricular depolarization and the end of repolarization is represented by this interval.[28] Research on *S apella* showed that the association of xylazine and ketamine had as response severe bradycardia that consequently prolonged the QT interval compared to the protocol that did not include α2-agonist agents.[10]

In the ventricular repolarization phrase, a QT interval was observed that decreased as the in heart rate increased, corroborating with the literature, that states that one of the main influences on the PR and QT intervals is the inverse relationship with heart rate and that these intervals decrease with increase in the HR and increase with its decrease. This effect occurs due to sympathetic stimulation.[30] Thus, the QTc was shown to be a better form of assessing the QT interval due to its variation with this parameter.

Some animals in the present study showed QRS complex with morphology similar to that found in humans and other non-human primates.[10,20,25] Two animals exhibited low amplitude QRS complex and T wave bigger than 1/4 R wave. The QRS wave complex represents ventricle activation, generated by ventricular depolarization, that is immediately followed by the T wave, caused by the ventricular repolarization.[28] Morphological alterations in the QRS allow identification of ventricular hypertrophies and branch blockages. Low amplitude QRS is observed in patients with obesity, lung emphysema, myocarditis, myxedema, and pericardial effusion, while a T wave with increased amplitude indicates delay in perfusion and may cause myocardial ischemia.[16] The animals with altered QRS complex were above the weight proposed for the species,[15] that may explain the occurrence.

The T wave polarity was positive in all the animals, similar to that found in humans[29] and black-tufted marmosets.[22] T wave inversion or other alterations in this morphology were not observed in the present research, different from reports by other authors in Japanese monkeys.[23] In a study on *S apella*, the T wave polarity was positive in 77.8% of the animals.[7] Research with protocols comparing tiletamine-zolazepam with midazolam-propofol and ketamine did not show larger influences on this parameter and positive T wave was reported in most of the animals.[8,9] In general, ventricular repolarization will always generate a T wave, and the appearance of irregular behavior is normal. The exact reason is not known for the wide variation in this wave, but it is known that its behavior can occur in other animal species, so that it is not used as an index for heart problems.

The mean duration of the variables of the P wave, P-R interval, QRS complex, and the Q-T interval in the *S libidinosus* in the present study were superior compared to the parameters investigated in *Cebus apella* immobilized with ketamine[8,9] but were similar to the study with midazolam and propofol in *S apella*.[7] The shorter duration of these variables in animals anesthetized with ketamine can be attributed to the fact that isolated dissociative anesthetics cause premature ventricular and sinus depolarization.[31]

The electrical axis corresponds to the position of the heart in relation to the thorax, hence, the shape of the thorax of the animal can affect this axis. The value recorded for *S libidinosus* ranged from −11° to 83°, different from the other primates described in the literature, including rhesus monkeys and Japanese monkeys, that presented 50-100° axis variation.[23] Research on animals of the genus *Sapajus* and *Cebus*[7-9] showed a higher incidence of 50-90° electrical axis. Considering the normality pattern attributed to animals of the genus in question, four animals in the present study presented axis deviation to the left, while the others were within the standard. Thus, an axle between 50° and 90° can be considered normal for the species.

It was observed that immobilization with intramuscular tiletamine-zolazepam showed less potential for heart rate alterations (Table 2) in relation to the physiologic values of this species, that range from 165 to 225 bpm,[17] that does not occur in protocols where the α2-agonist agents were used, that caused severe bradycardia in *C apella*.[10]

However, there are a series of factors involved in the HR variation in these animals, including restraint stress, mean group weight, age, and metabolic state (under the effect of anesthetic or not). A comparison demonstrates the difference in HR observed among primates of different sizes such as Japanese monkeys (*Macaca cyclopis*; 126 bpm)

without anesthesia,[23] cynomolgus monkeys (*Macaca fascicularis*) anesthetized with ketamine (182 bpm),[20] and black-tufted marmosets sedated with tiletamine-zolazepam (264 ± 73 bpm).[22] Thus, the smaller the animal, the bigger the heart rate tends to be, as is the case of the species under study *S libidinosus* (189 bpm), that had 2.95 kg mean weight, compared to the other report of Japanese monkeys (6.8 kg),[24] cynomolgus monkeys (5-6 kg),[2] and black-tufted marmosets (305 g).[22] Corroborating this research, primates of the species *Cebus paella* showed 212 ± 23.7 bpm mean heart rate under the effect of sedation with tiletamine-zolazepam[10] and a study on *S apella* using midazolam and propofol as anesthetic showed 198.4 ± 22.9 mean HR.[7]

Another factor discussed deals with the effect of anesthesia on the cardiovascular system.[21] The sinus tachycardia observed in one animal in the present study was also observed in research where only ketamine was used as inducing agent.[8,9] This occurred because the dissociative anesthetics simulated the cardiovascular system indirectly, including sympathomimetic effects and consequently raised the HR. Tiletamine is characterized by rapid induction and catalepteroid-type anesthesia, so that its use must be associated with zolazepam, a benzodiazepine that produces hypnosis and muscular relaxation.[31] The cardiovascular effects of tiletamine-zolazepam vary considerably among the species; in cats and dogs, it produces global cardiovascular simulation while depression usually occurs in monkeys.[3] Thus, the occurrence of sinus tachycardia in only one animal in the present study may have been associated with excitation or fear, as individual characteristics of the animal, since heart rate is little modified with the use of benzodiazepines.[32]

The *S libidinosus* in the present study, similar to that observed in black-tufted marmosets[22] and Japanese monkeys,[23] did not present electrocardiographic tracings with large alterations, such as manifestation of arrhythmia, as premature ventricular complexes, found in other non-human primates.[24,26,30]

The tiletamine-zolazepam latency period and useful anesthetic period were close to that reported on the effects of the association in (*Allouatta guariba clamitans*), that showed that the latency period these animals was 3.1 ± 0.7 minutes and that the useful anesthetic period was 38 ± 7 minutes, using 3.6 mg/kg dose.[33] It is emphasized that smaller species require higher doses of the drug.[2] For *S libidinosus*, no study was found in the literature reporting the tiletamine-zolazepam latency period and useful anesthetic period, although it is an excellent drug that is easily administered, an important consideration when dealing with a wild animal, and has gentle induction and good muscle relaxation, offering ample safety in use and providing a good anesthetic return.[17]

The study showed more similarities and greater proximity to the results found in research on *S apella*, in which benzodiazepine midazolam and propofol were used[7] that also caused fewer alterations in the electrocardiographic tracing.

In conclusion, the ECG values obtained in the present study on *S libidinosus* serve as base for a standard source of information for professional primatologists, appearing as a reference medium, because tiletamine-zolazepam administration was safe and efficacious at the concentration and dose used, with minimal cardiovascular effects.

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CONFLICTS OF INTEREST

All the authors declare that there are no conflicts of interest in the present study.

AUTHOR CONTRIBUTIONS

A.Y.F.L.S, J.K.A., K.K.A.L., T.S.F.C, M.P.O.S.R., and D.J.A.M. conception, design, or acquisition of data during the experimental period. A.Y.F.L.S, D.J.A.M., and A.P.S. analysis and interpretation of data. A.Y.F.L.S., A.N.C., J.G.S., A.P.S., and D.J.A.M. have been involved in drafting the manuscript or revising it critically.

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