Comparison of medetomidine-ketamine and medetomidine-Zoletil®
anaesthetic on cardiovascular parameters in chimpanzees (Pan troglodytes)

by

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Declaration

I, Chantell Greyling, student number 10014765, hereby declare that this dissertation, “Comparison of medetomidine-ketamine and medetomidine-Zoletil® anaesthetic on cardiovascular parameters in chimpanzees (Pan troglodytes)” is submitted in accordance with the requirements for the Master of Science degree at the University of Pretoria, is my own original work and has not previously submitted to any other institution of higher learning. All sources cited or quoted in this research paper are indicated and acknowledged with a comprehensive list of references.

Chantell Greyling

November 2021
# Table of Contents

[Declaration](#) ii

Table of Contents iii

1. Ethics statement v
2. Acknowledgements v
3. List of tables vi
4. List of figures vii
5. List of Abbreviations x
6. Abstract 1
7. Key terms 2
8. Introduction and Literature Review 3

8.1. Chimpanzee Biology 3
8.2. Chimpanzee Anaesthesia 4
8.3. Anaesthetic protocols 6

8.3.1. Choosing anaesthetic agents 6
8.3.2. Injectable versus inhalant anaesthesia 7
8.3.3. Pre-anaesthetic assessments 9
8.3.4. Drug delivery and induction of anaesthesia 15
8.3.5. Agents used for the induction of anaesthesia 16
8.3.6. Analgesics 28

8.4. Management and monitoring Anaesthesia 29

8.4.1. Intubation and IV lines 29
8.4.2. Monitoring Anaesthesia 30

8.5. Recovery 32

9. Aim and Objective 34

10. Materials and Methods 35

10.1. Induction and Monitoring 35
10.2. Statistical Analysis 40

11. Results 42

11.1. Induction and maintenance of anaesthesia 42
11.2. Duration of anaesthesia 45
11.3. Anaesthetic maintenance 45
11.4. Physiological parameters 47

11.4.1. Cardiovascular parameters 49
11.4.2. Rectal temperature 53
11.4.3. Pulmonary characteristics 53
11.4.4. Arterial blood gas analysis 56
11.4.5. Recovery time 60

12. Discussion 61
13. Conclusion ......................................................................................................................... 78
13. References .......................................................................................................................... 79
14. Annexures ........................................................................................................................ 91
1. Ethics statement

I, Chantell Greyling, declare that ethics approval was obtained for research described in this thesis, and this research adheres to the ethical standards laid out in the University of Pretoria’s Code of Ethics for researchers and Policy guidelines for responsible research. In addition, principles of honesty, objectivity, care of duty and appropriate acknowledgements of the work of others have been observed.

2. Acknowledgements

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3. List of tables

Table 1 Physiological parameters described for adult chimpanzees (*Pan troglodytes*). .......................... 31

Table 2 Population characteristics (age and sex), time data (induction and recovery time) and drug doses administered for 22 chimpanzees (*Pan troglodytes*) in which anaesthesia was induced using either induced with a medetomidine-Zoletil® (MedZol, n = 12) combination or a medetomidine-ketamine (MedKet, n = 10) combination. Anaesthetic agents were administered via hand injection. However, one individual from the MedKet group was darted due to unwillingness to allow hand injection.......................................................... 43

Table 3 Administration of intravenous ketamine top-up and arterial blood gas (ABG) sample collection from 22 chimpanzees in which anaesthesia was induced using either a medetomidine-Zoletil® combination (MedZol; n = 12) or medetomidine-ketamine combination (MedKet; n = 10). Intravenous ketamine top-ups were administered to 3 individuals (MedZol = 1; MedKet = 2) before 20 minutes, 12 individuals (MedZol = 8; MedKet = 4) at 20 minutes and 1 individual (MedZol), while one individual from the MedKet anaesthetic protocol received an intramuscular MedKet top-up after the monitoring period. Four chimpanzees did not receive any top-up during the monitoring period (MedZol = 2, MedKet = 2. ABG samples was collected from 12 individuals (MedZol = 8, MedKet = 4) before ketamine top-up was administered and collected from 6 individuals (MedZol= 2, MedKet= 4) after ketamine administration in................................. 46

Table 4 Physiological parameters measured for chimpanzees (n = 22) at individual time points after recumbency for two anaesthetic protocols, medetomidine-Zoletil® (MedZol; n = 12) and medetomidine-ketamine (MedKet; n = 10). Heart rate (bpm), respiratory rate (breaths/min), percentage oxygen saturation (SpO2; %), end tidal carbon dioxide (EtCO2, mmHg), rectal temperature (˚C), non-invasive systolic, diastolic and mean arterial blood pressure (SBP, DBP and MAP, mmHg) reported as mean ± standard deviation. Ketamine top-ups to maintain anaesthetic depth was administered at 10 min (MedZol = 1; MedKet = 2), 20 min (MedZol = 8; MedKet = 4) and 30 min (MedZol = 1; MedKet = 0). The effects of ketamine will only be observed at the next point of monitoring................................................................. 48

Table 5 Arterial blood gas values (mean ± standard deviation) for 22 chimpanzees obtained using a portable EPOC gas analyser in which anaesthesia was either induced with a medetomidine-Zoletil® (MedZol, n = 12) combination or a medetomidine-ketamine (MedKet, n = 10) combination. Arterial blood samples were collected approximately 22.27 ± 6.31 minutes after anaesthesia was induced. Blood samples were collected from n = 12 individuals before the administration of top-up (MedZol, n = 8, MedKet, n = 4), while blood samples were obtained from 6 individuals (MedZol, n = 2 and MedKet, n = 4) after the top-up was administered. No marked statistical significance was observed in any of the parameters measured (p > 0.05). 58
4. List of figures

Figure 1 Ideal recovery position for chimpanzees during recovery. The head of the individual was placed in the crook of the arm to raise the head and keep the neck straight. The knee of the upper leg was bent for stability. .......................................................... 33

Figure 2 Placement of handheld Meditech vital sign monitor, Meditech blood pressure monitor and blood pressure cuff to obtain cardiovascular and respiratory parameters during chimpanzee anaesthesia. .......................................................... 38

Figure 3 Placement of an arterial catheter for arterial blood collection and invasive IntraTorr blood pressure monitoring during chimpanzees under anaesthesia. Rectal thermometer (Checktemp1) was also inserted to obtain temperature reading during monitoring. .............. 39

Figure 4 After the completion of all procedures, chimpanzees were returned to the night rooms and placed in lateral recumbency for recovery. The endotracheal tube was removed as soon as the swallow reflex had returned. .................................................................................. 40

Figure 5 Box-and-whiskers plot of the overall induction time (min) observed in chimpanzees when anaesthesia was induced with either a medetomidine-Zoletil® (MedZol; n = 12) or a medetomidine-ketamine (MedKet; n = 10) combination. Overall induction time was observed from the time that the anaesthetic agents were administered to the individual until the individual was recumbent. Even though the overall induction time observed (mean [95% confidence intervals]) was longer when anaesthesia was induced with a MedKet combination (7.52 min [95% CI: 3.76, 11.27]) compared to when anaesthesia was induced with MedZol combination (6.54 min [95% CI: 4.29, 8.79]), no statistical difference was observed (p > 0.05). Some individuals in both anaesthetic protocols had longer induction times as indicated by the outliers. .................................................................................. 45

Figure 6 The observed mean heart rate (beats per minute, bpm) that was measured at predetermined time points after recumbency (min) in chimpanzees that received either a medetomidine-Zoletil® (MedZol, n = 12) or a medetomidine-ketamine (MedKet, n = 10) anaesthetic combination. Heart rate was displayed on the pulse oximeter, or by counting the number of auscultations over 20 seconds (multiplied by 3 to obtain the count over 1 minute). Overall heart rate (mean ± standard deviation) in individuals treated with MedKet (58.48 ± 7.43 bpm) was significantly higher than in individuals treated with MedZol, (53.80 ± 10.92 bpm; p = 0.0096). Ketamine top-ups were administered at 10 min (MedZol = 1; MedKet =2), 20 min (MedZol = 8; MedKet = 4), and 30 min (MedZol = 1; MedKet = 0). Two individuals only received top-up (ketamine = 1; MedKet = 1) after completion of the anaesthetic monitoring and did not affect the measured heart rate. Mixed linear model with a Bonferroni adjustment indicated no significant change in HR irrespective of the time the ketamine top-up was administered (p < 0.05). ............... 51

Figure 7 Effect of medetomidine-Zoletil® (MedZol; n = 12) and medetomidine-ketamine (MedKet, n = 10) anaesthesia on A) systolic blood pressure (SBP, mmHg), B) diastolic blood pressure (DBP, mmHg) and C) mean arterial blood pressure (MAP, mmHg) in chimpanzees measured over a phase ...
period of 30 minutes using a non-invasive blood pressure cuff and monitor. The mean (± standard deviation) for SBP (MedZol = 139.84 ± 23.35 mmHg; MedKet = 126.98 ± 16.74 mmHg), DBP (MedZol = 75.19 ± 17.25 mmHg; MedKet = 66.63 ± 15.84 mmHg) and MAP (MedZol = 100.17 ± 20.55 mmHg; MedKet = 90.26 ± 18.67 mmHg). The overall blood pressure parameters (SBP, DBP and MAP) were all significantly higher when anaesthesia was induced with MedZol compared to when individuals were treated with MedKet (p < 0.05). No statistical difference at any of the individual time points were observed when the two anaesthetic protocols were compared using a t-test (p > 0.05). Ketamine top-ups (n = 16) were administered at 10 min (MedZol = 1; MedKet =2), 20 min (MedZol = 8; MedKet = 4), and 30 min (MedZol = 0; MedKet =1) of monitoring. Mixed linear models and Bonferroni adjustment indicated that there was a significant decrease in MAP after the administration if a ketamine top-up was administered at 10 min after recumbency (p = 0.000). No changes in SBP and DBP were observed after the administration of ketamine top-up (p < 0.05).................................

Figure 8 Respiratory rate (RR, breaths/min) observed over a 30-minute time period after chimpanzees received either a medetomidine-Zoletil® (MedZol, n = 12) or a medetomidine-ketamine (MedKet, n = 10) anaesthetic combination. Overall RR (mean ± standard deviation) was significantly higher when anaesthesia was induced with MedZol (26.46 ± 14.17 breaths/min) compared to MedKet (20.73 ± 10.12 breaths/min); p = 0.016). Mixed linear models indicated no significant differences were observed between the anaesthetic protocols at the different time points (p > 0.05). Ketamine top-ups were administered at 10 min (MedZol = 1; MedKet = 2), 20 min (MedZol = 8; MedKet = 4), and 30 min (MedZol = 1; MedKet = 0). The administration of ketamine did not have an immediate effect and the ketamine effect were only observed at the next monitoring point. Mixed linear model and Bonferroni adjustment did indicate a decrease in RR if ketamine top-up were administered before 20 minutes of monitoring, but the difference was not significant (n = 3; p = 0.091)..................................................52

Figure 9 Oxygen saturation (mean ± standard deviation; SpO₂, %) observed over time after recumbency (min) when anaesthesia was induced in chimpanzees with either a medetomidine-Zoletil® (MedZol; n = 12; 85.68 ± 8.41 %) or medetomidine-ketamine (MedKet; n = 10; 87.85 ± 5.56 %) combination as determined by pulse oximetry. Although SpO₂ remained higher in the MedKet group over time, no statistical differences were observed between the two treatments (p = 0.119). Ketamine top-ups were administered to 16 individuals at 10 min (MedZol = 1; MedKet = 2), 20 min (MedZol = 8; MedKet = 4), and 30 min (MedZol = 1; MedKet = 0). No statistical differences were observed in SpO₂ after the ketamine top-up was administered when mixed linear models with Bonferroni adjustments were conducted (p < 0.05)...........................54

Figure 10 End tidal carbon dioxide (EtCO₂, mmHg) in 22 chimpanzees as determined by capnography monitoring. Individuals treated with medetomidine-Zoletil® (MedZol; n = 12; 43.92 ± 10.53 mmHg) had a significantly higher overall EtCO₂ when compared to medetomidine-ketamine combination (MedKet; n = 10; 37.74 ± 11.21 mmHg; p = 0.009). Mixed linear model corrected with a Bonferroni adjustment indicated no statistical differences between the two anaesthetic
protocols or at the different time point ($p < 0.05$). Ketamine top-ups were administered at 10 min (MedZol = 1; MedKet =2), 20 min (MedZol = 8; MedKet = 4), and 30 min (MedZol = 1; MedKet = 0). The administration of ketamine top-up ($n = 16$) at 10 minutes of monitoring did indicate a decrease in EtCO$_2$ when mixed linear models were conducted with a Bonferroni adjustment. However, this decrease was not significant ($p = 0.193$).

\textbf{Figure 11} A box-and-whisker plot indicating the mean partial pressure of oxygen (PaO$_2$, mmHg, A) and the mean partial pressure of carbon dioxide (PaCO$_2$, mmHg, B) measured in chimpanzees in which anaesthesia was either induced with a medetomidine-Zoletil® (MedZol, $n = 12$) or a medetomidine-ketamine (MedKet, $n = 10$) combination. PaO$_2$ and PaCO$_2$ values were obtained for each individual after collecting and analysing an arterial blood sample using portable gas analyser Data indicated that MedZol anaesthesia produced a higher PaO$_2$ (59.79 mmHg [95% CI: 52.53, 67.05]) but a slightly lower PaCO$_2$ (43.23 mmHg [95% CI: 40.12, 46.33]) when compared to MedKet induced anaesthesia (PaO$_2$: 60.95 mmHg [95% CI: 54.67, 67.19] and PaCO$_2$: 43.85 mmHg [42.01, 45.85]). No statistically significant difference between the two anaesthetic protocols for these variables were observed ($p > 0.05$).

\textbf{Figure 12} Mean A-a gradient observed when anaesthesia was induced in chimpanzees using either a medetomidine-Zoletil® (MedZol, $n = 12$) or a medetomidine-ketamine (MedKet, $n = 10$) anaesthetic combination. The minimum-maximum range and interquartile range is also displayed on the box-and-whiskers plot. The A-a gradient (mean [95% confidence intervals]) for MedKet induced anaesthesia (32.67 mmHg [22.85, 42.49]) was higher but not statistically different ($p = 0.904$) compared to when anaesthesia was induced with MedZol (31.85 mmHg [20.39, 42.49]).

\textbf{Figure 13} A box-and-whiskers plot indicating the for the overall recovery time (min) when two anaesthetic protocols were used to induce anaesthesia in chimpanzees ($n = 22$) with either a medetomidine-Zoletil® (MedZol, $n = 12$) or a medetomidine-ketamine (MedKet, $n = 10$) combination. Overall recovery time was measured from the time at which atipamezole was administered to the time at which the individual lifts its head. The total recovery time (mean [95% confidence intervals]) was longer when anaesthesia was induced with MedKet (22.57 min [15.69, 29.46]) combination when compared to when anaesthesia was induced with a MedZol (20.29 min [15.31, 25.27]) combination. No significant difference in recovery time was observed between the two anaesthetic protocols ($p > 0.05$).
5. List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>°C</td>
<td>Degrees Celsius</td>
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<tr>
<td>A-a gradient</td>
<td>Alveolar-arterial oxygen gradient</td>
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<tr>
<td>ABG</td>
<td>Arterial blood gas</td>
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<tr>
<td>BP</td>
<td>Blood pressure</td>
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<tr>
<td>bpm</td>
<td>Beats per minute</td>
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<tr>
<td>Breaths/min</td>
<td>Breaths per minute</td>
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<tr>
<td>CNS</td>
<td>Central Nervous System</td>
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<tr>
<td>CRT</td>
<td>Capillary refill time</td>
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<tr>
<td>DBP</td>
<td>Diastolic blood pressure</td>
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<tr>
<td>ETCO₂</td>
<td>End tidal carbon dioxide</td>
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<tr>
<td>GABA</td>
<td>Gamma-aminobutyric acid</td>
</tr>
<tr>
<td>HCO₃⁻</td>
<td>Bicarbonate</td>
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<tr>
<td>HR</td>
<td>Heart rate</td>
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<tr>
<td>IBP</td>
<td>Invasive blood pressure</td>
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<tr>
<td>IM</td>
<td>Intramuscular</td>
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<tr>
<td>IV</td>
<td>Intravenous</td>
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<tr>
<td>kg</td>
<td>Kilogram</td>
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<tr>
<td>MAP</td>
<td>Mean arterial blood pressure</td>
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<tr>
<td>MedKet</td>
<td>Medetomidine-ketamine</td>
</tr>
<tr>
<td>MedZol</td>
<td>Medetomidine-Zoletil®</td>
</tr>
<tr>
<td>mg/kg</td>
<td>Milligram per kilogram</td>
</tr>
<tr>
<td>min</td>
<td>Minutes</td>
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<tr>
<td>ml</td>
<td>Millilitres</td>
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<tr>
<td>mmHg</td>
<td>Millimeters Mercury</td>
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<tr>
<td>Mmol/L</td>
<td>Millimoles per litre</td>
</tr>
<tr>
<td>NDMA</td>
<td>N-methyl-D-aspartate</td>
</tr>
<tr>
<td>NHP</td>
<td>Non-human primate</td>
</tr>
<tr>
<td>NIBP</td>
<td>Non-invasive blood pressure</td>
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<tr>
<td>PaCO₂</td>
<td>Arterial pressure of CO₂</td>
</tr>
<tr>
<td>PaO₂</td>
<td>Arterial pressure of O₂</td>
</tr>
<tr>
<td>PO</td>
<td>Orally</td>
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<tr>
<td>RR</td>
<td>Respiratory rate</td>
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<tr>
<td>SaO₂</td>
<td>Arterial hemoglobin oxygen saturation</td>
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<tr>
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<td>Standard deviation</td>
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<tr>
<td>SpO₂</td>
<td>Oxygen saturation</td>
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6. Abstract

Habitat loss, disease and conflict with humans have resulted in an increase in the number of chimpanzees held in captivity, where anaesthesia may be required for a variety of reasons. Ineffective anaesthetic protocols may pose a severe threat to human safety due to the physical strength of chimpanzees. In this study, anaesthesia was induced in a group of 22 captive chimpanzees during their annual health assessments using either medetomidine-Zoletil® (MedZol: 0.03 ± 0.01 mg/kg; 1.52 ± 0.19 mg/kg; n = 12) or medetomidine-ketamine (MedKet: 0.05 ± 0.01 mg/kg; 5.50 ± 1.12 mg/kg; n = 10). Chimpanzees were premedicated with midazolam (15 mg) orally prior to being hand injected (n = 21) or darted (n = 1) with either combination. Additionally, intravenous ketamine (1.5 mg/kg) was administered to 16 individuals at approximately 17.54 ± 11.86 min (mean ± standard deviation) of monitoring to maintain anaesthetic depth. One individual did receive a MedKet top-up instead of a ketamine top-up, but this was done after the period of monitoring and this should not affect the physiological parameters. Induction and recovery times were recorded, while the quality of induction, ease of intubation and muscle relaxation was subjectively scored. Physiological parameters (heart rate, respiratory rate, oxygen saturation, end-tidal CO₂, and blood pressure) were recorded at five-minute intervals over a 30-minute monitoring period. An arterial blood sample for blood gas analysis was collected at approximately 20 min (mean 22.27 ± 6.31 min) after recumbency. The recorded results were statistically analysed using a paired t-test and mixed linear models. The results from the study indicated that the induction (MedZol = 6.54 ± 3.54 min; MedKet = 7.52 ± 5.26 min) and recovery times (MedZol = 20.29 ± 7.83 min; MedKet (22.57 ± 9.63 min) did not differ significantly between the two treatments. Result from monitoring physiological parameters indicated that the mean blood pressure, respiratory rate and EtCO₂ were all significantly higher (p < 0.05) when anaesthesia was induced with MedZol, while the mean HR is higher when anaesthesia was induced with MedKet. The observed mean PaO₂, PaCO₂ and A-a gradient for both anaesthetic protocols were similar and observed mean PaO₂ and A-a gradient values for both anaesthetic protocols indicated that some degree of hypoxemia and ventilation-perfusion mismatching occurred. Results indicated that both protocols produce safe and effective short-term anaesthesia in chimpanzees. However, the cardiovascular and
respiratory systems may be affected during anaesthesia and physiological parameters should be closely monitored.

7. **Key terms**
Arterial blood gasses, atipamezole, anaesthesia, cardiovascular parameters, chimpanzee, ketamine, medetomidine, midazolam, respiratory parameters, Zoletil®
8. Introduction and Literature Review

Anaesthesia is defined as the reversible loss of sensation to the entire or any part of the body that is accompanied by a loss of consciousness, immobility and provides effective analgesia in individuals that undergo surgery (Chinnadurai et al., 2016; Dodds, 1999; Fahlman, 2005; Ferreira, 2016; Murphy et al., 2012). The main goal of anaesthesia is to reduce physiological stress and pain whilst ensuring that tissues are properly oxygenated (Fahlman, 2008; Horne, 2001). Adequate tissue oxygenation occurs when oxygen uptake is optimal, the cardiovascular system functions effectively, and blood is properly saturated with oxygen (Horne, 2001).

Non-human primates often require handling for physical examinations; placement of contraceptive implants; dental and surgical procedures; translocations; and disease screening, thereby necessitating the induction of anaesthesia to safely handle of these dangerous animals (Adami et al., 2013, 2012; Adams et al., 2003; April et al., 1982; Brainard and Darrow, 2013; Unwin et al., 2009; Young et al., 1999). Working with chimpanzees and other nonhuman primates carries an increased risk of zoonotic disease transmission including tuberculosis, hepatitis, herpesvirus, and simian immunodeficiency virus (SIV) to their human caregivers, occurring via scratches, bites and contact with blood (Adams et al., 2003; April et al., 1982; Johnson-Delaney, 1994; Murphy, 2008; Murphy et al., 2012; Rothschild, 2015; Sun et al., 2003; Young et al., 1999). Hence, effective anaesthetic protocols are critical in order to improve the safety for both humans and animals, avoid any pain or distress animals may experience during handling and minimise the risk of transmitting zoonotic disease (Adami et al., 2013, 2012; Murphy, 2008; Naples et al., 2010; Unwin et al., 2009).

8.1. Chimpanzee Biology

Wild chimpanzees naturally occur in forested and savanna-woodland habitats of equatorial Africa (Humle et al., 2016; Kalan et al., 2020; Sesink Clee et al., 2015). Wild chimpanzee populations across their natural range are under severe threat due to extensive habitat loss, disease, and conflict with humans that often leave babies orphaned (Adams et al., 2003; Fultz, 2017; Humle et al., 2016; Hyeroba et al., 2013; Wobber and Hare, 2011), attributing to their endangered status on the IUCN Red List of Threatened species (Adams et al., 2003; Humle et al., 2016; Miyabe-Nishiwaki et
al., 2021). Captive chimpanzees often become unwanted pets (Fultz, 2017), which turn leads to an increase in the number of individuals in institutions such as sanctuaries or rehabilitation centres, ultimately increasing the need for veterinary interventions (Hyeroba et al., 2013; Miyabe-Nishiwaki et al., 2021; Wobber and Hare, 2011). In the wild, chimpanzees weigh between 50 – 70 kg and a large male can reach a height of 1.8 m when standing upright (Sleeman, 2007; Videan and McGrew, 2002). Wild chimpanzees spend approximately 30 - 60 % of their day foraging for food which includes ripe fruit, leaves, bark, and other herbaceous vegetation (Curry, 2020). In captivity, chimpanzees have a stable, high-calorie diet (high sugar, low fibre diet) and a significant decrease in activity which often leads to captive chimpanzees reaching unnatural weights of up to 90 kg (Curry, 2020; Reamer et al., 2014; Sleeman, 2007; Thompson and Sabbi, 2020; Videan and McGrew, 2002). The usual life span of chimpanzees in the wild is between 30 and 40 yrs but in captivity they can live for up to 50 – 60 yrs (Havercamp et al., 2019; Reamer et al., 2014; Thompson and Sabbi, 2020). Their increased life expectancy in captivity is associated with having a steady supply of highly nutritional foods, a lack of natural predators, and a high level of veterinary care (Havercamp et al., 2019; Reamer et al., 2014; Thompson and Sabbi, 2020).

8.2. CHIMPANZEE ANAESTHESIA

Anaesthesia in chimpanzees can be particularly challenging. The agility, strength, and intelligence of this species often contributes to potential human injury during anaesthesia (Adami et al., 2013, 2012; Adams et al., 2003; Brainard and Darrow, 2013; Murphy et al., 2012; Naples et al., 2010; Sleeman, 2007; Tribe and Spielman, 1996; Wallace et al., 1960). When threatened, chimpanzees may attempt to avoid and distract the darting veterinarian and may even become aggressive towards the veterinarian (Burrows et al., 2021; Murphy, 2008; Sleeman, 2007; Unwin et al., 2009). Anaesthesia in chimpanzees is further complicated by the high frequency of occurrence of cardiovascular disease like cardiomyopathy and obesity among captive individuals (Adami et al., 2012; Burrows et al., 2021; Seller et al., 2009; Sleeman, 2007; Strong et al., 2018). Underlying cardiovascular disease is of particular concern as it is the leading cause of mortality in captive chimpanzees, especially when
anaesthesia is induced with alpha-2-adrenergic agonists (Seller et al., 2009; Sleeman, 2007; Strong et al., 2018). Significant differences in weights observed between captive chimpanzees could lead to weights being over- or underestimated increasing the occurrence of complications during anaesthesia and the development of respiratory distress (Sleeman, 2007; Thompson and Sabbi, 2020).

Anaesthetic protocols vary greatly with regards to the type of agent used and why these agents are administered (Atencia et al., 2017). Some anaesthesia protocols have severe side effects and can be dangerous or even fatal to animals and their caretakers (Fahlman, 2005; Melis et al., 2012; Murphy et al., 2012). The choice of anaesthetic agents should aim to reduce stress in the most effective and safest way possible; have a short induction period; provide good muscle relaxation and analgesia; and ensure smooth recovery (Brainard and Darrow, 2013; Chinnadurai et al., 2016; Fahlman, 2005; Ferreira, 2016; Murphy, 2008; Naples et al., 2010). The availability of antagonists will also greatly impact the choice of anaesthetic drugs (Chinnadurai et al., 2016; Ferreira, 2016; Grimm and Lamont, 2007; Melis et al., 2012; Wenger, 2004). Anaesthetic agents may have a range of side effects such as changes in blood pressure, heart rate, oxygen supply, and body temperature (Atencia et al., 2017; Fahlman, 2008). Limited availability and financial constraints often force primate care facilities to be pragmatic about the anaesthetic drugs that are used (Atencia et al., 2017).

Various anaesthetic agents can be used to induce anaesthesia in chimpanzees including carfentanil and fentanyl; ketamine; tiletamine-zolazepam (Zoletil®); alpha-2 agonists -such as medetomidine and xylazine--; and various combinations thereof (Adami et al., 2013; Capuano III et al., 1999; Murphy, 2008; Unwin, 2005; Unwin et al., 2009; Wenger, 2004). Most of these agents are potent and have a relatively wide therapeutic margin, while some are partially or fully reversible (Adami et al., 2012; Adams et al., 2003; Fahlman, 2008, 2005; Fahlman et al., 2006; Lewis, 1993; Loeffler, 1992; Naples et al., 2010; Randell and Kyytä, 1998; Unwin et al., 2009; Young et al., 1999).
8.3. Anaesthetic protocols

8.3.1. Choosing anaesthetic agents

According to Unwin et al., (2009), the choice of anaesthetic agent is a compromise between safety and efficiency. Most veterinarians base their choice of anaesthesia on the length of the procedure to be conducted and the amount of pain resulting from the procedure (Chinnadurai et al., 2016; Dodds, 1999; Wenger, 2004). Factors that are often considered in selecting anaesthetic agents are the safety margin, the minimum volume required for effective anaesthesia, the side-effects they produce, and the availability of a reversal agent (Klein and Klide, 1989; Melis et al., 2012; Murphy et al., 2012; Unwin et al., 2009; Wenger, 2004). Additional characteristics that should also be considered include quick tissue absorption, rapid onset of pharmacological effects, and the rapid excretion of the agent from the body (Klein and Klide, 1989; Wenger, 2004). Anaesthetic agents with a wide therapeutic range is often preferred as assessments before anaesthesia is difficult and diseases or health issues that increase anaesthesia risk may be unknown prior to anaesthesia (Adams et al., 2003).

Since no single agent has all the preferred properties, agents are often combined to achieve the required effects (Klein and Klide, 1989). Furthermore, the choice of an anaesthetic agent will also be limited by the terrain in which it will be used and the routes through which these agents will be delivered (Fahlman, 2008; Ølberg, 2007; Zenker, 2004). Routes in which agents can be delivered include intramuscular (IM), intravenous (IV), subcutaneous (SC) and orally (PO); however, administering anaesthetic agents via the IM route seems to be the most reliable and practically feasible route of administration in chimpanzees (Murphy, 2008). This tends to limit the choice of anaesthetic agent, as the acidity of some drugs, like ketamine, may cause minor discomfort and damage to the muscles surrounding the injection site in small primate species like marmosets (Bakker et al., 2013; Murphy et al., 2012; Sun et al., 2003; Unwin et al., 2009). The myotoxicity related to the administration of ketamine may lead to an increased awareness of pain around the injection site (Sun et al., 2003). Administering smaller doses of ketamine or combining it with additional anaesthetic agents like medetomidine limits the amount of tissue damage (Unwin et al., 2009). Delivery by hand injection or by remote-drug delivery systems also limits the volume of agent that can be administered, making only highly concentrated agents suitable for IM administration (Fahlman, 2005).
8.3.2. Injectable versus inhalant anaesthesia

8.3.2.1. Inhalant anaesthesia

Inhalant anaesthetics have a non-selective mechanism of action that can be delivered to individuals as vapours (Grimm and Lamont, 2007; Murphy et al., 2012). The use of inhalants is relatively safe with several advantages that include a rapid onset of action and controlled anaesthetic depth in response to stimuli during procedures (Grimm and Lamont, 2007; Horne, 2001; Miyabe-Nishiwaki et al., 2021; Murphy et al., 2012; Wenger, 2004). This makes the use of inhalant anaesthesia useful for maintaining longer periods of anaesthesia (Adami et al., 2012; Adams et al., 2003; Miyabe-Nishiwaki et al., 2021). Even though inhalant agents are safe to use, the administration of inhalants must be carefully monitored (Grimm and Lamont, 2007; Randell and Kyttä, 1998). The uptake, distribution, and elimination of inhalant anaesthetics are determined by the solubility of the specific agent in the blood – the more soluble the agent, the longer it will take to affect the body (Horne, 2001), while more insoluble agents will produce predictable, rapid inductions and recoveries (Dodds, 1999). It may therefore be counterintuitive that anaesthetic agents with a higher solubility have a slower onset of action and result in slower recovery (Khan et al., 2014). However, the alveolar partial pressure of inhalant anaesthetic agents may be the determining factor of the speed of induction and recovery from anaesthesia, as less soluble agents are associated with a higher alveolar partial pressure and a more rapid occurrence of anaesthetic action (Bezuidenhout, 2020; Khan et al., 2014). Inhalant anaesthetics are rapidly eliminated through respiration, allowing for effective adjustments to anaesthetic depth as well as rapid recovery after the administration of inhalant anaesthetics are ceased (Chinnadurai et al., 2016; Grimm and Lamont, 2007; Murphy et al., 2012). Unfortunately, the use of inhalants anaesthetics requires a specialised and well-maintained gas anaesthetic machine for delivery, making it difficult to use under field conditions (Chinnadurai et al., 2016; Grimm and Lamont, 2007; Murphy et al., 2012). Although inhalant anaesthetics are not often used in inducing anaesthesia in wild animals, they can be used to maintain anaesthesia when delivered at low levels with supplemental oxygen once the anaesthesia has been induced with parenteral anaesthetics (Murphy et al., 2012; Randell and Kyttä, 1998; Unwin et al., 2009; Wenger, 2004).
One of the most popular inhalant anaesthetics agents, isoflurane, is often used to maintain general anaesthesia in large primates, like chimpanzees (Brainard and Darrow, 2013; Chinnadurai et al., 2016; Horne, 2001; Murphy et al., 2012; Naples et al., 2010; Sleeman, 2007; Unwin et al., 2009). Compared to other inhalant anaesthetics agents (like sevoflurane), isoflurane is the most cost-effective inhalant anaesthetics (Horne, 2001). Even though isoflurane causes a stable and potent form of anaesthesia, the administration of high isoflurane concentrations through an endotracheal tube with supplemental oxygen may irritate the airways (Grimm and Lamont, 2007; Horne, 2001; Murphy et al., 2012; Unwin, 2005; Unwin et al., 2009). The administration of isoflurane after anaesthetic induction may lead to a depression of both the cardiovascular and respiratory systems; causing a decrease in both blood pressure and body temperature (Burrows et al., 2021; Chinnadurai et al., 2016; Horne, 2001; Horne et al., 1998; Naples et al., 2010; Sleeman, 2007; Unwin, 2005; Unwin et al., 2009). These side effects have been attributed to the vasodilatory effects of isoflurane administration (Atencia et al., 2017; Horne, 2001; Naples et al., 2010; Sleeman, 2007). As there is a decrease in blood pressure, monitoring blood pressure is extremely important when isoflurane is administered (Horne, 2001; Naples et al., 2010). Hypotension caused by isoflurane can be treated by adjusting the concentration of isoflurane and providing supplemental intravenous fluids (Naples et al., 2010; Unwin et al., 2009).

8.3.2.2. Injectable anaesthesia

Injectable anaesthetics that can be formulated in highly concentrated solutions can provide a quick and accurate means of producing anaesthesia in large animals (Fahlman, 2008; Unwin et al., 2009). Injectable anaesthetics often have high potencies which, combined with their formulation in highly concentrated solutions, means that they can be used in small injection volumes that are suitable for administration via remote delivery systems (Fahlman, 2008; Melis et al., 2012; Zenker, 2004). Commonly used injectable agents include dissociative agents, barbiturates, propofol, and opioids that can either be used alone or in combination with various sedatives or tranquilisers like alpha-adrenergic agonists or benzodiazepines to increase the speed and reliability of onset of action (Grimm and Lamont, 2007; Murphy et al., 2012).
8.3.3. Pre-anaesthetic assessments

The choice of drugs used to anaesthetise chimpanzees is often based on the body weight and overall physical condition of the individual, which can be difficult to assess prior to anaesthesia (Adams et al., 2003; Unwin et al., 2009; Videan and McGrew, 2002). The dose administered to each individual will also depend on the size, overall health, and behaviour of the animal (Murphy, 2008; Unwin et al., 2009). However, the exact weight of an individual is usually not known at the time of induction and is often estimated based on previous anaesthetic records (Adams et al., 2003; Atencia et al., 2017; Chinnadurai et al., 2016; Fahman, 2008; Sleeman, 2007; Unwin et al., 2009; Zenker, 2004). This means that an anaesthetic drug with a wide safety margin needs to be used to prevent overdosing (Fahlman, 2008; Unwin et al., 2009). Furthermore, if available, it is important to have a record of each individual’s medical history and previous anaesthetic complications to minimize the risks of anaesthesia (Murphy, 2008; Unwin et al., 2009).

Ideally, captive chimpanzees that require anaesthesia should be fasted for 12 – 24 hours and water should be removed one hour beforehand (Brainard and Darrow, 2013; Naples et al., 2010; Sleeman, 2007; Unwin, 2005; Unwin et al., 2009; Williams et al., 2003). Fasting is necessary as some anaesthetic agents could cause vomiting that can be aspirated (Unwin et al., 2009). Isolating the individual from the rest of the group is also an important step in the anaesthetic process (Adami et al., 2013; Sleeman, 2007; Unwin et al., 2009). This will prevent attacks on the individual experiencing the initial effects of the anaesthetic agent(s) (Cunningham et al., 2015; Zenker, 2004), and ensure safe access to the individual once unresponsive to stimuli (Lambeth et al., 2006). It is important to remember that the isolation process and anaesthesia may cause behavioural disruptions and upset group dynamics (Unwin et al., 2009). Chimpanzees are intelligent and extremely sensitive to changes in their environment and daily routine which can lead to an increased level of stress, excitement and aggression (Adams et al., 2003; Naples et al., 2010; Pomerantz, 2009; Sleeman, 2007; Unwin et al., 2009).

8.3.3.1. Premedication

Providing a pre-anaesthetic drug is a critical phase associated with anaesthetising primates (Adami et al., 2013). The administration of pre-anaesthetic medication will significantly reduce the level of stress the patient experiences before anaesthesia and
will assist in inducing sedation (Cashman et al., 1987; Loeffler, 1992; Maaly et al., 2019). Even though stress is a normal response to any unpleasant stimuli, significant levels of stress associated with anaesthetic induction may increase the occurrence of injuries and physiological abnormalities during anaesthesia (Burrows et al., 2021; Naples et al., 2010). Additionally, administering pre-anaesthetic medication may also allow for some individuals to be hand injected, which will significantly reduce the level of excitement, pain and stress associated with the darting process (Unwin et al., 2009). For an ideal anaesthetic protocol, pre-anaesthetic medication should be administered approximately 30 min before the full administration of the anaesthesia protocol occurs (Murphy, 2008; Unwin et al., 2009). Ideally, the pre-anaesthetic medication should be suspended in the smallest possible volume of liquid to prevent vomiting and aspiration during anaesthesia (Adams et al., 2003; Murphy, 2008; Sleeman, 2007). Over time, some primate species, especially chimpanzees, do become reluctant to consume the premedication suspension when offered on subsequent health assessments (Naples et al., 2010; Sleeman, 2007). Premedicating agents that have been regularly used in reducing the anxiety of chimpanzees before darting include benzodiazepines, phenothiazines and butyrophenone (Adami et al., 2013; Murphy, 2008; Naples et al., 2010; Unwin et al., 2009). Even though pre-medicating agents are effective in reducing stress, the uptake of these agents may be unpredictable (Adams et al., 2003).

8.3.3.1.1. Benzodiazepines

Benzodiazepines produce effective anxiolysis, hypnosis, and amnesia, but also prevent convulsions, provide good muscle relaxation, and result in changes in behaviour (Ferreira, 2016; Klein and Klide, 1989; Ølberg, 2007; Paterson, 2007; Pieri, 1983; Randell and Kyttä, 1998; Sleeman, 2007). When benzodiazepines are administered as pre-anaesthetic agents, some degree of amnesia may occur (Ferreira, 2016; Loeffler, 1992; Maaly et al., 2019; Roth et al., 1984; Uzun et al., 2010; Veselis et al., 1997), which is especially useful if multiple anaesthesia events are required (Ferreira, 2016). Benzodiazepines are often combined with other anaesthetic agents (like opioids and cyclohexamines) to improve the quality of anaesthesia and to reduce the side effects that are associated with these anaesthetic agents (Horne, 2001; Klein and Klide, 1989; Randell and Kyttä, 1998). The effect that
Benzodiazepines have on the body is related to the receptors that are affected when these agents are administered (Klein and Klide, 1989; Lin et al., 1992). The hypnotic and anticonvulsant effects are produced when benzodiazepines interact with γ-aminobutyric acid (GABA) production (Fahlman, 2008; Griffin III et al., 2013; Klein and Klide, 1989; Loeffler, 1992; Veselis et al., 1997; Wenger, 2004). The receptor complex of interest has a gating function for chloride ion flux in excitatory neurons (Griffin III et al., 2013; Klein and Klide, 1989; Loeffler, 1992). When GABA binds to the receptor complex, the ion channels open, allowing the influx of chloride and causing the cell membrane to become hyperpolarised and less excitable (Griffin III et al., 2013; Klein and Klide, 1989; Loeffler, 1992). With the introduction of benzodiazepines to the receptor complex, the GABA affinity for the receptors is enhanced and the inhibitory function is improved (Klein and Klide, 1989). Benzodiazepines also interact with the glycine-mediated pathways of the central nervous system (CNS) and reduce anxiety and increase the degree of muscle relaxation that is observed (Klein and Klide, 1989; Wenger, 2004).

Benzodiazepines are considered to be safe for use in primates, have minimal effects on the cardiopulmonary systems, and the development of tolerance or dependence to benzodiazepines is rare (Horne, 2001; Klein and Klide, 1989; Lin et al., 1992; Ølberg, 2007; Randell and Kyttä, 1998; Sleeman, 2007). Even though benzodiazepines have a rapid onset and short duration of action, these agents have no analgesic effects (Ferreira, 2016; Griffin III et al., 2013; Lin et al., 1992; Pieri, 1983). The effect that benzodiazepines have on the body also depends on the route of administration, absorption, and distribution (Griffin III et al., 2013). Routes of administration of benzodiazepines include transmucosal, IM, IV and PO (Griffin III et al., 2013; Loeffler, 1992; Pieri, 1983). Intravenous administration of benzodiazepines leads to rapid absorption and distribution to the CNS, while the absorption of IM administered benzodiazepines varies depending on the agent that is administered (Griffin III et al., 2013). Oral administration and transmucosal absorption of benzodiazepines tends to be a less stressful method of anaesthetic induction (Naples et al., 2010; Sleeman, 2007). Benzodiazepines are readily absorbed from the gastrointestinal system (Griffin III et al., 2013; Loeffler, 1992), but have a slow onset of action and the effect that is produced may be unpredictable (Adams et al., 2003; Randell and Kyttä, 1998; Sleeman, 2007). The absorption of benzodiazepines across the mucosa tends to be rapid and the availability of the drug is increased as the initial
metabolisation of the drug in the liver is avoided (Randell and Kyttä, 1998). Transmucosal induction of anaesthesia would require an extended period of mucosal contact and the anaesthetic effect produced remains unpredictable (Naples et al., 2010; Randell and Kyttä, 1998; Sleeman, 2007; Unwin et al., 2009). Common benzodiazepines used in wild animals include diazepam, zolazepam (used in combination with tiletamine) and midazolam (Griffin III et al., 2013; Murphy et al., 2012; Paterson, 2007; Sleeman, 2007; Unwin, 2005; Unwin et al., 2009; Wenger, 2004; Zenker, 2004).

Midazolam is a short-acting benzodiazepine, with a rapid onset that produces effective sedation, anxiolysis, and amnesia (Griffin III et al., 2013; Horne, 2001; Johnson et al., 2003; Klein and Klide, 1989; Maaly et al., 2019; Randell and Kyttä, 1998; Strong et al., 2018). The amnesia effect caused by midazolam is considered to be a beneficial characteristic as it assists in individuals failing to remember the darting and induction of anaesthesia (Johnson et al., 2003; Loeffler, 1992; Roth et al., 1984). This may assist in making animals more tractable for future darting and anaesthetic events. Midazolam is water-soluble and can be mixed with other agents without the addition of other solvents (Amrein et al., 1988; Griffin III et al., 2013; Horne, 2001; Klein and Klide, 1989; Lin et al., 1992; Loeffler, 1992; Pieri, 1983; Prommer, 2020; Smith et al., 1981). The solubility of midazolam allows for rapid absorption when administered IM making it suitable for administration via remote delivery (Ferreira, 2016; Griffin III et al., 2013; Klein and Klide, 1989; Loeffler, 1992; Smith et al., 1981). The metabolism of midazolam is faster when compared to diazepam, but unlike diazepam, metabolism of midazolam produces no active metabolites (Griffin III et al., 2013; Klein and Klide, 1989; Pieri, 1983). In primates, midazolam can be administered at a dose of 0.1 – 0.5 mg/kg either IM or IV , and at a dose of 0.7 -1.2 mg/kg PO (Hess et al., 2010; Murphy, 2008; Unwin, 2005; Unwin et al., 2009; Wenger, 2004; Williams et al., 2003). Midazolam, at an oral dose of 0.5 mg/kg, has been successfully used as a pre-anaesthetic drug in children (Maaly et al., 2019).

Benzodiazepines can be effectively antagonised by sarmazenil and flumazenil (Randell and Kyttä, 1998; Unwin et al., 2009; Wenger, 2004). Sarmazenil is a competitive benzodiazepine antagonist acting at the GABA receptor (Johnson et al., 2003; Müller et al., 2005; Walzer and Huber, 2002), used to reverse the effects of midazolam when used in combination with other anaesthetic agents (Johnson et al.,
Sarmazenil has a relatively short half-life and must be administered frequently to maintain active drug levels (Müller et al., 2005). Another benzodiazepine antagonist, flumazenil, is often used to reverse the sedation effects of zolazepam, diazepam, and midazolam (Ferreira, 2016; Johnson et al., 2003; Klein and Klide, 1989; Loeffler, 1992; Murphy et al., 2012; Papich, 2015; Randell and Kyttä, 1998; Short and Galletly, 1989; Walzer and Huber, 2002). The administration of flumazenil will not necessarily reduce the recovery time or increase the quality of recovery, but it will increase the alertness of the individual during recovery (Ferreira, 2016; Sleeman, 2007; Unwin et al., 2009). Generally, flumazenil is a short-acting antagonist and when used to reverse high doses of long-acting benzodiazepines such as midazolam, re-sedation can occur (Klein and Klide, 1989; Loeffler, 1992; Randell and Kyttä, 1998; Short and Galletly, 1989). Even though the use of flumazenil is relatively safe, reversing the effect of benzodiazepines is limited by the costs that are associated with flumazenil (Klein and Klide, 1989; Short and Galletly, 1989; Walzer and Huber, 2002).

8.3.3.1.2. Tranquilisers – Phenothiazines and Butyrophenones

Phenothiazines and butyrophenones, are commonly used as anti-psychotic agents in humans (Ayano, 2016; James et al., 2000; Ohlow and Moosmann, 2011; Tobin and Ballard, 1979; Uesono et al., 2008), but have also been effectively applied for wildlife tranquilisation (Grimm and Lamont, 2007; Tobin and Ballard, 1979). These tranquilisers may decrease the level of stress that is experienced while having minimal effects on the consciousness, coordination and spontaneous movement of the animals (Ferreira, 2016; Tobin and Ballard, 1979; Wenger, 2004). The use of phenothiazine and butyrophenones in nonhuman primates are limited but they have been used to reduce aggression, abnormal behaviour and anxiety (Redrobe, 2008).

Phenothiazine and butyrophenone tranquilisers mainly act on the D2-dopamine receptors in the CNS, reducing the release and transmission of dopamine (Ashoor et al., 2011; Ayano, 2016; Ferreira, 2016; Grimm and Lamont, 2007; Grinchii and Dremencov, 2020; Hernández-Godínez et al., 2019; Ohlow and Moosmann, 2011; Tobin and Ballard, 1979; Wenger, 2004). These agents will also have an effect on other dopaminergic, serotonergic and alpha-2-adrenergic receptors (Ferreira, 2016; Grimm and Lamont, 2007; Tobin and Ballard, 1979; Wenger, 2004). These agents are readily absorbed when administered orally or parentally (Ayano, 2016), however less
predictable absorption is observed when administered PO (Ayano, 2016). Disadvantages associated with using these tranquilising agents include the occurrence extrapyramidal and neurological side effects (Ayano, 2016; Ferreira, 2016; Grinchii and Dremencov, 2020; Redrobe, 2008; Tobin and Ballard, 1979), changes in thermoregulation and a decrease in blood pressure (Ferreira, 2016; Grinchii and Dremencov, 2020; Tobin and Ballard, 1979). Additionally, these agents have no analgesic effects and the effect produced cannot be reversed (Ferreira, 2016).

8.3.3.1.2.1. Phenothiazines

Phenothiazines consist of more than 40 compounds that block a range of receptors that will result in decreased alertness and responsiveness as well as produce a range of effects that include enhancing the anaesthetic effect, sedation, antiemetic, antihistamine, analgesia, antidepressants, antipsychotic agents and even possess some antimicrobial and anthelminthic properties (Ayano, 2016; Owens, 2012; Tobin and Ballard, 1979; Uesono et al., 2008; Wenger, 2004). Administration of phenothiazine tranquilisers (IM) may result in reduced respiration, but may also cause vasodilation, reducing blood pressure and increase heart rate (Tobin and Ballard, 1979; Wenger, 2004).

The phenothiazine tranquiliser, acepromazine, is commonly used in a variety of species (including nonhuman primates) to effectively reduce stress, excitement and motor activity, as well as having a sedative effect on individuals (Ferreira, 2016; Hernández-Godínez et al., 2019; Murphy, 2008; Papich, 2015; Paterson, 2007; Schneiders et al., 2012; Wenger, 2004), but is rarely used to induce anaesthesia on its own (Wenger, 2004). When combined with opioids and cyclohexamines, acepromazine assists in reducing the induction time, reduces the dose of anaesthesia required, and reduces the side effects associated with anaesthetic agents (Ferreira, 2016; Grimm and Lamont, 2007; Wenger, 2004). The approximate dose of acepromazine given to nonhuman primates as a pre-anaesthetic medication and tranquillization is 0.2 – 1.0 mg/kg for PO, IM, or SC routes of administration (Murphy, 2008).
8.3.3.1.2.2. Butyrophenones

Butyrophenone tranquilisers are chemically different from the phenothiazines, but the pharmacological effects produced are similar to that of phenothiazines (Tobin and Ballard, 1979; Wenger, 2004). Butyrophenones are short-acting tranquilisers that can be administered either IM or IV (Grimm and Lamont, 2007). Butyrophenones that are commonly used in wildlife include azaperone, droperidol and haloperidol (Wenger, 2004). Droperidol is a potent antiemetic and anti-psychotic agent, which can be administered to adult humans to control agitation at a dose of 2.5 to 10 mg IM or 0.625 to 10 mg IV (Ayano, 2016; Prommer, 2020; Randell and Kyttä, 1998). In chimpanzees, the administration of oral droperidol has shown to provide effective sedation before IM delivery of anaesthetic agents (Kearns et al., 2000; Sleeman, 2007) and has the ability to reduce the respiratory depression associated with the use of other anaesthetic agents (Kearns et al., 2000; Wenger, 2004). Redrobe (2008) noted that the use of haloperidol reduces intraspecific aggression and abnormal behaviours exhibited by gorillas. The effect produced when azaperone is administered alone in primates is lacking (Bäckström, 2020), but it has been used in combination with butorphanol and medetomidine (butorphanol-azaperone-medetomidine; BAM) to induce reliable anaesthesia in rhesus macaques and chacma baboons (Bäckström, 2020; Malinowski et al., 2019).

8.3.4. Drug delivery and induction of anaesthesia

The remote delivery of anaesthetic drugs through darting can be stressful for individuals. One disadvantage of remote drug delivery in primates is that an individual may remove the dart before the full dose is completely injected (Adami et al., 2012; Adams et al., 2003; Naples et al., 2010; Sleeman, 2007; Unwin et al., 2009). Removal of the dart increases the need for potent anaesthetic agents that can be administered in small volumes (Adams et al., 2003). Failure to discharge the full dose may increase the time it takes for the individual to become recumbent and can often lead to the requirement of the administration of a second dose to safely handle the individual (Naples et al., 2010; Unwin et al., 2009). Care should also be taken to not excite the individual prior to darting, as excited and aggressive individuals would require higher anaesthetic doses which can affect recovery time if not all the components of the
anaesthetic protocol are reversible (Murphy, 2008; Sleeman, 2007; Unwin et al., 2009). Individuals that are severely stressed, excited, or agitated show increased stress hormone levels (i.e. adrenaline, norepinephrine and cortisol) in the bloodstream, which will influence heart rate, blood pressure and cardiac output during anaesthesia and prevent an effective anaesthetic depth (Adams et al., 2003; Brivio et al., 2015; Burrows et al., 2021; Ferreira, 2016; Klein and Klide, 1989; Maaly et al., 2019; Unwin et al., 2009; Wenger, 2004; Whitten et al., 1998). Increased stress levels and the release of stress hormones can influence the effectivity of many sedatives and anaesthetic agents (like alpha-2 agonists), resulting in prolonged inductions, ineffective levels of anaesthesia and anaesthetic complications (Brivio et al., 2015; Burrows et al., 2021; Maaly et al., 2019; Unwin et al., 2009).

The route of administration may also influence the level of stress experienced by the individual. Hand injection has a significant advantage over darting as it will assist in preventing the increase in physiological parameters, for example heart rate, blood pressure, SpO₂, and temperature, that is often seen in when animals are stressed during darting (Burrows et al., 2021; Ferreira, 2016). Induction is defined as the time between the drugs being administered to the first signs of sedation, and recumbency or the loss of consciousness (Caulkett and Cattet, 1997; Fahlman, 2008, 2005; Ferreira, 2016; Jacquier et al., 2006; Melis et al., 2012). Only once the individual is recumbent (lying down) and showing no response to external stimuli, can the individual be approached and handled (Atencia et al., 2017; Young et al., 1999).

8.3.5. Agents used for the induction of anaesthesia

8.3.5.1. Alpha-2-adrenergic agonists

Alpha-2-adrenergic agonists have sedative, muscle relaxing, and analgesic properties (Ranheim et al., 1999). The level of sedation that is provided by these agents significantly depends on the dose that has been administered (Wenger, 2004). Even though, alpha-2-adrenergic agonists are highly lipophilic (increasing absorption into the brain), they will not produce complete anaesthesia and should be paired with other anaesthetic agents (Chinnadurai et al., 2016; Horne, 2001; Jalanka and Roekken, 1990; Klein and Klide, 1989; Wenger, 2004). The effects that are produced by alpha-2-adrenergic agonists are related to the affinity that these drugs have for pre- and post-
synaptic alpha-2-receptors (Fahlman, 2008; Ferreira, 2016; Grimm and Lamont, 2007; Klein and Klide, 1989; Naples et al., 2010). Alpha-2-adrenergic agonists have widespread effects based on their interactions with a variety of alpha-2-adrenergic receptors located throughout the CNS, respiratory, cardiovascular, and gastrointestinal systems, as well as affecting the thermoregulatory centres (Chinnadurai et al., 2016; Ferreira, 2016; Grimm and Lamont, 2007; Sinclair, 2003; Wenger, 2004). Alpha-2 adrenergic agonists that affect the receptors in the brain, tend to regulate stages of awareness, arousal and vigilance (Sinclair, 2003). Due to the various locations of alpha-2-adrenergic receptors, the administration of alpha-2-adrenergic agonists may cause cardiopulmonary depression, affect thermoregulatory abilities and cause changes in ocular pressure (Grimm and Lamont, 2007). The use of several alpha-2-adrenergic agonists – such as xylazine, detomidine, naphthylmedetomidine, and medetomidine – has been described in various primate species (Adami et al., 2013; Ancrenaz et al., 2003; Brainard and Darrow, 2013; Chinnadurai et al., 2016; Grimm and Lamont, 2007; Hess et al., 2010; Horne, 2001; Horne et al., 1998; Sleeman, 2007; Wenger, 2004).

8.3.5.1.1. Medetomidine

Medetomidine can be used in various wildlife species and has been proven effective for anaesthesia in nonhuman primates (Melis et al., 2012; Murphy, 2008; Murphy et al., 2012). Medetomidine cannot be used as a complete anaesthetic on its own, but the effect that medetomidine has on the body will be enhanced when combined with other anaesthetic drugs (Williams et al., 2003). Medetomidine is a highly selective alpha-2-adrenergic receptor agonist that modulates the release of noradrenaline from sympathetic neurons, reducing the affinity of the receptors and producing adequate sedation, muscle relaxation, and analgesia (Baker et al., 2011; Ferreira, 2016; Grimm and Lamont, 2007; Horne, 2001; Jalanka and Roeken, 1990; Klein and Klide, 1989; Lee et al., 2010; Murphy, 2008; Naples et al., 2010; Papich, 2015; Sinclair, 2003; Unwin, 2005; Unwin et al., 2009; Williams et al., 2003). The reduction in the release of norepinephrine, results in a decrease in efferent CNS activity that will lead to bradycardia, changes in blood pressure (initial hypertension followed by hypotension is observed), hyperthermia, vigilance, and reduced anaesthetic requirements (Baker et al., 2011; Deem and Citino, 1998; Ferreira, 2016; Grimm and Lamont, 2007; Horne,
The side effects of medetomidine seem to be less severe in nonhuman primates when compared to other animal species (Naples et al., 2010). Medetomidine is rapidly absorbed when administered IM and has a relatively short elimination half-life (Sinclair, 2003). In nonhuman primates, like chimpanzees, medetomidine is preferred over detomidine and xylazine due to its potency and selectivity for alpha-2-adrenergic receptors and its ability to produce longer anaesthesia and analgesia (Brainard and Darrow, 2013; Ferreira, 2016; Klein and Klide, 1989; Naples et al., 2010; Sinclair, 2003). Like other alpha-2-adrenergic agonists, medetomidine has the advantage that it has a specific antagonist (atipamezole) that will effectively reverse the effect of medetomidine (Ferreira, 2016; Grimm and Lamont, 2007; Murphy, 2008; Naples et al., 2010; Unwin et al., 2009).

8.3.5.1.2. Dexmedetomidine

Dexmedetomidine is the D-enantiomer of medetomidine (Horne, 2001; Horne et al., 1998; Naples et al., 2010; Romagnoli et al., 2016); due to the greater potency of dexmedetomidine, it will produce a similar effect as medetomidine but at half the dose (Maaly et al., 2019; Romagnoli et al., 2016; Sinclair, 2003). Dexmedetomidine has been used in veterinary anaesthesia but marked motor activity has been observed during induction (Dodds, 1999). The use of dexmedetomidine has the benefit of decreasing the overall anaesthetic requirements (Dodds, 1999; Lee et al., 2010). As with other alpha-2 adrenergic agonists, side effects that are associated with the use of dexmedetomidine include bradycardia, hypotension and hypertension (Grimm and Lamont, 2007; Romagnoli et al., 2016; Sleeman, 2007). In nonhuman primates, dexmedetomidine can be combined with ketamine to effectively induce anaesthesia and provide sufficient analgesia during painful procedures (Murphy et al., 2012; Romagnoli et al., 2016; Selmi et al., 2004). In a study conducted on golden-headed lion tamarins (Leontopithecus chrysomelas), Selmi et al. (2004) noted that when dexmedetomidine-ketamine was compared to medetomidine-ketamine, the former produced not only a longer anaesthetic effect, but also greater sedation and analgesia during the earlier stages of anaesthesia.
8.3.5.1.3. Xylazine

Xylazine, like medetomidine, will not provide effective anaesthesia when used alone and must be used in combination with other anaesthetic agents (Alsobayil et al., 2018; Klein and Klide, 1989). This alpha-2-adrenergic agonist has a short duration of action but will produce effective muscle relaxation and anaesthesia for short procedures (Ferreira, 2016; Grimm and Lamont, 2007). Generally, xylazine is administered IM to nonhuman primates as a pre-medicating agent prior to administering anaesthetic agents or in combination with ketamine to induce anaesthesia for minor procedures (Alsobayil et al., 2018; April et al., 1982; Hernández-Godínez et al., 2019; Melis et al., 2012; Murphy et al., 2012; Sun et al., 2003; Young et al., 1999). Xylazine is readily available and reasonably priced (Ferreira, 2016; Grimm and Lamont, 2007). However, side effects that are associated with the use of xylazine include hypersalivation, respiratory depression, muscle tremors, bradycardia and hypertension followed by hypotension (Ferreira, 2016).

8.3.5.2. Alpha-2-antagonists

The use of alpha-2-adrenergic agonists has a significant advantage over the use of dissociative anaesthetic agents since they have specific antagonists that can reverse the effects that the alpha-2-adrenergic agonists produce (Chinnadurai et al., 2016; Ferreira, 2016; Klein and Klide, 1989; Murphy et al., 2012; Wenger, 2004). When alpha-2-antagonists are administered, they block the agonist at the synapses, allowing for the normal release of norepinephrine and normal neural transmission to take place (Wenger, 2004). The administration of an antagonist will not only reverse the effect that is produced by the agonist, but will also reverse the analgesic effects associated with these agents (Ferreira, 2016).

8.3.5.2.1. Atipamezole

Anaesthetic protocols that involve the use of alpha-2-adrenergic agonists (medetomidine, dexmedetomidine, or xylazine) in combination with other anaesthetic agents can be partially reversed using atipamezole that is injected into the muscle to
reverse the alpha-2-adrenergic agonists part of the protocol (Adams et al., 2003; Ashley et al., 2021; Atencia et al., 2017; Ferreira, 2016; Grimm and Lamont, 2007; Jalanka and Roeken, 1990; Klein and Klide, 1989; Murphy et al., 2012; Naples et al., 2010; Romagnoli et al., 2016; Unwin et al., 2009). The half-life of atipamezole is much shorter than the half-life of medetomidine, so administering atipamezole too early may lead to re-sedation (Baker et al., 2011; Ranheim et al., 1997). The administration of atipamezole should be done 15 – 60 min after medetomidine was administered to prevent this re-sedation effect (Jalanka and Roeken, 1990; Ranheim et al., 1999). For the same reason, it is not advisable to administer atipamezole IV – IV administration can also result in rapid changes in cardiovascular function which may additionally be dangerous to the animal (Ranheim et al., 1999; Sinclair, 2003). The prescribed dose of atipamezole is 4 – 5 times the medetomidine dose in primates (Fahlman, 2008, 2005; Fahlman et al., 2006; Lewis, 1993; Murphy, 2008; Ranheim et al., 1997; Sinclair, 2003; Unwin, 2005; Unwin et al., 2009). Recovery times after the atipamezole is administered can vary significantly based on the administration of any pre-anaesthetic agents, the type of concomitant agent given, and the dose of the concomitant agent (Klein and Klide, 1989; Melis et al., 2012; Naples et al., 2010; Sleeman, 2007; Unwin et al., 2009). Rapid, full recoveries have been observed in primates when anaesthesia is induced with MedKet within 10 – 13 min after IM administration of atipamezole and approximately 6 min after IV administration of atipamezole (Horne, 2001; Lewis, 1993; Sleeman, 2007). Animals that receive atipamezole administered at high doses may become overly alert upon recovery (Jalanka and Roeken, 1990). Other alpha-2-antagonists are available, but atipamezole has the greatest affinity for alpha-2-receptors, making it more effective in reversing the effects of alpha-2-agonists with fewer side effects (Ferreira, 2016; Klein and Klide, 1989; Papich, 2015; Sinclair, 2003; Unwin et al., 2009).

8.3.5.2.2. Yohimbine

Yohimbine is a plant-derived compound that is commonly used as a performance-enhancing agent in humans as well as an alpha-2-antagonist in animals (Grimm and Lamont, 2007; Klein and Klide, 1989). It may cause excitement upon recovery due to its stimulatory effects (Grimm and Lamont, 2007; Papich, 2015; Vasa et al., 2009). Reversal effects of the alpha-2-adrenergic agonist occur when yohimbine blocks the
central and peripheral alpha-2-receptors (Papich, 2015). Yohimbine commonly increases heart rate and blood pressure (Klein and Klide, 1989) but will also affect the behaviour of the individual it is administered to (Klein and Klide, 1989; Papich, 2015). The increase in norepinephrine, autonomic activity and awareness when yohimbine is administered to humans may result in severe anxiety, while the observed effects in animals (cats, dogs and sheep) may include excitement and behavioural depressions (Klein and Klide, 1989; Vasa et al., 2009). Yohimbine is commonly used for the reversal of xylazine and is not as effective in reversing newer agents like medetomidine (Ferreira, 2016; Grimm and Lamont, 2007; Klein and Klide, 1989; Murphy et al., 2012; Papich, 2015; Unwin et al., 2009).

8.3.5.3. Dissociative anaesthetic agents

Dissociative anaesthetic agents can produce both anaesthesia and analgesia (Bush et al., 1977; Unwin et al., 2009). These effects are produced by interrupting both the transmission of sensory signals to the brain and communication between different parts of the CNS (Lin et al., 1992; Wenger, 2004). The anaesthetic and analgesic properties of dissociative anaesthetics, like ketamine and tiletamine, are produced when these agents interact with phencyclidine sites of the N-methyl-D-aspartate (NMDA) receptors which will inhibit the transport of serotonin transport (Baker et al., 2011; Brainard and Darrow, 2013; Fahlman, 2008; Ferreira, 2016; Grimm and Lamont, 2007; Horne, 2001; Kohrs and Durieux, 1998; Murphy et al., 2012; Papich, 2015; Unwin et al., 2009; Zanos et al., 2018). The degree of anaesthesia that is produced depends on the dose that is administered (Fahlman, 2005).

The administration of dissociative anaesthetic agents alone will produce poor muscle relaxation and may cause convulsions, excessive salivation, and hyperthermia (Fahlman, 2008; Ferreira, 2016; Jacquier et al., 2006; Wenger, 2004). As dissociative anaesthetic agents may cause hyperthermia, temperature during anaesthesia should be carefully monitored (Wenger, 2004). Pharyngeal and laryngeal reflexes, as well as cardiovascular functions are well-maintained when dissociative agents are administered (Bush et al., 1977; Ferreira, 2016; Wenger, 2004). The maintenance of laryngeal and pharyngeal reflexes could complicate intubation (Fahlman, 2005; Horne, 2001; Jalanka and Roeken, 1990; Kohrs and Durieux, 1998; Murphy et al., 2012;
but can also be considered an advantage if pre-anaesthetic fasting was not possible (Murphy et al., 2012; Ølberg, 2007; Sleeman, 2007; Unwin et al., 2009). Unlike alpha-2-adrenergic agonists, dissociative agents cannot be effectively reversed (Ferreira, 2016). Ketamine and tiletamine are commonly used dissociative agents in primate anaesthesia (Fahlman, 2008, 2005; Lee et al., 2003; Lin et al., 1992; Wenger, 2004). Both of these agents are suspected to induce a stress response in unhabituated individuals, and may cause disorientation upon induction and emergence from anaesthesia (Whitten et al., 1998).

8.3.5.3.1. Ketamine

Ketamine is an injectable dissociative anaesthetic drug, widely used in nonhuman primates (Alsobayil et al., 2018; Chinnadurai et al., 2016; Fahlman, 2008; Horne, 2001; Jalanka and Roeken, 1990; Lee et al., 2010; Lewis, 1993; Murphy, 2008; Murphy et al., 2012; Unwin et al., 2009; Wenger, 2004; Young et al., 1999). When ketamine is administered, it not only produces effective anaesthesia and analgesia, but also amnestic, psychotomimetic and neuroprotective effects (Fahlman, 2008; Ferreira, 2016; Horne, 2001; Kohrs and Durieux, 1998; Murphy et al., 2012).

Even though ketamine mainly interacts with NMDA receptors, ketamine also interacts with various other receptors including the cholinergic receptors; certain opioid receptors; and voltage-dependent ion channels (Jalanka and Roeken, 1990; Kohrs and Durieux, 1998). However, Kohrs and Durieux (1998) noted that the interaction with these receptors only plays a minor role in the effects that ketamine has on the body.

Ketamine administration has several advantages and disadvantages. Ketamine has a relatively wide safety margin, but is a short-acting anaesthetic agent and is not recommended for use in procedures that require extended anaesthetic periods (Alsobayil et al., 2018; Brainard and Darrow, 2013; Cunningham et al., 2015; Ferreira, 2016; Grimm and Lamont, 2007; Horne, 2001; Jalanka and Roeken, 1990; Melis et al., 2012; Naples et al., 2010; Unwin et al., 2009; Wenger, 2004; Young et al., 1999). Generally, ketamine is administered IM, but can be administered through any other routes, IV, PO and rectally (Brainard and Darrow, 2013; Lee et al., 2010). The administration of ketamine IM causes rapid induction provides effective analgesia.
during short procedures and recovery that is in most cases smooth (Chinnadurai et al., 2016; Fahlman, 2008, 2005; Ferreira, 2016; Jalanka and Roeken, 1990; Kearns et al., 1999; Lewis, 1993; Murphy, 2008; Naples et al., 2010; Randell and Kyttä, 1998; Sleeman, 2007; Unwin et al., 2009). Even though ketamine has little effect on the respiratory system (Grimm and Lamont, 2007; Horne, 2001; Jalanka and Roeken, 1990; Kohrs and Durieux, 1998; Lewis, 1993; Unwin et al., 2009; Zanos et al., 2018), it may produce a temporary apneustic breathing pattern in which there is a prolonged inspiration period that is followed by a short expiration period (Brainard and Darrow, 2013; Grimm and Lamont, 2007; Lin et al., 1992). Ketamine seems to slightly stimulate rather than depress the cardiovascular system – leading to an increase in heart rate and mean arterial pressure (Brainard and Darrow, 2013; Ferreira, 2016; Grimm and Lamont, 2007; Horne, 2001; Jalanka and Roeken, 1990; Kohrs and Durieux, 1998; Lewis, 1993). The slight stimulation of the cardiovascular system is associated with an increase of norepinephrine and epinephrine levels in the blood (Baker et al., 2011; Horne, 2001; Kohrs and Durieux, 1998).

Anaesthetic induction using ketamine at high doses could lead to increased muscle spasms, increased blood flow to the brain, and occasionally even seizures (Bakker et al., 2013; Chinnadurai et al., 2016; Ferreira, 2016; Hyeroba et al., 2013; Lee et al., 2010; Murphy, 2008; Naples et al., 2010; Unwin et al., 2009). Like with other dissociative anaesthetic agents, ketamine induces excessive salivation that can be controlled by the use of anticholinergic agents (Brainard and Darrow, 2013; Fahlman, 2008, 2005; Horne, 2001; Jalanka and Roeken, 1990; Lewis, 1993; Sleeman, 2007).

Preventing spontaneous arousal during anaesthesia is key to ensuring the safety of personnel and the patient (Murphy et al., 2012; Unwin, 2005). The sudden arousal of chimpanzees during ketamine-induced anaesthesia may necessitate the need for additional doses of ketamine (1 – 2 mg/kg IM) to maintain anaesthetic depth (Melis et al., 2012). Under normal circumstances, the recovery of an anaesthetised individual that has been induced with ketamine is approximately 40 – 60 min (Sleeman, 2007). The incremental administration of ketamine for maintenance purposes will significantly increase recovery time (Lewis, 1993; Murphy, 2008; Sleeman, 2007; Zenker, 2004). In humans, recovery from ketamine-induced anaesthesia may also result in hallucinations upon emergence (Alsobayil et al., 2018; Ferreira, 2016).
Chimpanzees that are sedated with ketamine on a regular basis may develop a tolerance to the drug and will consequently require higher or more frequent additional ketamine doses to induce and maintain anaesthesia (Lewis, 1993; Naples et al., 2010; Sleeman, 2007; Unwin et al., 2009; Zanos et al., 2018). Ketamine use in individuals with kidney or liver disease should be avoided due to the inability of these organs to adequately metabolise and eliminate the anaesthetic agents (Cunningham et al., 2015; Horne, 2001; Jalanka and Roeken, 1990; Kohrs and Durieux, 1998; Wenger, 2004).

8.3.5.3.2. Ketamine combinations

The dose of ketamine that is recommended for chimpanzees is based on the size and weight of the individual but generally ranges between 5 – 20 mg/kg, producing a range of pharmacological effects including motor stimulation, ataxia, sedation, catalepsy, analgesia, amnesia, and anaesthesia (Horne, 2001; Murphy, 2008; Murphy et al., 2012; Sleeman, 2007). The combination of ketamine with other sedatives or tranquilisers will not only reduce the volume of ketamine that is required for induction, but may also increase the degree of muscle relaxation, and attenuate some of the side effects that are associated with ketamine (Bakker et al., 2013; Fahlman, 2008, 2005; Wenger, 2004). Ketamine doses in combination with other anaesthetic agents have been described in various literature. Ketamine-medetomidine (2 – 5 mg/kg ketamine: 0.02 – 0.05mg/kg medetomidine) is the most frequently used combination to induce anaesthesia in chimpanzees, but combinations of ketamine-midazolam (5 – 15 mg/kg ketamine: 0.05 – 0.15 mg/kg midazolam) and ketamine-xylazine (10 – 20 mg/kg ketamine: 1 mg/kg xylazine) have also been described (Klein and Klide, 1989; Murphy et al., 2012; Sleeman, 2007).

8.3.5.3.2.1. Ketamine-medetomidine combination

The combination of medetomidine and ketamine is commonly used for effective NHP anaesthesia (Fahlman, 2008; Horne, 2001; Murphy, 2008; Naples et al., 2010; Unwin et al., 2009). Ketamine-medetomidine combinations can be used to achieve reliable, short-term reversible anaesthesia, with limited effects on the cardiovascular systems (Adami et al., 2013, 2012; Capuano III et al., 1999; Horne, 2001; Hyeroba et
Medetomidine-ketamine induced anaesthesia produce rapid and smooth inductions with significant muscle relaxation that is useful for intubation (Horne, 2001; Jalanka and Roeken, 1990; Lee et al., 2010; Lewis, 1993; Sleeman, 2007; Unwin et al., 2009). Horne (2001) noted that the effects of sedation in NHPs are generally seen within 3 – 5 min after the administration of a ketamine-medetomidine combination, while complete sedation generally occurs within 10 – 15 min. Due to the short duration of action that is associated with the ketamine-medetomidine combination, the combination provides effective anaesthesia for 30 – 45 min, after which the maintenance of anaesthesia becomes unpredictable (Unwin et al., 2009). For this reason, induction using a ketamine-medetomidine combination should be followed by the administration of other maintenance anaesthetics such as isoflurane when longer procedures are required (Murphy, 2008; Williams et al., 2003).

The combination of ketamine-medetomidine has several advantages over using ketamine alone (Horne, 2001). Firstly, the ketamine portion can be significantly lowered when combined with medetomidine, while still causing rapid inductions (Cunningham et al., 2015; Fahlman, 2005; Hyeroba et al., 2013; Klein and Klide, 1989; Lee et al., 2010; Lewis, 1993; Murphy, 2008; Sleeman, 2007; Unwin et al., 2009; Wenger, 2004; Young et al., 1999). Side effects are also limited (increased muscle tone and excessive salivation), and tissue damage that is associated with the use of ketamine is reduced (Bakker et al., 2013; Cunningham et al., 2015; Fahlman, 2005; Hyeroba et al., 2013; Klein and Klide, 1989; Lee et al., 2010; Lewis, 1993; Murphy, 2008; Sleeman, 2007; Sun et al., 2003; Unwin et al., 2009; Wenger, 2004; Young et al., 1999). However, the addition of medetomidine to ketamine may mask insufficient ketamine doses that will result in sudden recoveries without any noticeable warning signs (Caulkett and Cattet, 1997; Fahlman, 2008, 2005; Horne, 2001; Jalanka and Roeken, 1990; Lee et al., 2010; Lewis, 1993; Murphy et al., 2012; Sleeman, 2007; Unwin et al., 2009; Williams et al., 2003; Young et al., 1999). It is best to leave the individual alone for at least 10 min after the drugs have been administered before attempting to move the individual to prevent spontaneous arousal (Horne, 2001; Horne et al., 1998; Melis et al., 2012; Sleeman, 2007; Zenker, 2004).

Secondly, the medetomidine portion in the combination is completely reversible when atipamezole is injected, ensuring smooth and shortened recoveries (Bakker et al., 2013; Cunningham et al., 2015; Fahlman, 2008; Klein and Klide, 1989; Lee et al.,
2010; Lewis, 1993; Murphy, 2008; Murphy et al., 2012; Sleeman, 2007; Unwin et al., 2009; Williams et al., 2003; Young et al., 1999). Unwin et al. (2009) recommended waiting at least 30 min before administering atipamezole when using a ketamine-medetomidine combination. If the reversal is given earlier, the individual will remain under the residual effects of ketamine, causing turbulent recoveries that may last longer and leave the individual disorientated (Unwin et al., 2009; Williams et al., 2003).

8.3.5.3.3. Tiletamine-zolazepam

The tiletamine-zolazepam combination (a 1 part cyclohexylamine and 1 part benzodiazepine combination; Zoletil®) can be used independently or in combination with other drugs as an anaesthetic for various nonhuman primate species (Brainard and Darrow, 2013; Chinnadurai et al., 2016; Deem and Citino, 1998; Fahlman, 2008, 2005; Ferreira, 2016; Horne, 2001; Jacquier et al., 2006; Lin et al., 1992; Naples et al., 2010; Papich, 2015; Sleeman, 2007; Unwin et al., 2009; Walzer and Huber, 2002; Wenger, 2004; Wilson et al., 1993). Trading as Zoletil® or Telazol®, this anaesthetic combination has a wide safety margin, provides rapid induction, good muscle relaxation and has the potential to induce longer anaesthetic periods (Chinnadurai et al., 2016; Cunningham et al., 2015; Fahlman, 2008; Ferreira, 2016; Lee et al., 2003; Lin et al., 1992; Naples et al., 2010; Sleeman, 2007; Unwin et al., 2009). The anaesthetic effects of Zoletil® are produced by the individual pharmacology of the agents in the combination. The dissociative agent, tiletamine, in the combination provides effective sedation, and analgesia (Deem and Citino, 1998; Lin et al., 1992). The pharmacology of tiletamine is similar to that of ketamine (Lin et al., 1992; Walzer and Huber, 2002; Wenger, 2004), producing the same effects on the body (Fahlman, 2008; Grimm and Lamont, 2007). However, compared to ketamine, tiletamine is a more potent, faster-acting agent that produces anaesthesia that lasts longer (Cunningham et al., 2015; Fahlman, 2008; Lin et al., 1992; Wenger, 2004). Tiletamine may have significant effects on the CNS that may range from excitement and ataxia to severe seizures (Fahlman, 2008; Grimm and Lamont, 2007; Horne, 2001; Lin et al., 1992; Wenger, 2004). As severe seizures occur at higher tiletamine doses, tiletamine is often combined with zolazepam because of the anti-convulsant properties of the latter (Ferreira, 2016; Grimm and Lamont, 2007; Lin et al., 1992; Walzer and Huber, 2002; Wenger, 2004; Wilson et al., 1993). Zolazepam, a benzodiazepine derivative,
provides sedation, produce amnesia, assists in muscle relaxation and mitigates the side effects associated with the use of tiletamine (Deem and Citino, 1998; Ferreira, 2016; Lin et al., 1992; Naples et al., 2010; Walzer and Huber, 2002; Wenger, 2004; Wilson et al., 1993). Additional advantages of zolazepam include a relatively wide safety margin, minimal effects on the cardiopulmonary system, minimal chance of developing dependence, and can be effectively antagonised using flumazenil (Chinnadurai et al., 2016; Lin et al., 1992).

The tiletamine-zolazepam combination has an advantage over the use of ketamine as it eases the process of intubation without the additional administration of anaesthetic agents (Horne, 2001; Naples et al., 2010). The increased potency of tiletamine over ketamine considerably reduces the volume of tiletamine-zolazepam that is needed for effective anaesthesia which is beneficial when drugs are delivered by remote injection (Bush et al., 1977; Ferreira, 2016; Horne, 2001; Melis et al., 2012; Murphy et al., 2012; Unwin et al., 2009; Wenger, 2004). Even though tiletamine-zolazepam is sometimes preferred over the use of ketamine in order to avoid the side effects associated with ketamine use (Grimm and Lamont, 2007; Naples et al., 2010; Unwin et al., 2009; Wenger, 2004), tiletamine-zolazepam has its own side effects depending on the species that it is given to, the dose administered and the period of anaesthesia (Naples et al., 2010; Unwin et al., 2009; Wilson et al., 1993). Side effects associated with tiletamine-zolazepam include hypothermia, a decrease in ventilation (respiratory depression), a decrease in heart rate, certain neurological effects (like seizures and changes to behaviour) and potential gastrointestinal complications (Caulkett and Cattet, 1997; Ferreira, 2016; Lin et al., 1992; Naples et al., 2010; Wilson et al., 1993). Other studies found that the use of tiletamine-zolazepam has little effect on the cardiopulmonary and thermoregulatory systems (Horne, 2001; Jacquier et al., 2006; Sleeman, 2007; Unwin et al., 2009). As respiratory depression is a common side effect associated with the use of tiletamine-zolazepam, oxygen should be supplied to the individuals during anaesthesia (Jacquier et al., 2006).

The standard tiletamine-zolazepam dose is between 2 – 6 mg/kg depending on the specific primate species (Brainard and Darrow, 2013; Murphy, 2008; Unwin et al., 2009). Chimpanzees, in particular, generally require a dose of 3 – 4 mg/kg to provide adequate anaesthetic depth for approximately 60 min (Murphy, 2008; Unwin et al., 2009). Recovery from tiletamine-zolazepam anaesthesia is dose-dependent and the
combination is often associated with longer recovery times as it can only be partially reversed by reversing the zolazepam portion of the combination (Brainard and Darrow, 2013; Horne, 2001; Jacquier et al., 2006; Melis et al., 2012; Murphy, 2008; Naples et al., 2010; Unwin et al., 2009; Wenger, 2004; Wilson et al., 1993). Ketamine can also be used as a top-up in tiletamine-zolazepam anaesthesia. The additional administration of ketamine will have an impact on the recovery period, increasing the time until the individual has recovered (Unwin et al., 2009).

8.3.5.3.4. Tiletamine-zolazepam and medetomidine combination

In chimpanzees and most Southeast Asian primates, tiletamine–zolazepam–medetomidine produces complete and partially reversible anaesthesia with less of the side effects that are associated with the individual agents (Fahlman et al., 2006; Ferreira, 2016; Murphy, 2008; Naples et al., 2010). Additionally, the dose that is required for effective sedation is further reduced when medetomidine is added to a tiletamine-zolazepam combination (Ferreira, 2016; Naples et al., 2010). A tiletamine-zolazepam-medetomidine combination (administered at 3mg/kg IM or 0.1mg/kg PO) produces smooth inductions, excellent muscle relaxation, leads to smooth recoveries, reduces stress, and is ideal for anaesthetic periods longer than 30 min (Naples et al., 2010; Unwin et al., 2009). Side effects associated with this combination can vary from species to species. For example, hypertension and mild acidosis are characteristic side effects when using a tiletamine-zolazepam-medetomidine combination to anaesthetise black bears (Caulkett and Cattet, 1997), while bradycardia associated with medetomidine is not as pronounced when the tiletamine-zolazepam-medetomidine is used in cheetahs (Deem and Citino, 1998). Tiletamine-zolazepam-medetomidine-induced anaesthesia can be partially reversed using atipamezole and flumazenil (Ferreira, 2016). Recoveries from a tiletamine-zolazepam-medetomidine may be characterised by drowsiness, ataxia, and vomiting (Sleeman, 2007).

8.3.6. Analgesics

Analgesia is the process by which pain is relieved without the loss of consciousness (Machin, 2007). Good analgesia is considered an important part of an effective anaesthetic protocol as it will assist in the recovery of the individual after anaesthesia,
especially if potentially painful procedures have been performed (Machin, 2007; Murphy, 2008; Murphy et al., 2012; Unwin et al., 2009). Analgesics can be administered at any time during the procedures, but the most effective pain relief will be observed if analgesics are given before anaesthesia (Chinnadurai et al., 2016; DiVincenti Jr, 2013; Machin, 2007; Murphy et al., 2012). Recognising signs of pain and distress in primates are often difficult and the expression thereof may differ between species and individuals (DiVincenti Jr, 2013; Machin, 2007; Murphy et al., 2012). Signs of pain in chimpanzees may include changes in posture or behaviour; reluctance to move; facial grimaces; and distress vocalisations (DiVincenti Jr, 2013; Machin, 2007; Murphy et al., 2012). Administering oral analgesics may be beneficial in the long-term treatment of pain, preventing repeated injections and reducing the stress and discomfort experienced by the individual (Murphy, 2008; Murphy et al., 2012). However, oral analgesics may be refused by primates over time, making injectable analgesic more feasible (Chinnadurai et al., 2016; Murphy, 2008). Analgesic agents that are often used to treat pain in chimpanzees include opioids (buprenorphine, morphine, and fentanyl), nonsteroidal anti-inflammatories (NSAIDs like carprofen, and meloxicam), alpha-2-adrenergic agonists and other agents like ketamine (Brainard and Darrow, 2013; Chinnadurai et al., 2016; DiVincenti Jr, 2013; Ferreira, 2016; Horne, 2001; Machin, 2007; Murphy, 2008; Murphy et al., 2012; Unwin et al., 2009; Wenger, 2004; Zanos et al., 2018). These agents can also be combined to potentiate the side effects (including bradycardia, hypotension, respiratory depression and development of dependence) that may be observed when certain agents are used independently (Chinnadurai et al., 2016; DiVincenti Jr, 2013; Horne, 2001; Murphy, 2008; Randell and Kyttä, 1998; Wenger, 2004).

8.4. Management and monitoring Anaesthesia

8.4.1. Intubation and IV lines

Even though short-term anaesthesia in chimpanzees does not usually require intubation, intubation is always preferred to ensure that the airways remain open and unobstructed (Murphy et al., 2012; Unwin et al., 2009). Great apes have short tracheas and are prone to the occurrence of laryngospasms, complicating the intubation process which should be done while the animal is in dorsal or lateral recumbency (Adami et al., 2012; Brainard and Darrow, 2013; Horne, 2001; Murphy et al., 2012;
Sleeman, 2007; Unwin et al., 2009). Unfortunately, intubation can only be successful if all reflexes are absent and the animal is sedated enough to have a slack jaw tone (Brainard and Darrow, 2013). It is also recommended that a catheter with an IV line is attached to the femoral or saphenous veins for IV injection of anaesthetic agents for anaesthetic maintenance and preventing sudden arousals during longer anaesthetic procedures (Brainard and Darrow, 2013; Chinnadurai et al., 2016; Horne, 2001; Murphy et al., 2012; Sleeman, 2007; Unwin, 2005; Unwin et al., 2009). This will also assist in administering IV fluids used in the treatment of dehydration, hypotension and blood loss during procedures (Brainard and Darrow, 2013; Chinnadurai et al., 2016; DiVincenti Jr, 2013; Horne, 2001; Murphy et al., 2012; Unwin, 2005; Unwin et al., 2009). However, care should be taken not to overload individuals with fluids as it may result in complications such as hypervolemia, bladder distension, oedemas and death (Malbrain et al., 2020; Murphy et al., 2012; Navarro et al., 2015).

8.4.2. Monitoring Anaesthesia

It is important to monitor the individual during anaesthesia, regardless of the anaesthetic agent used (Ferreira, 2016; Heard, 2007; Murphy et al., 2012). Adequate monitoring will ensure that the procedure is carried out safely without excessive anaesthetic depth (Chinnadurai et al., 2016; Heard, 2007; Horne, 2001; Unwin et al., 2009). Adequate monitoring will identify respiratory depression and other instabilities during anaesthesia, decreasing the risk of anaesthesia related mortalities or complications (Chinnadurai et al., 2016; Grimm and Lamont, 2007; Sleeman, 2007). Any sudden changes in physiological parameters may indicate inadequate anaesthetic depth or lack of analgesia, increasing the risk of human injury (Ferreira, 2016; Heard, 2007; Unwin et al., 2009).
Table 1 Physiological parameters described for adult chimpanzees (*Pan troglodytes*).

<table>
<thead>
<tr>
<th>Physiological parameter</th>
<th>Ranges*</th>
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<tbody>
<tr>
<td>Body temperature (°C)</td>
<td>35.5 – 37.8 2,3,6</td>
</tr>
<tr>
<td>Heart Rate (bpm)</td>
<td>60 – 200 1,2,3,6</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>126 – 147 4</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>63 – 84 4</td>
</tr>
<tr>
<td>Respiratory rate (breaths/min)</td>
<td>10 – 15 5</td>
</tr>
</tbody>
</table>

*Ranges are based on a variety of literature: 1 Horne (2001), 2 Melis *et al.* (2012), 3 Johnson-Delaney (1994), 4 Ely *et al.* (2011), 5 Murphy *et al.* (2012), 6 Sleeman (2007). Abbreviations: bpm = beats per minute, °C = degrees Celsius mmHg= millimetres mercury*

Inadequate anaesthetic depth can be corrected by increasing the anaesthetic dose (either by increasing the vaporised setting of inhalant anaesthetics or injecting additional doses of the relevant anaesthetics), preventing painful stimuli, or administering an opioid analgesic (Adami *et al.*, 2012; Heard, 2007). Other methods that are often employed to assess anaesthetic depth also include a pinch response; palpebral and corneal reflexes; eye position, jaw tone; as well as respiration rate and depth (Adami *et al.*, 2012; Heard, 2007; Murphy *et al.*, 2012). Some of these methods are superficial (like palpebral reflexes and jaw pinch) and may vary significantly between individuals under anaesthesia (Horne, 2001), while other methods are more accurate when monitoring anaesthetic effects like mucous membrane colour, capillary refill time and physiological parameters (Ferreira, 2016; Heard, 2007; Horne, 2001; Sleeman, 2007; Unwin *et al.*, 2009; Zenker, 2004).

Most anaesthetic drug combinations will have also dose-dependent side effects that will affect temperature, respiration, and cardiovascular function (Unwin *et al.*, 2009). Some anaesthetic agents - like alpha-2-adrenergic agonists and acepromazine - may suppress the normal thermoregulatory ability of the individual, leading to significant changes in body temperature (Capuano *et al.*, 1999; Heard, 2007; Jalanka and Roeken, 1990; Klein and Klide, 1989; Murphy *et al.*, 2012; Unwin *et al.*, 2009; Wenger, 2004). Routine collection of arterial blood samples for blood gas analysis is useful when monitoring individuals during anaesthesia (Sleeman, 2007). Arterial blood gas (ABG) analysis also provides an accurate means of assessing cardiovascular and respiratory function under anaesthesia, as well as assessing oxygenation (PaO2), acid-base status (pH, lactate, base excess and HCO3-), and adequate ventilation...
(PaCO\textsubscript{2}) - thereby assessing the effect that anaesthesia has on physiological parameters (Bush \textit{et al.}, 1977; Fahlman, 2008, 2005; Heard, 2007; Horne, 2001; Murphy \textit{et al.}, 2012; Nakayama \textit{et al.}, 2018, 2017). Additional values that can be obtained from analysing ABG samples include the concentrations of various minerals (Ca\textsuperscript{++}, Cl\textsuperscript{-}, Na\textsuperscript{+} etc.) in the blood (Nakayama \textit{et al.}, 2017), which is particularly important to manage long-term anaesthesia (Murphy \textit{et al.}, 2012). Anaesthetic agents tend to interfere with normal respiratory function which result in respiratory depression, hypoxemia and respiratory acidosis caused by an increase in CO\textsubscript{2} in the blood (Fahlman, 2008; Heard, 2007). It is therefore valuable to assess the quality of respiration during anaesthesia (Fahlman, 2005). The duration of anaesthesia should be kept as short as possible to prevent the occurrence of complications (Paterson, 2007).

8.5. Recovery

After all procedures have been completed, the individual should be moved back to a quiet, separate, and secure room where it should be placed in lateral recumbency to recover (Ancrenaz \textit{et al.}, 2003; Brainard and Darrow, 2013; Ferreira, 2016; Murphy, 2008; Murphy \textit{et al.}, 2012; Sleeman, 2007; Unwin \textit{et al.}, 2009). Ideally, during recovery the head should be placed on the arm to raise the head, keeping the neck straight and bending the upper knee for stability (Figure 1) (Tribe and Spielman, 1996). Placing the individual in the correct position for recovery prevents the airway from being blocked as great apes tend to drop their chins towards their chest (Brainard and Darrow, 2013; Murphy, 2008). The period of recovery can be shortened if the anaesthetic agents were administered intravenously or intramuscularly instead of the oral route and can be further shortened if a specific antagonist is available (Bakker \textit{et al.}, 2013; Chinnadurai \textit{et al.}, 2016; Ferreira, 2016; Unwin \textit{et al.}, 2009; Young \textit{et al.}, 1999). Fluid and heat loss during procedures are unavoidable, but replacing fluids and providing heat during post-operative care can also reduce the recovery period (Díaz and Becker, 2010; Unwin, 2005). The endotracheal tube should only be removed once the swallow reflex and jaw tone have returned, while ensuring human safety (Chinnadurai \textit{et al.}, 2016; Murphy, 2008; Murphy \textit{et al.}, 2012; Unwin \textit{et al.}, 2009). The extubating of chimpanzees may lead to coughing, nausea and vomiting (Miyabe-Nishiwaki \textit{et al.}, 2021). In some cases, arousal may occur relatively quickly after protective reflexes
have returned (Murphy et al., 2012). All physiological parameters should still be monitored until the individual is able to it up (Unwin et al., 2009).

Figure 1 Ideal recovery position for chimpanzees during recovery. The head of the of the individual placed in the crook of the arm to raise the head and keep the neck straight. The knee of the upper leg bent for stability.
9. Aim and Objective
The aim of this study was to assess the effects that two anaesthetic combinations (medetomidine-ketamine or medetomidine-Zoletil®) have on the physiological parameters, heart rate, blood pressure, respiratory rate, oxygen saturation, end tidal CO₂, rectal temperature, arterial blood gas values and A-a gradients of chimpanzees (Pan troglodytes). This study also aimed to compare data on induction and recovery times, overall anaesthetic depth, and muscle relaxation between the two anaesthetic protocols, to determine if there is a difference in one of the anaesthetic combinations producing better anaesthetic characteristics. Even though we expected the measured physiological parameters to remain close to normal values described for chimpanzees, any differences between the two anaesthetic protocols was observed. We hypothesised that one of these anaesthetic combinations included into this study was going to produce better quality of anaesthesia while having minimal effects on physiological parameters and minimising the occurrence of sudden arousal.
10. Materials and Methods

An anaesthetic protocol comparison study was conducted on chimpanzees housed at the Jane Goodall Institute South Africa Chimp Eden (JGISA, Mbombela, South Africa). JGISA is a member of PASA (Pan African Sanctuary Alliance) - all housing and care requirements and all health assessments for all individuals at JGISA were managed in accordance with guidelines set out in the PASA operations manual (Bettinger et al., 2016). Apart from two individuals born at the sanctuary, all chimpanzees housed at JGISA were wild-born individuals that were orphaned by poachers, sold as illegal pets, or kept in zoos across the world. Individuals at the sanctuary are housed in three separated mixed-sex groups ranging in ages from 4 to 75 yrs old. All three groups have access to outside enclosures but are housed indoors at night for safety, observational and feeding purposes. The diet of the chimpanzees at JGISA mainly consists of fruits and vegetables, but the animals are also provided with porridge, cooked beans and hard-boiled eggs as supplementary food on a scheduled basis determined by sanctuary management. All staff working near the chimpanzees are required to wear masks and gloves to prevent the spread of zoonotic diseases. A footbath is present at the entrance to all night room facilities.

10.1. Induction and Monitoring

During November 2019, April 2021 and August 2021, a total of 22 chimpanzees were anaesthetised for their annual health assessments. Anaesthesia in these individuals was induced using either a medetomidine (Wildlife Pharmaceuticals, White River 1240, South Africa; 0.05 mg/kg) and ketamine (Kyron Laboratories, Johannesburg, 2094, South Africa; 5 mg/kg) combination (MedKet, n = 10) or a medetomidine (0.03 mg/kg) and tiletamine-zolazepam (Zoletil®, Pfitzer, Johannesburg, 2196, South Africa; 1.5 mg/kg) combination (MedZol, n = 12). The evening prior to each health assessment, all individuals received their normal evening feed after which food was withheld for approximately 12 – 18 hours prior to anaesthesia. Unfortunately, two individuals (one from each of the anaesthetic protocols) had access to food after they were let out of the night rooms due to logistical reasons. These individuals were successfully returned to the night rooms before anaesthesia was induced. Each chimpanzee was separated from the rest of the group
after they were premedicated with midazolam (Dormicum®, Roche, Johannesburg, 2196, South Africa; 15 mg) dissolved in a warm water and honey solution. Individuals received their premedication solution approximately 30 – 60 min before injection of the anaesthetic combinations. The volume of the solution in which midazolam was dissolved was kept as small as possible (approximately 30 ml) to prevent aspiration during anaesthesia.

All doses required to induce anaesthesia in each chimpanzee was estimated based on weights obtained during previous health assessments. All population characteristics, drug doses and anaesthetic durations are described in Table 1. Anaesthetic agents were administered to cooperative individuals via hand injection using a 3 ml syringe aimed at the muscles surrounding the wrist, shoulder, or thigh. Individuals were only darted if they remained uncooperative after several attempts to hand inject anaesthetic agents (n = 1). Darting was done using a 1 cc gel collar dart (Pneudart; Williamsport, PA, USA) with a 0.75-inch needle, delivered into the quadricep muscles by a Pneudart X-calibre CO₂ dart gun (Pneudart; Williamsport, PA, 17701, USA).

The time at which anaesthetic agents were administered and the time at which the first signs of sedation, ataxia, lowering of the head and open mouth breathing, were observed and recorded. The individual was moved from the night rooms to the on-site clinic or procedure table once it had been confirmed that the individual was recumbent and unresponsive to any external stimuli. Before conducting any health assessments (dental procedures; collection of blood and urine samples; tuberculosis [TB] screening and vasectomies), accurate weights for each individual were obtained using a hanging scale (Tianchen OCS-L Mini Portable Hanging Scale, Hangzhou Tianchen Scale Equipment, Hangzhou, China) and stretcher. Thereafter, the anaesthetised individual was placed in sternal recumbency and intubated using an 8 – 9 mm endotracheal tube to protect the airways during all procedures. Once intubated, the individual was placed in either lateral or dorsal recumbency on the procedure table depending on the procedure being conducted. The individual was repositioned as and when needed. Individuals were blindfolded to protect the eyes and reduce reaction to external stimuli. An IV catheter was inserted in either the cephalic or antebrachial veins for the administration of supplemental intravenous fluids and the administration of additional ketamine doses (1.5 mg/kg). Intravenous ketamine was administered at approximately
20 min of anaesthetic monitoring unless there were concerns that the anaesthetic depth was too light prior to that time point (Table 2). However, four individuals did not receive an additional ketamine dose as there was no concerns regarding anaesthetic depth. Anaesthetic depth was assessed by observing muscle tone, palpebral reflexes, and reaction to painful stimuli. Anaesthesia for longer procedures was maintained by placing the individual on 1 % to 2 % isoflurane after the physiological parameters were recorded over a period of 30 min of monitoring. The quality of induction, ease of intubation and muscle tone was subjectively scored (Appendix A).

The pulse-oximeter sensor of a handheld Meditech vital sign monitor (PC100 Handheld Vital Sign Monitor, Meditech, Wuhan, China) or a Cardell Veterinary Monitor (9500 HD, Midmark Cooperation, Versailles, USA) was attached to either the tongue or the lip, while a wide-stream capnography meter was attached to the endotracheal tube. The information displayed on these monitors included peripheral oxygen saturation (SpO₂), end tidal carbon dioxide (EtCO₂), heart rate (HR) and respiratory rate (RR) - these parameters were recorded on the anaesthetic sheet. If the vital signs monitor failed to measure HR and RR, data was obtained by counting the number of auscultations and number of breaths over a 20 second period (count multiplied by three to obtain the amount per minute). A blood pressure cuff was placed over the brachial artery, just above the elbow fold on the opposite arm in which the IV catheter was placed. The blood pressure cuff was attached to a non-invasive blood pressure monitor (Meditech, Wuhan, China). Systolic, diastolic, and mean arterial blood pressure displayed were recorded on the anaesthetic sheet. A rectal thermometer (Checktemp1, Hanna Instruments, Woonsocket, USA) was used to measure the temperature which was recorded on the anaesthetic sheet. All physiological data were recorded every 5 min starting approximately 10 min after the individual was removed from the enclosure.
An arterial catheter (22G) was placed in the dorsal, pedal, or tibial arteries to collect arterial blood samples (Figure 3). If the arterial catheter placement was unsuccessful, a direct arterial blood sample was collected from the femoral artery using a 23G needle and a 1 ml heparinised syringe. An arterial blood sample was taken before the administration of the ketamine top-up (MedZol = 8; MedKet = 4). However, in several individuals (MedZol, \( n = 2 \); MedKet, \( n = 4 \)) this was not possible before the ketamine top-up was administered (Table 3). Arterial samples were immediately analysed using a portable blood gas analyser (EPOC Blood Analysis System; Epocal; ON; Canada) and EPOC BGEM test cards (BGEM smart cards; Epocal; ON; Canada). A single set of blood gas analysis values (pH, partial pressures of oxygen, partial pressure of CO\(_2\), packed cell volume, as well as the concentration of sodium, calcium, potassium, chloride, glucose, lactate and creatinine) were obtained for each individual during the monitoring period. All variables were measured at 37 °C. The alveolar-arterial oxygen partial pressure gradient ((A-a)O\(_2\)) was calculated for an open system with constant pressure using the formula reported by Buss et al., (2015):

\[
(A-a)O_2 = \text{FiO}_2 \times (P_b - P_{H_2O}) - \text{PaCO}_2 - \text{PaO}_2
\]

where:

\(\text{FiO}_2\) = fractional inspired O\(_2\) (0.209)
P_b = measured barometric pressure (mmHg)
P_{H2O} = water vapour pressure of saturated air in alveoli (mmHg)
PaCO_2 = partial pressure of alveolar oxygen
PaO_2 = partial pressure of arterial carbon dioxide

The \( P_{H2O} \) was calculated as follows (Buss et al., 2015):
\[ P_{H2O} = 4.58 \times e^{[(17.27T_b)/(237.3 + T_b)]} \]

where:
\[ T_b = \text{Body temperature (°C)} \]

If an arterial catheter could successfully be placed, invasive blood pressure (diastolic, systolic, and mean arterial pressure) was continuously measured using a Deltran II pressure transducer (Utah Medical, Utah, United Stated) attached to the arterial catheter which was connected to an IntraTorr blood pressure monitor (IntraTorr, IntraVitals, United Kingdom). The transducer was placed on the chest in line with the base of the heart.

Figure 3 Placement of an arterial catheter for arterial blood collection and invasive IntraTorr blood pressure monitoring during chimpanzees under anaesthesia. Rectal thermometer (Checktemp1) was also inserted to obtain temperature reading during monitoring.

The arterial and IV catheters; non-invasive blood pressure monitor and cuff, pulse oximeter sensor; IntraTorr blood pressure monitor and pressure transducer; and rectal thermometer were all removed once procedures related to the health assessments
had been completed. The individual was returned to the night rooms and placed in lateral recumbency with an arm placed underneath the head while still isolated from the rest of the group (Figure 4). The medetomidine portion of both the anaesthetic protocols was reversed by hand injecting atipamezole (Wildlife Pharmaceuticals, White River, 1240, South Africa) into the semitendinosus or semimembranosus muscles at five times the initial medetomidine dose (in mg). The time that the atipamezole was injected was recorded. Monitoring continued until the swallow reflex and jaw tone returned, whereupon the endotracheal tube was removed. The safety of the observer was ensured by attaching a string to the tube which enabled the observer to remove the endotracheal tube from the outside of the enclosure, preventing injury and the potential of disease transmission. Further signs of recovery (blinking, lifting of the head, standing, and regaining full coordination) were monitored from the outside of the enclosure and recorded. A recovery score was also subjectively given and recorded on the anaesthetic sheet (refer to Appendix A for scoring). The individual was only re-introduced back into the rest of the group once the individual had regained full consciousness and mobility.

**Figure 4** After the completion of all procedures, chimpanzees were returned to the night rooms and placed in lateral recumbency for recovery. The endotracheal tube was removed as soon as the swallow reflex had returned.

### 10.2. Statistical Analysis

All data is reported as a mean ± standard deviation and was statistically analysed on Stata 14 (StataCorp, College Station, TX, USA). Normality of all descriptive data - heart rate, respiration rate, oxygen saturation, end tidal carbon dioxide, blood pressure
measurements, pH, partial pressure of oxygen and carbon dioxide, and other blood
gas analysis values (refer to Table 4) was estimated using histograms and a Shapiro-
Wilk’s test was used to assess the normality for all physiological parameters at the
different time points. Normally distributed physiological data, weights, induction time,
and recovery time were statistically compared between the two anaesthetic protocols
using a paired t-test with unequal variances. A series of mixed linear models were
conducted to compare the measured physiological parameters at the different time
points for the anaesthetic protocols. Furthermore, mixed linear models were applied
to determine whether the age, sex and body weight had any influence on physiological
data and to determine if the time at which the ketamine top-up was given had any
influence parameters. All p-values for mixed linear models were evaluated for
significance using Bonferroni adjustment for multiple comparisons to determine
significant differences within the same treatment and among different times. Statistical
significance for both the t-test, and Bonferroni adjustment for mixed linear models were
accepted for $p < 0.05$. 
11. Results

11.1. Induction and maintenance of anaesthesia

A preliminary assessment before anaesthesia determined all individuals included into this study were in good overall health. Cardiac function of all individuals are assessed biennially and individuals with known cardiac disease were not considered for this study. All individuals in the study voluntarily took the complete oral midazolam solution approximately 100.64 ± 92.41 minutes before anaesthesia was induced. However, one individual did receive an additional midazolam dose (approximately 5.5 hours after the initial dose) as anaesthesia could not be induced before the effects of midazolam wore off (Amrein et al., 1988; Prommer, 2020). The observed pre-anaesthetic activity level in most individuals were subjectively assessed as low to moderate (scored 2 – 3, refer to Appendix A). Most individuals were assessed to be either alert and aware of external stimuli, but remained relaxed; or attempted to prevent the administration of the anaesthetic agents (pre-anaesthetic demeanor scored 2 – 3, refer to Appendix A).

The observed induction time to achieve a safe plane of anaesthesia did not differ significantly between the two anaesthetic protocols ($p = 0.625$; MedKet = 7.52 ± 5.26 min, MedZol = 6.54 ± 3.54 min; Table 2, Figure 5). The time between administering the anaesthetic agents and the animal showing signs of sedation and the time from showing signs of sedation and recumbency was equally long ($p > 0.05$) for both anaesthetic protocols (refer to Table 2). Both anaesthetic treatments produced a light to surgical plane of anaesthesia (scored 3 – 4, refer to Appendix A) in all individuals with a good degree of muscle relaxation (scored 1 – 2, refer to Appendix A) with overall muscle twitching limited or only occurring in focal areas of the body like the eyes, hands, and feet. Intubation in all individuals was assessed to be easy to moderate (scored 1 – 2, refer to Appendix A) with the occurrence of limited coughing, and no additional administration of anaesthetic agents was not needed to facilitate intubation. However, no difference ($p > 0.05$) was observed between the two anaesthetic protocols when the ease of intubation scores were compared. Mucous membrane colour and capillary refill time remained satisfactory throughout anaesthesia. The body weight for individuals for the MedZol group (58.32 ± 9.95 kg) was also not statistically different ($p = 0.288$) when compared to the MedKet group (54.29 ± 7.34 kg). The weights of the individuals included into the study were over- or underestimated by a
mean of 8%. Furthermore, the correlation between estimated and actual weight was good \((R = 0.91; p < 0.05)\).

**Table 2** Population characteristics (age and sex), time data (induction and recovery time) and drug doses administered for 22 chimpanzees (*Pan troglodytes*) in which anaesthesia was induced using either a medetomidine-Zoletil® (MedZol, \(n = 12\)) or a medetomidine-ketamine (MedKet, \(n = 10\)) combination. Anaesthetic agents were administered via hand injection. However, one individual from the MedKet group was darted due to unwillingness to allow hand injection.

<table>
<thead>
<tr>
<th>Anaesthetic protocol</th>
<th>MedZol</th>
<th>MedKet</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number of individuals ((n))</strong></td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>(4 females; 8 males)</td>
<td>(7 females; 3 males)</td>
</tr>
<tr>
<td><strong>Age (yrs)</strong></td>
<td>18.17 ± 6.63</td>
<td>18.90 ± 7.08</td>
</tr>
<tr>
<td></td>
<td>Range: 8 – 30</td>
<td>Range: 11 – 33</td>
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<tr>
<td><strong>Estimated body weight (kg)</strong></td>
<td>57.09 ± 11.21 d</td>
<td>54.28 ± 8.20 d</td>
</tr>
<tr>
<td></td>
<td>Range: 41.85 – 76.30</td>
<td>Range: 42.10 – 64.60</td>
</tr>
<tr>
<td><strong>Actual body weight (kg)</strong></td>
<td>58.32 ± 9.95 d</td>
<td>54.29 ± 7.34 d</td>
</tr>
<tr>
<td></td>
<td>Range: 35.40 – 76.30</td>
<td>Range: 42.30 – 64.60</td>
</tr>
<tr>
<td><strong>Midazolam (mg)</strong></td>
<td>16.25 ± 4.33 a</td>
<td>15.00 ± 0.00</td>
</tr>
<tr>
<td><strong>Midazolam (mg/kg)</strong></td>
<td>0.28 ± 0.08</td>
<td>0.28 ± 0.04</td>
</tr>
<tr>
<td><strong>Total injection volume (ml)</strong></td>
<td>0.94 ± 0.28</td>
<td>1.55 ± 0.29</td>
</tr>
<tr>
<td><strong>Induction medetomidine dose (mg/kg)</strong></td>
<td>0.03 ± 0.01</td>
<td>0.05 ± 0.01</td>
</tr>
<tr>
<td><strong>Induction Zoletil® dose (mg/kg)</strong></td>
<td>1.52 ± 0.19</td>
<td>-</td>
</tr>
<tr>
<td><strong>Induction ketamine dose (mg/kg)</strong></td>
<td>-</td>
<td>5.50 ± 1.12</td>
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<tr>
<td><strong>Top-up Ketamine dose (total mg)</strong></td>
<td>82.80 ± 14.50 b</td>
<td>80.79 ± 21.36 b</td>
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<tr>
<td></td>
<td>-</td>
<td>242.72 ± 0.00 c</td>
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<tr>
<td>Top-up ketamine dose (mg/kg)</td>
<td>1.23 ± 0.58&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.13 ± 0.69&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>-----------------------------------</td>
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<td>--------------------------</td>
</tr>
<tr>
<td>Top-up medetomidine dose (total mg)</td>
<td>5.74 ± 0.00&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.43 ± 0.00&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>Top-up medetomidine dose (mg/kg)</td>
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<tr>
<td>Total medetomidine dose (mg/kg)</td>
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<td>0.06 ± 0.02</td>
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<tr>
<td>Total Zoletil® dose (mg/kg)</td>
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<tr>
<td>Total Ketamine dose (mg/kg)</td>
<td>1.22 ± 0.58&lt;sup&gt;e&lt;/sup&gt;</td>
<td>7.10 ± 1.96</td>
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<td>Atipamezole dose (total mg)</td>
<td>9.15 ± 2.77</td>
<td>13.24 ± 2.38</td>
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<tr>
<td>Atipamezole dose (mg/kg)</td>
<td>0.16 ± 0.03</td>
<td>0.24 ± 0.03</td>
</tr>
<tr>
<td>Overall induction time (min)</td>
<td>6.54 ± 3.54&lt;sup&gt;d&lt;/sup&gt;</td>
<td>7.52 ± 5.26&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>Range: 2 – 14</td>
<td>Range: 1 – 8</td>
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<tr>
<td>First sign of sedation (min)</td>
<td>2.33 ± 1.05&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3.6 ± 4.02&lt;sup&gt;d&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>Range: 1 – 5</td>
<td>Range: 0.5 – 14.5</td>
</tr>
<tr>
<td>Time to recumbency (min)</td>
<td>4.21 ± 3.33&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3.91 ± 2.04&lt;sup&gt;d&lt;/sup&gt;</td>
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<td></td>
<td>Range: 1 – 12</td>
<td>Range: 1 – 8</td>
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<tr>
<td>Overall recovery time (min)</td>
<td>20.29 ± 7.83&lt;sup&gt;d&lt;/sup&gt;</td>
<td>22.57 ± 9.63&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>Range: 9 – 32</td>
<td>Range: 10 – 38</td>
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<tr>
<td>First sign of recovery (min)</td>
<td>15.12 ± 7.51&lt;sup&gt;d&lt;/sup&gt;</td>
<td>13.11 ± 8.37&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>Range: 1 – 27</td>
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<td>Time to head up &amp; sitting (min)</td>
<td>13.14 ± 6.78&lt;sup&gt;d&lt;/sup&gt;</td>
<td>18.87 ± 10.67&lt;sup&gt;d&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>Range: 4 – 23.5</td>
<td>Range: 7 – 38</td>
</tr>
</tbody>
</table>

<sup>a</sup> One individual received an additional midazolam dose approximately 5 hours after the initial dose was administered.
<sup>b</sup> Individuals that only received an IV ketamine dose (<i>n</i> = 17)
<sup>c</sup> One individual received an IM Medket top-up instead of IV ketamine top-up, administered after the pre-determined monitoring period
<sup>d</sup> Difference between two treatments not significant (<i>p</i> > 0.05)
<sup>e</sup> Total dose (mg/kg) refers to the entire dose of agents administered to individuals (induction dose + top-up dose)
11.2. Duration of anaesthesia

Total anaesthesia time was defined as the time at which the individual was approached and removed from the enclosure until the time at which the reversal agent (atipamezole) was administered. The mean anaesthetic time observed was statistically insignificant between the two anaesthetic protocols (MedKet: 80.6 ± 42.84 min; MedZol: 56.75 ± 13.12 min; \( p = 0.081 \)) were compared. Individuals were placed on isoflurane to maintain anaesthesia during extensive dental procedures.

Figure 5 Box-and-whiskers plot of the overall induction time (min) observed in chimpanzees when anaesthesia was induced with either a medetomidine-Zoletil® (MedZol; \( n = 12 \)) or a medetomidine-ketamine (MedKet; \( n = 10 \)) combination. Overall induction time was observed from the time that the anaesthetic agents were administered to the individual until the individual was recumbent. Even though the overall induction time observed (mean [95% confidence intervals]) was longer when anaesthesia was induced with a MedKet combination (7.52 min [95% CI: 3.76, 11.27]) compared to when anaesthesia was induced with MedZol combination (6.54 min [95% CI: 4.29, 8.79]), no statistical difference was observed (\( p > 0.05 \)). Some individuals in both anaesthetic protocols had longer induction times as indicated by the outliers.

11.3. Anaesthetic maintenance

In this study, anaesthetic depth was maintained throughout monitoring using the administration of additional anaesthetic agents. Apart from four individuals, all individuals received a top-up of anaesthetic agents to maintain anaesthesia. Intravenous ketamine top-ups (1.5 mg/kg) were administered to most individuals at 20 min after the individual was approached (MedZol = 8, MedKet = 4, refer to Table 3),
while some received their top-ups before 20 min (MedZol = 1; MedKet = 2) or after 20 min (MedZol = 1; Table 3). In addition to the IV ketamine top-ups administered, one individual from the MedKet group, received an IM MedKet top-up at a similar dose as the induction dose after the predetermined monitoring time, which would not have affected the readings for physiological monitoring. Anaesthesia for longer procedures was maintained by the administration of 1 - 2% isoflurane which commenced only once the 30 min monitoring period had ended (MedKet = 4; MedZol = 4).

Table 3 Administration of intravenous ketamine top-up and arterial blood gas (ABG) sample collection from 22 chimpanzees in which anaesthesia was induced using either a medetomidine-Zoletil® combination (MedZol; n = 12) or medetomidine-ketamine combination (MedKet; n = 10). Intravenous ketamine top-ups were administered to 3 individuals (MedZol = 1; MedKet = 2) before 20 minutes, 12 individuals (MedZol = 8; MedKet = 4) at 20 minutes and 1 individual (MedZol), while one individual from the MedKet anaesthetic protocol received an intramuscular MedKet top-up after the monitoring period. Four chimpanzees did not receive any top-up during the monitoring period (MedZol = 2, MedKet = 2. ABG samples was collected from 12 individuals (MedZol = 8, MedKet = 4) before ketamine top-up was administered and collected from 6 individuals (MedZol= 2, MedKet= 4) after ketamine administration in.

<table>
<thead>
<tr>
<th>Time after recumbency</th>
<th>MedZol N = 12</th>
<th>MedKet N = 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Top-up given</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ABG sample collected</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>ABG sample collected</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Top-up given</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>ABG sample collected</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Top-up given</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ABG sample collected</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Top-up given</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>ABG sample collected</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Top-up given</td>
<td>2</td>
<td>1 (1*)</td>
</tr>
<tr>
<td>ABG sample collected</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>After monitoring</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

* One individual received a ketamine top-up (IV), while one individual received a MedKet top-up (IM)
11.4. Physiological parameters

According to the results from the Shapiro-Wilk’s test, all physiological data were normally distributed for both anaesthetic protocols ($p > 0.05$). Analysis using a mixed linear model, indicated that age and sex had no significant impact on any of the physiological parameters measured in this study, while body weight significantly ($p < 0.05$) impacted the HR, SBP and SpO$_2$ measurements in this study. The physiological parameters observed and recorded for chimpanzees under MedKet and MedZol anaesthesia have been summarized in Table 4.
Table 4: Physiological parameters measured for chimpanzees \( (n = 22) \) at individual time points after recumbency for two anaesthetic protocols, medetomidine-Zoletil® (MedZol; \( n = 12 \)) and medetomidine-ketamine (MedKet; \( n = 10 \)). Heart rate \( (\text{bpm}) \), respiratory rate \( (\text{breaths/min}) \), percentage oxygen saturation \( (\text{SpO}_2; \%) \), end tidal carbon dioxide \( (\text{EtCO}_2, \text{mmHg}) \), rectal temperature \( (^\circ \text{C}) \), non-invasive systolic, diastolic and mean arterial blood pressure \( (\text{SBP, DBP and MAP, mmHg}) \) reported as mean ± standard deviation. Ketamine top-ups to maintain anaesthetic depth was administered at 10 min \( (\text{MedZol} = 1; \text{MedKet} = 2) \), 20 min \( (\text{MedZol} = 8; \text{MedKet} = 4) \) and 30 min \( (\text{MedZol} = 1; \text{MedKet} = 0) \). The effects of ketamine will only be observed at the next point of monitoring.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Time after recumbency</th>
<th>MedZol</th>
<th>MedKet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart Rate (bpm)</td>
<td>Overall: 53.80 ± 10.92</td>
<td>58.45 ± 7.43</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10: 54.17 ± 9.54</td>
<td>60.63 ± 7.65</td>
<td></td>
</tr>
<tr>
<td></td>
<td>15: 53.92 ± 9.51</td>
<td>58.10 ± 6.82</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20: 53.25 ± 12.89</td>
<td>60.50 ± 7.68</td>
<td></td>
</tr>
<tr>
<td></td>
<td>25: 52.58 ± 12.67</td>
<td>58.30 ± 9.12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30: 55.08 ± 11.33</td>
<td>55.3 ± 5.87</td>
<td></td>
</tr>
<tr>
<td>Respiratory Rate (RR) (breaths/min)</td>
<td>Overall: 26.46 ± 14.17</td>
<td>20.73 ± 10.12</td>
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</tr>
<tr>
<td></td>
<td>10: 29.83 ± 10.70</td>
<td>20.44 ± 12.59</td>
<td></td>
</tr>
<tr>
<td></td>
<td>15: 32.58 ± 23.74</td>
<td>21.5 ± 9.95</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20: 22.64 ± 6.07</td>
<td>21.90 ± 12.11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>25: 21.36 ± 5.77</td>
<td>19.90 ± 9.49</td>
<td></td>
</tr>
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<td>30: 25.17 ± 14.28</td>
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</tr>
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<td>SpO₂ (% saturation)</td>
<td>Overall: 85.68 ± 8.41</td>
<td>87.85 ± 5.56</td>
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<td>10: 84.09 ± 7.53</td>
<td>85.63 ± 5.97</td>
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</tr>
<tr>
<td></td>
<td>15: 83.83 ± 10.21</td>
<td>89.78 ± 6.22</td>
<td></td>
</tr>
<tr>
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<td>20: 87.00 ± 7.51</td>
<td>87.10 ± 4.36</td>
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<td>25: 86.36 ± 9.06</td>
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<td></td>
<td>30: 87.17 ± 8.23</td>
<td>88.00 ± 5.50</td>
<td></td>
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<tr>
<td>EtCO₂ (mmHg)</td>
<td>Overall: 43.92 ± 10.53</td>
<td>37.74 ± 11.21</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10: 44.00 ± 13.41</td>
<td>35.29 ± 12.51</td>
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</tr>
<tr>
<td></td>
<td>15: 45.55 ± 12.55</td>
<td>37.63 ± 11.34</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20: 43.10 ± 9.48</td>
<td>37.67 ± 11.16</td>
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<td></td>
<td>25: 43.10 ± 9.54</td>
<td>38.78 ± 12.20</td>
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<tr>
<td></td>
<td>30: 43.73 ± 8.87</td>
<td>38.78 ± 11.56</td>
<td></td>
</tr>
<tr>
<td>Rectal temperature (°C)</td>
<td>Overall: 36.58 ± 0.89</td>
<td>36.51 ± 0.68</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10: 37.05 ± 0.85</td>
<td>36.63 ± 0.60</td>
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</table>

48
<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Non-invasive systolic blood pressure (mmHg)</th>
<th>Overall</th>
<th>139.84 ± 23.35</th>
<th>126.98 ± 16.74</th>
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<tr>
<td>10</td>
<td>148.42 ± 25.13</td>
<td>127.29 ± 27.96</td>
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</tr>
<tr>
<td>15</td>
<td>143.08 ± 21.18</td>
<td>128.56 ± 17.10</td>
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<td></td>
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<tr>
<td>25</td>
<td>137.09 ± 22.45</td>
<td>125.9 ± 13.35</td>
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</tr>
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<td>30</td>
<td>131.17 ± 27.28</td>
<td>122.3 ± 12.02</td>
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</table>

<table>
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<th>Time (min)</th>
<th>Non-invasive diastolic blood pressure (mmHg)</th>
<th>Overall</th>
<th>75.19 ± 17.25</th>
<th>66.63 ± 15.84</th>
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<td>15</td>
<td>78.33 ± 14.93</td>
<td>68.11 ± 14.4</td>
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<td>20</td>
<td>75.33 ± 17.76</td>
<td>67.8 ± 15.84</td>
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<tr>
<td>25</td>
<td>70.27 ± 17.23</td>
<td>66.2 ± 16.69</td>
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<tr>
<td>30</td>
<td>69.33 ± 19.50</td>
<td>60.6 ± 13.64</td>
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</table>

<table>
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<tr>
<th>Time (min)</th>
<th>Non-invasive mean arterial blood pressure (mmHg)</th>
<th>Overall</th>
<th>100.17 ± 20.55</th>
<th>90.26 ± 18.67</th>
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<tr>
<td>30</td>
<td>92.58 ± 23.31</td>
<td>83.00 ± 12.74</td>
<td></td>
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</tr>
</tbody>
</table>

* Significant difference observed between the two anaesthetic protocols.

Statistical analysis was conducted for each physiological parameter at the different time points did not show any statistical significance \((p > 0.05)\). Abbreviations: bpm = beats per minute; breaths/min = breaths per minute; mmHg = millimeters mercury; °C = degrees Celsius.

### 11.4.1. Cardiovascular parameters

The overall mean HR observed (Figure 6) for individuals treated with MedKet (58.48 ± 7.43 bpm) was significantly higher \((p = 0.0096)\) when compared to individuals treated with MedZol (53.80 ± 10.92 bpm). Comparing HR using a mixed linear model and Bonferroni adjustment indicated that there was no statistical difference in HR at 20 min in the MedKet group \((p = 0.089)\) compared to the MedZol group, even though HR was higher in the MedKet group. Furthermore, a decrease in HR was also observed in the MedKet group at 30 minutes when time points were compared to the initial reading at 10 minutes \((p = 0.145)\), but was not observed in the MedZol group.
The overall non-invasive systolic, diastolic, and mean blood pressures (SBP, DBP, and MAP) measured during monitoring was significantly higher \((p < 0.05)\) when anaesthesia was induced with MedZol \((139.84 \pm 23.35\) mmHg; \(75.19 \pm 17.25\) mmHg; and \(100.17 \pm 20.55\) mmHg, respectively) compared to when anaesthesia was induced using MedKet \((126.98 \pm 16.74\) mmHg; \(66.63 \pm 15.84\) mmHg; and \(90.26 \pm 18.67\) mmHg, respectively). Blood pressure (SBP, DBP and MAP) when anaesthesia was induced with MedZol was initially elevated and decreased over time (Figure 7 A – C). Invasive blood pressure readings using an arterial catheter and the IntraTorr blood pressure monitor could not be consistently obtained for all 22 chimpanzees included into the study \((\text{MedZol: } n = 6; \text{MedKet: } n = 5)\).

Mixed linear model analysis indicated significant decreases in SBP for the MedZol group at time points 15, 20, 25 and 30 minutes and for MedKet at 25 and 30 minutes \((p < 0.05)\). The mixed linear model also indicated that significant decreases occurred when time points were compared to the initial readings at 10 for MedZol at 20, 25 and 30 minutes \((p < 0.05)\) while SBP only significantly decreased when time point 30 minutes was compared to 10 minutes \((p = 0.000)\). Diastolic blood pressure (DBP) showed significant decreases for both anaesthetic protocols at 20, 25 and 30 minutes \((p < 0.05)\) and significant change occurred when the time points 20, 25 and 30 minutes were compared to the initial readings for both the treatments \((p < 0.05)\). Mean arterial pressure (MAP) for the MedZol protocol showed significant decreases at 15, 25 and 30 minutes \((p < 0.05)\), and MedKet protocol showed significant decrease at 15 and 30 minutes \((p < 0.05)\). Mixed linear model also indicated that there was a significant decrease in MedZol when time points 25 and 30 were compared to initial readings \((p < 0.05)\) and in the MedKet treatment when 30 min were compared to 10 min \((p = 0.005)\).

Mixed linear models and Bonferroni adjustment for multiple comparisons were used to assess the influence that the ketamine top-up had on cardiovascular parameters. Statistical analysis indicated that even though HR, SBP and DBP were not influenced by ketamine top-up irrespective of the time administered, there was a significant decrease \((p = 0.000)\) in MAP if ketamine top-up was administered before 15 minutes of monitoring \((n = 3)\).
The observed mean heart rate (beats per minute, bpm) that was measured at predetermined time points after recumbency (min) in chimpanzees that received either a medetomidine-Zoletil® (MedZol, $n = 12$) or a medetomidine-ketamine (MedKet, $n = 10$) anaesthetic combination. Heart rate was displayed on the pulse oximeter, or by counting the number of auscultations over 20 seconds (multiplied by 3 to obtain the count over 1 minute). Overall heart rate (mean ± standard deviation) in individuals treated with MedKet ($58.48 \pm 7.43$ bpm) was significantly higher than in individuals treated with MedZol, ($53.80 \pm 10.92$ bpm; $p = 0.0096$). Ketamine top-ups were administered at 10 min (MedZol = 1; MedKet = 2), 20 min (MedZol = 8; MedKet = 4), and 30 min (MedZol = 1; MedKet = 0). Two individuals only received top-up (ketamine = 1; MedKet = 1) after completion of the anaesthetic monitoring and did not affect the measured heart rate. Mixed linear model with a Bonferroni adjustment indicated no significant change in HR irrespective of the time the ketamine top-up was administered ($p < 0.05$).
Figure 7 Effect of medetomidine-Zoletil® (MedZol; *n* = 12) and medetomidine-ketamine (MedKet, *n* = 10) anaesthesia on A) systolic blood pressure (SBP, mmHg), B) diastolic blood pressure (DBP, mmHg) and C) mean arterial blood pressure (MAP, mmHg) in chimpanzees measured over a period of 30 minutes using a non-invasive blood pressure cuff and monitor. The mean (± standard deviation) for SBP (MedZol = 139.84 ± 23.35 mmHg; MedKet = 126.98 ± 16.74 mmHg), DBP (MedZol = 75.19 ± 17.25 mmHg; MedKet = 66.63 ± 15.84 mmHg) and MAP (MedZol = 100.17 ± 20.55 mmHg; MedKet = 90.26 ± 18.67 mmHg). The overall blood
pressure parameters (SBP, DBP and MAP) were all significantly higher when anaesthesia was induced with MedZol compared to when individuals were treated with MedKet ($p < 0.05$). No statistical difference at any of the individual time points were observed when the two anaesthetic protocols were compared using a t-test ($p > 0.05$). Ketamine top-ups ($n = 16$) were administered at 10 min (MedZol = 1; MedKet = 2), 20 min (MedZol = 8; MedKet = 4), and 30 min (MedZol = 0; MedKet = 1) of monitoring. Mixed linear models and Bonferroni adjustment indicated that there was a significant decrease in MAP after the administration if a ketamine top-up was administered at 10 min after recumbency ($p = 0.000$). No changes in SBP and DBP were observed after the administration of ketamine top-up ($p < 0.05$).

11.4.2. Rectal temperature

Observed rectal temperature for MedZol was 36.58 ± 0.89 °C while temperature for MedKet was 36.51 ± 0.68 °C. Rectal temperature remained stable over the monitoring time and was not significant different between treatments ($p = 0.64$).

11.4.3. Pulmonary characteristics

Overall respiratory rate (breaths/min) was higher ($p = 0.016$) when anaesthesia was induced with MedZol (26.46 ± 14.17 breaths/min) when compared to anaesthesia induced with MedKet (20.73 ± 10.12 breaths/min). Respiratory rate for MedKet remained relatively stable over time (Figure 8).

Peripheral oxygen saturation (SpO$_2$) measured indicated that MedKet (87.85 ± 5.56 %) did not differ significantly ($p = 0.119$) to MedZol (85.68 ± 8.41 %; Figure 9). Overall end tidal carbon dioxide (EtCO$_2$) measurements were significantly higher ($p = 0.009$) when anaesthesia was induced in individuals with MedZol (43.92 ± 10.53 mmHg) compared to when anaesthesia was induced with MedKet (37.74 ± 11.21 mmHg). EtCO$_2$ for both anaesthetic treatments followed a similar trend (Figure 10).

Mixed linear models with Bonferroni adjustment for multiple comparisons indicated no significant differences ($p < 0.05$) when RR, SpO$_2$ and EtCO$_2$ were compared between the two anaesthetic protocols at the different time points. RR and EtCO$_2$ was higher when anaesthesia were induced with MedZol when compared to MedKet induced anaesthesia at 15 minutes of monitoring, but was not significantly different ($p = 0.209$ and $p = 0.210$).

Evaluating the influence that ketamine top-ups had on the pulmonary characteristics using a mixed linear model indicated that SpO$_2$ did not show any significant changes irrespective of the time that the ketamine top-up was administered ($p = 0.091$). Statistical testing, however, did not indicate a statistical difference in
EtCO$_2$ when ketamine top-up was administered before 15 minutes ($p = 0.193$) or in RR when the ketamine top-up was administered before 20 minutes ($p = 0.091$).

**Figure 8** Respiratory rate (RR, breaths/min) observed over a 30-minute time period after chimpanzees received either a medetomidine-Zoletil® (MedZol, $n = 12$) or a medetomidine-ketamine (MedKet, $n = 10$) anaesthetic combination. Overall RR (mean ± standard deviation) was significantly higher when anaesthesia was induced with MedZol (26.46 ± 14.17 breaths/min) compared to MedKet (20.73 ± 10.12 breaths/min; $p = 0.016$). Mixed linear models indicated no significant differences were observed between the anaesthetic protocols at the different time points ($p > 0.05$). Ketamine top-ups were administered at 10 min (MedZol = 1; MedKet = 2), 20 min (MedZol = 8; MedKet = 4), and 30 min (MedZol = 1; MedKet = 0). The administration of ketamine did not have an immediate effect and the ketamine effect were only observed at the next monitoring point. Mixed linear model and Bonferroni adjustment did indicate a decrease in RR if ketamine top-up were administered before 20 minutes of monitoring, but the difference was not significant ($n = 3; p = 0.091$).
Figure 9 Oxygen saturation (mean ± standard deviation; SpO_2, %) observed over time after recumbency (min) when anaesthesia was induced in chimpanzees with either a medetomidine-Zoletil® (MedZol; n = 12; 85.68 ± 8.41 %) or medetomidine-ketamine (MedKet; n = 10; 87.85 ± 5.56 %) combination as determined by pulse oximetry. Although SpO_2 remained higher in the MedKet group over time, no statistical differences were observed between the two treatments (p = 0.119). Ketamine top-ups were administered to 16 individuals at 10 min (MedZol = 1; MedKet = 2), 20 min (MedZol = 8; MedKet = 4), and 30 min (MedZol = 1; MedKet = 0). No statistical differences were observed in SpO_2 after the ketamine top-up was administered when mixed linear models with Bonferroni adjustments were conducted (p < 0.05).
Figure 10 End tidal carbon dioxide (EtCO₂, mmHg) in 22 chimpanzees as determined by capnography monitoring. Individuals treated with medetomidine-Zoletil® (MedZol; n = 12; 43.92 ± 10.53 mmHg) had a significantly higher overall EtCO₂ when compared to medetomidine-ketamine combination (MedKet; n = 10; 37.74 ± 11.21 mmHg; p = 0.009). Mixed linear model corrected with a Bonferroni adjustment indicated no statistical differences between the two anaesthetic protocols or at the different time point (p < 0.05). Ketamine top-ups were administered at 10 min (MedZol = 1; MedKet =2), 20 min (MedZol = 8; MedKet = 4), and 30 min (MedZol = 1; MedKet = 0). The administration of ketamine top-up (n = 16) at 10 minutes of monitoring did indicate a decrease in EtCO₂ when mixed linear models were conducted with a Bonferroni adjustment. However, this decrease was not significant (p = 0.193).

11.4.4. Arterial blood gas analysis

A single arterial blood (ABG) sample was collected at approximately 22.27 ± 6.31 min (mean ± standard deviation) after anaesthesia was induced from each individual included in the study. The time at which ABG samples are reported in Table 5. ABG samples were collected from 12 chimpanzees (MedZol = 8; MedKet = 4) before the ketamine top-up was administered and 6 chimpanzees (MedZol = 2; MedKet = 4) after the ketamine top-up was administration. Four individuals (MedZol = 2; MedKet = 2) did not receive a ketamine top-up and their ABG samples were not affected by the ketamine top-up.

Arterial blood samples were analysed and values for the two anaesthetic protocols were statistically compared. None of the blood gas analysis values showed any significant difference between treatments, and all values fell within the clinically
acceptable reference ranges for chimpanzees (Table 5) (Howell et al., 2003). The mean partial pressure of oxygen (PaO₂, Figure 11A) and carbon dioxide (PaCO₂, Figure 11B) were not significantly different ($p > 0.05$) when anaesthesia was induced MedKet (60.95 ± 7.69 mmHg and 43.82 ± 2.59 mmHg, respectively) compares to when anaesthesia was induced with MedZol (59.79 ± 11.43 mmHg and 43.23 ± 4.89 mmHg, respectively). The overall A-a gradient also did not differ significantly between treatments ($p = 0.904$; Table 4, Figure 12). Barometric pressure during the health assessments varied between 670 - 770 mmHg.
Table 5 Arterial blood gas values (mean ± standard deviation) for 22 chimpanzees obtained using a portable EPOC gas analyser in which anaesthesia was either induced with a medetomidine-Zoletil® (MedZol, n = 12) combination or a medetomidine-ketamine (MedKet, n = 10) combination. Arterial blood samples were collected approximately 22.27 ± 6.31 minutes after anaesthesia was induced. Blood samples were collected from n = 12 individuals before the administration of top-up (MedZol, n = 8, MedKet, n = 4), while blood samples were obtained from 6 individuals (MedZol, n = 2 and MedKet, n = 4) after the top-up was administered. No marked statistical significance was observed in any of the parameters measured (p > 0.05).

<table>
<thead>
<tr>
<th>Parameter</th>
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<th>MedKet</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.40 ± 0.02</td>
<td>7.39 ± 0.02</td>
<td>0.131</td>
</tr>
<tr>
<td>PaCO₂ (mmHg)</td>
<td>43.23 ± 4.89</td>
<td>43.85 ± 2.59</td>
<td>0.645</td>
</tr>
<tr>
<td>PaO₂ (mmHg)</td>
<td>59.79 ± 11.43</td>
<td>60.95 ± 7.69</td>
<td>0.872</td>
</tr>
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<td>HCO₃⁻ (mmol/L)</td>
<td>26.99 ± 2.43</td>
<td>26.79 ± 1.88</td>
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<tr>
<td>BE (mmol/L)</td>
<td>2.27 ± 2.4</td>
<td>1.86 ± 2.11</td>
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<td>SO₂ (%)</td>
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<td>90.09 ± 3.43</td>
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</tr>
<tr>
<td>Na (mmol/L)</td>
<td>140.58 ± 7.29</td>
<td>141.88 ± 3.91</td>
<td>0.867</td>
</tr>
<tr>
<td>K (mmol/L)</td>
<td>3.72 ± 0.45</td>
<td>3.71 ± 0.45</td>
<td>0.639</td>
</tr>
<tr>
<td>Ca (mmol/L)</td>
<td>1.09 ± 0.13</td>
<td>1.14 ± 0.05</td>
<td>0.187</td>
</tr>
<tr>
<td>Cl⁻ (mmol/L)</td>
<td>103.73 ± 7.14</td>
<td>105 ± 4.21</td>
<td>0.669</td>
</tr>
<tr>
<td>TCO₂</td>
<td>28.35 ± 2.55</td>
<td>28.13 ± 1.98</td>
<td>0.586</td>
</tr>
<tr>
<td>Agap (mmol/L)</td>
<td>9.73 ± 1.35</td>
<td>10.13 ± 2.30</td>
<td>0.928</td>
</tr>
<tr>
<td>Hct (% PCV)</td>
<td>41.58 ± 4.36</td>
<td>41.0 ± 5.24</td>
<td>0.928</td>
</tr>
<tr>
<td>Glu (mmol/L)</td>
<td>6.88 ± 1.27</td>
<td>7.08 ± 1.90</td>
<td>0.720</td>
</tr>
<tr>
<td>Lac (mmol/L)</td>
<td>0.80 ± 0.35</td>
<td>0.73 ± 0.29</td>
<td>0.504</td>
</tr>
<tr>
<td>Crea (mg/kg)</td>
<td>88.75 ± 16.87</td>
<td>78.00 ± 24.71</td>
<td>0.335</td>
</tr>
<tr>
<td>A-a gradient</td>
<td>31.85 ± 5.20</td>
<td>32.67 ± 4.34</td>
<td>0.904</td>
</tr>
</tbody>
</table>

Abbreviations: PaO₂ = partial pressure of oxygen; PaCO₂ = partial pressure of carbon dioxide; HCO₃⁻ = bicarbonate; BE = base excess; SO₂ = sulfur dioxide; Na = sodium; K = potassium; Ca = calcium; Cl⁻ = chloride; TCO₂ = total carbon dioxide; Agap = anion gap; Hct = hematocrit concentration; Glu = glucose concentration; Lac = lactate concentration; Crea = creatinine; mmol/L = millimole per liter; mmHg = millimeters mercury; PCV = packed cell volume.
Figure 11 A box-and-whisker plot indicating the mean partial pressure of oxygen (PaO$_2$, mmHg, A) and the mean partial pressure of carbon dioxide (PaCO$_2$, mmHg, B) measured in chimpanzees in which anaesthesia was either induced with a medetomidine-Zoletil® (MedZol, \( n = 12 \)) or a medetomidine-ketamine (MedKet, \( n = 10 \)) combination. PaO$_2$ and PaCO$_2$ values were obtained for each individual after collecting and analysing an arterial blood sample using portable gas analyser. Data indicated that MedZol anaesthesia produced a higher PaO$_2$ (59.79 mmHg [95% CI: 52.53, 67.05]) but a slightly lower PaCO$_2$ (43.23 mmHg [95% CI: 40.12, 46.33]) when compared to MedKet induced anaesthesia (PaO$_2$: 60.95 mmHg [95% CI: 54.67, 67.19] and PaCO$_2$: 43.85 mmHg [42.01, 45.85]). No statistically significant difference between the two anaesthetic protocols for these variables were observed (\( p > 0.05 \)).

Figure 12 Mean A-a gradient observed when anaesthesia was induced in chimpanzees using either a medetomidine-Zoletil® (MedZol, \( n = 12 \)) or a medetomidine-ketamine (MedKet, \( n = 10 \)) anaesthetic combination. The minimum-maximum range and interquartile range is also displayed on the box-and-whiskers plot. The A-a gradient (mean [95% confidence intervals]) for MedKet induced anaesthesia (32.67 mmHg [22.85, 42.49]) was higher but not statistically different (\( p = 0.904 \)) compared to when anaesthesia was induced with MedZol (31.85 mmHg [20.39, 42.49]).
11.4.5. Recovery time

Overall recovery time for anaesthesia induced with MedKet (22.57 ± 9.63 min) was not statistically different to \((p = 0.556)\) the recovery time seen with MedZol (20.29 ± 7.83 min; Figure 13). Time to first sign of recovery was also not significantly different between treatments \((p < 0.05;\) MedZol =15.12 ± 7.51 min; MedKet =13.11 ± 8.37 min). Similarly, time to lifting heads did not differ significantly between treatments \((p < 0.05;\) MedKet = 18.87 ± 10.67 min; MedZol = 13.14 ± 6.78 min).

![Box-and-whiskers plot indicating the for the overall recovery time (min) when two anaesthetic protocols were used to induce anaesthesia in chimpanzees \((n = 22)\) with either a medetomidine-Zoletil® (MedZol, \(n = 12\)) or a medetomidine-ketamine (MedKet, \(n = 10\)) combination.](https://example.com/figure13)

**Figure 13** A box-and-whiskers plot indicating the for the overall recovery time (min) when two anaesthetic protocols were used to induce anaesthesia in chimpanzees \((n = 22)\) with either a medetomidine-Zoletil® (MedZol, \(n = 12\)) or a medetomidine-ketamine (MedKet, \(n = 10\)) combination. Overall recovery time was measured from the time at which atipamezole was administered to the time at which the individual lifts its head. The total recovery time (mean [95% confidence intervals]) was longer when anaesthesia was induced with MedKet (22.57 min [15.69, 29.46]) combination when compared to when anaesthesia was induced with a MedZol (20.29 min [15.31, 25.27]) combination. No significant difference in recovery time was observed between the two anaesthetic protocols \((p > 0.05)\).
12. Discussion

The purpose of the study was to assess the effects that two anaesthetic protocols, a MedZol combination or a MedKet combination, had on cardiovascular and respiratory parameters and to determine the effectiveness of these combinations for chimpanzee anaesthesia.

Chimpanzees are extremely sensitive to changes in their daily routine and signs of stress and agitation may be observed in response to these changes (Adams et al., 2003; Burrows et al., 2021; Naples et al., 2010; Tribe and Spielman, 1996; Unwin et al., 2009). This could prolong the induction period and increase the risk of physiological abnormalities occurring when anaesthetic protocols are used (Burrows et al., 2021; Naples et al., 2010). Administering benzodiazepines (such as midazolam) as premedicating agents before anaesthetic induction can assist in reducing stress and mitigating anaesthetic complications (Atencia et al., 2017; Cashman et al., 1987; Lee et al., 2010; Loeffler, 1992; Maaly et al., 2019; Unwin et al., 2009), while improving the overall quality of anaesthesia (Tribe and Spielman, 1996).

Even though midazolam is rapidly absorbed and distributed when administered intramuscularly (IM) (Amrein et al., 1988; Ferreira, 2016; Griffin III et al., 2013; Klein and Klide, 1989; Loeffler, 1992), the uptake of these agents, when given orally, may be unpredictable (Adams et al., 2003). When midazolam (7.5 – 15 mg) is administered to human adults, the elimination half-life is approximately 1.5 to 3 hours after which the effects of midazolam are decreased (Amrein et al., 1988; Griffin III et al., 2013; Prommer, 2020) - this necessitated the administration of a second dose of midazolam to a single individual. The fast elimination of midazolam and decreased activity over time suggest that administering a second midazolam dose would have had a minimal effect on physiological parameters. The level of sedation observed when midazolam is administered increases in a dose-dependent and time-dependent manner (Bulach et al., 2005; Kain et al., 2000; Loeffler, 1992; Weldon et al., 1992), meaning that when midazolam is administered at a higher dose and longer before the anaesthetic event, the level of sedation will increase. Even though the effects produced by midazolam in this study varied among individuals, it was found that chimpanzees that received midazolam showed definite signs of sedation (relaxation and incoordination), and reacted significantly less to external stimuli which allowed for individuals to be hand
injected (Burrows et al., 2021; Hess et al., 2010). Although signs of sedation were observed, individuals remained aware of stimuli and they exhibited reduced activity to avoid the administration of anaesthetic agents. The diminished reactivity to external stimuli also allowed for anaesthetic agents to be administered via hand-injection in all individuals except for one individual from the MedZol group in which darting was required. Darting has a disadvantage over hand-injection in captive primates as it may cause a greater degree of stress and pain and can result in changes in certain physiological parameters like HR, SpO₂ and temperature (Burrows et al., 2021; Videan et al., 2005). The administration of both combinations of MedZol and MedKet, as were used in this study, have been reported to have wide safety margins, produce rapid, smooth inductions and provide good muscle relaxation during anaesthesia (Cunningham et al., 2015; Horne, 2001; Jalanka and Roeken, 1990; Lee et al., 2010; Lewis, 1993; Lin et al., 1992; Naples et al., 2010; Unwin, 2005; Unwin et al., 2009). In addition, these combinations produce minimal side effects and can be partially reversed due to the availability of an antagonist for medetomidine (Atencia et al., 2017; Fahlman, 2008; Klein and Klide, 1989; Melis et al., 2012; Murphy, 2008; Unwin et al., 2009; Wenger, 2004). MedKet combinations are often preferred over MedZol combinations as MedZol anaesthesia has been reported to result in prolonged recoveries and significant reductions in blood pressure (Adami et al., 2012; Lin et al., 1992; Naples et al., 2010). The preference of MedKet as anaesthetic agent in great apes is also related to its rapid absorption when administered IM (Adami et al., 2012).

The overall induction time observed with MedZol anaesthesia in this study ranged between 2 – 14 min (6.54 ± 3.54 min), with the first signs of sedation occurring within 1 – 5 min (2.33 ± 1.05 min). On the other hand, the overall induction time observed for MedKet anaesthesia ranged from 3.5 - 20.5 min (7.52 ± 5.26 min) with the first sign of sedation being observed between 0.5 - 14.5 min (3.60 ± 4.02 min). The observed induction times in the current study were consistent with the induction times described in literature for chimpanzees in which anaesthesia was induced with similar combinations. MedKet induced anaesthesia in chimpanzees has been reported to result in inductions within 3 - 15 min, with the first signs of sedation observed within 2 - 5 min after administration of the combination (Brainard and Darrow, 2013; Horne, 2001; Horne et al., 1998). On the other hand, a deeper plane of anaesthesia was achieved more rapidly when anaesthesia was induced with a MedZol combination in
chimpanzees (Horne et al., 1998). Inducing anaesthesia with either one of these combinations may therefore result in signs of sedations within 2 – 5 min after administration (Brainard and Darrow, 2013; Horne, 2001; Horne et al., 1998). A few individuals in the current study that were treated with MedKet showed a safe but lighter plane of anaesthesia (some twitching occurred when moved or stimulated) occurring within the first five min of monitoring, where after the anaesthetic depth increased. Ideally, stimuli should be kept to a minimum during the induction phase for both anaesthetic protocols to prevent spontaneous recovery occurring and extending the induction period.

The effective anaesthetic doses required by individuals may vary based on the size, overall health and observed behaviour of the animals (Adams et al., 2003; Unwin, 2005; Unwin et al., 2009; Videan et al., 2005). As the exact weight for individuals were unknown prior to induction in the current study, weight was estimated based on previous anaesthetic records to calculate induction doses (Adams et al., 2003; Atencia et al., 2017; Chinnadurai et al., 2016; Fahlman, 2008; Sleeman, 2007; Unwin et al., 2009; Zenker, 2004).

Due to logistical reasons, fasting was not possible in two individuals (one individual induced with MedKet and one individual induced with MedZol) in the current study, yet no adverse effects (like vomiting) were observed in either of these individuals. Some anaesthetic agents (Zoletil®, ketamine, medetomidine, opioids and combinations thereof) may also cause nausea and vomiting which may increase the risk of aspiration (Ferreira, 2016; Jacquier et al., 2006; Kohrs and Durieux, 1998; Sinclair, 2003; Sleeman, 2007; Unwin et al., 2009; Young et al., 1999; Zanos et al., 2018). The retention of the swallow reflex associated with the administration of dissociates – ketamine and tiletamine - is particularly beneficial when individuals could not be fasted before anaesthesia was induced as it can prevent vomiting and aspiration, although it can make intubation challenging (Cunningham et al., 2015; Fahlman, 2005; Jalanka and Roeken, 1990; Lewis, 1993; Murphy et al., 2012; Strong et al., 2018; Unwin, 2005; Unwin et al., 2009).

All individuals included into the current study were successfully intubated with limited coughing occurring. Even though coughing was observed in some individuals, intubation was successful without the need to administer additional anaesthetic agents. When dissociative agents are used, it often results in the requirement of the
administration of additional anaesthetic agents, like medetomidine, which assist in muscle relaxation and increase the success of intubation in chimpanzees (Horne, 2001; Jalanka and Roeken, 1990; Lee et al., 2010; Lewis, 1993; Unwin et al., 2009). The inclusion of medetomidine into anaesthetic protocols may explain the ease of intubation, good muscle relaxation and anaesthetic depth observed in the current study for both protocols (Bakker et al., 2013; Horne, 2001; Jalanka and Roeken, 1990; Klein and Klide, 1989; Lee et al., 2010; Lewis, 1993; Murphy et al., 2012; Strong et al., 2018; Unwin et al., 2009). It has been suggested that Zoletil® may have an added advantage over ketamine due to the inclusion of zolazepam which also assists in muscle relaxation and increases the ease of intubation (Bush et al., 1977; Fahlman, 2008; Klein and Klide, 1989; Lee et al., 2003; Lin et al., 1992; Naples et al., 2010).

The administration of ketamine and tiletamine when used alone may result in brief sedation, excessive salivation and convulsions (Bakker et al., 2013; Fahlman, 2005; Lewis, 1993; Lin et al., 1992; Naples et al., 2010). Medetomidine administered on its own may result in recumbency, but the anaesthesia that is produced is not reliable (Horne et al., 1998; Klein and Klide, 1989; Murphy et al., 2012; Williams et al., 2003). Combining these agents with medetomidine not only assist in eliminating these side effects, but also enhance effect and decrease the effective dose of the dissociative agents (Bakker et al., 2013; Cunningham et al., 2015; Fahlman, 2008, 2005; Ferreira, 2016; Hyeroba et al., 2013; Klein and Klide, 1989; Lee et al., 2010; Lewis, 1993; Murphy, 2008; Naples et al., 2010; Unwin et al., 2009; Wenger, 2004; Williams et al., 2003; Young et al., 1999). Ketamine administration may also cause some muscle damage around the injection site if large volumes are injected due to the acidity of ketamine (Murphy et al., 2012; Unwin et al., 2009). All side effects associated with dissociative agents can be attenuated by combining these agents with an alpha-2 agonist like medetomidine (Ancrenaz et al., 2003; Cunningham et al., 2015; Naples et al., 2010). None of these side effects were observed in this study probably due to the incorporation of medetomidine into both anaesthetic protocols. Unfortunately, medetomidine can cause its own set of side effects and its administration may depress cardiopulmonary function and affect the thermoregulation ability of individuals under anaesthesia (Chinnadurai et al., 2016; Ferreira, 2016; Grimm and Lamont, 2007; Ølberg, 2007; Sleeman, 2007; Wenger, 2004). In the current study, apart from a
reduction in RR observed when MedKet was used, no major cardiopulmonary depressions were observed in either of the anaesthetic protocols.

MedKet has shown to produce effective and predictable anaesthesia in primates for approximately 30 – 45 min, after which anaesthesia may become unpredictable (Unwin, 2005; Unwin et al., 2009). This means that the administration of additional anaesthetic agents during anaesthesia (like ketamine or isoflurane), is required to ensure adequate anaesthetic depth for longer anaesthetic procedures (Murphy, 2008; Williams et al., 2003). For this study, ketamine was administered intravenously at approximately 20 min after recumbency to most individuals or when there were concerns that the anaesthetic depth was inadequate for safe handling of the animal. Although ketamine is beneficial for maintaining anaesthetic depth, the administration of incremental ketamine doses may result in significantly prolonged recovery periods (Lewis, 1993; Murphy, 2008; Sleeman, 2007; Zenker, 2004). The fact that both anaesthetic protocols in this study received a single ketamine dose for maintenance, means that this dose was unlikely to have resulted in any prolonged recoveries. Additionally, the administration of a MedKet top-up to one individual after the predetermined monitoring period could have prolonged the recovery period observed. As only a single individual received a MedKet top-up, the influence on recovery should not be significant.

Research has indicated that the administration of ketamine has a stimulatory effect on cardiovascular function (increased HR, BP and cardiac output) and RR (Brainard and Darrow, 2013; Ferreira, 2016; Grimm and Lamont, 2007; Haas and Harper, 1992; Horne, 2001; Jalanka and Roeken, 1990; Kohrs and Durieux, 1998; Lee et al., 2003, 2010; Lewis, 1993; Naples et al., 2010). However, no significant stimulatory effects following ketamine administration were observed in either of the anaesthetic protocols in this study. Isoflurane was only administered after all physiological monitoring (30 min after onset of recumbency) was completed as part of the comparative anaesthetic study. Generally, inhalant anaesthetic agents should not have an impact on recovery as these agents are rapidly eliminated through the respiratory system when administration is ceased (Chinnadurai et al., 2016; Grimm and Lamont, 2007; Murphy et al., 2012).

Most agents used to induce anaesthesia may have an effect on cardiopulmonary function to some degree and may result in a range of side effects related to changes
in HR, BP, SPO2 and temperature (Atencia et al., 2017; Fahlman, 2008; Murphy et al., 2012). Normal reference ranges for chimpanzees described in literature are often distorted as values can only be obtained from individuals under anaesthesia and have therefore have been affected by stress and the anaesthetic agents themselves (Poliquin et al., 2017; Schapiro et al., 2012). In primates, normal HR ranges have been reported as 60 - 200 bpm in individuals under a variety of anaesthetic protocols (Horne, 2001; Melis et al., 2012), although, a range of 80 -150 bpm has also been suggested to be normal (Johnson-Delaney, 1994; Melis et al., 2012). The observed HR in our study (MedZol = 53.80 ± 10.92 bpm; MedKet = 58.48 ± 7.43 bpm) was slightly lower compared to values reported for chimpanzees by Horne (2001), Johnson-Delaney (1994) and Melis et al. (2012). In chimpanzees, a HR of 60 – 90 bpm has been reported for individuals when anaesthesia was induced with MedKet (Horne et al., 1998; Melis et al., 2012), which was similar to the HR that was observed in the present study when the MedKet combination was used. The higher HR observed in the MedKet induced anaesthesia group in this study compared to when MedZol was administered may be attributed to the stimulatory effect that ketamine has on the body (Jalanka and Roeken, 1990). Alpha-2 agonists (like medetomidine) are known to stimulate the central and peripheral adrenoreceptors, affecting the release of norepinephrine, resulting in the occurrence of bradycardia, BP changes and reduced cardiac output (Atencia et al., 2017; Baker et al., 2011; Bakker et al., 2013; Carter and Story, 2013; Deem and Citino, 1998; Ferreira, 2016; Grimm and Lamont, 2007; Horne, 2001; Jalanka and Roeken, 1990; Klein and Klide, 1989; Lee et al., 2010; Murphy, 2008; Murphy et al., 2012; Naples et al., 2010; Sinclair, 2003; Strong et al., 2018; Tribe and Spielman, 1996; Unwin et al., 2009; Wenger, 2004; Williams et al., 2003; Young et al., 1999). The lower than normal heart rate observed in the present study for both anaesthetic protocols, seems consistent with observations made in nonhuman primates (like chimpanzees and cynomolgus monkeys) when medetomidine is combined with dissociative agents (Lee et al., 2010; Strong et al., 2018; Young et al., 1999).

Monitoring arterial blood pressure is an effective method to assess cardiovascular function during anaesthesia (Rodriguez Lopez del Rio et al., 2014). Information on normal blood pressure in chimpanzees tends to be limited (Atencia et al., 2017; Naples et al., 2010) and reported values are inconsistent in literature (Naples et al., 2010).
Certain factors - sex, age and weight - contribute to the variations in blood pressure that are observed (Ely et al., 2011; Naples et al., 2010). Ely et al. (2011) noted that weight will have a greater influence on SBP, while age may impact DBP more severely. Even though mixed linear modelling in this study found that weight had an influence on SBP as described by Ely et al. (2011), the effect of age is expected to have on DBP was not observed. In the present study, the mean blood pressure observed for MedZol induced anaesthesia was 139/75 mmHg (SBP/DBP), while the mean blood pressure observed for MedKet induction was 127/67 mmHg. Blood pressure readings for both anaesthetic protocols fell within similar ranges described by Ely et al. (2011) for chimpanzees under Zoletil® (4.0 mg/kg) induced anaesthesia – the author reported SBP ranges between 126 - 147 mmHg, while normal DBP ranged between 63 - 84 mmHg (Ely et al., 2011). The inclusion of medetomidine into anaesthetic protocols has been reported to affect blood pressure to some degree (Baker et al., 2011; Drane et al., 2021; Fahlman et al., 2006; Grimm and Lamont, 2007; Hess et al., 2010; Ølberg, 2007; Sinclair, 2003; Sleeman, 2007). In captive orangutans, MedZol induced anaesthesia (at a dose of 0.02 mg/kg medetomidine and 1.1 mg/kg Zoletil® ) has been reported to result in lowered blood pressure, which is probably due to the inclusion of medetomidine in the combination (Fahlman et al., 2006). Pre-hypertension should be considered as a SBP between 148 - 153 mmHg, and DBP between 85 – 88 mmHg (Ely et al., 2011). Any blood pressure readings above these ranges would indicate hypertension (Ely et al., 2011), which is considered a significant risk factor as the cardiac output will be increased (Atencia et al., 2017; Ely et al., 2011; Rodriguez Lopez del Rio et al., 2014). Systolic blood pressure below 90 mmHg is indicative of hypotension (Adami et al., 2013), which was not observed in our study with either treatments. The values were highest at 10 min after recumbency but decreased over the monitoring period. Blood pressure values for the MedKet group showed an overall decrease over time with a slight increase around 20 – 25 min of monitoring, although these changes were not significant ($p = 0.08$). Mean arterial pressure (MAP) recorded in the current study was 100 mmHg and 90 mmHg for MedZol and MedKet, respectively. Normotensive MAP ranges for humans are described as 65 - 100 mmHg (Bernardis et al., 2020). Bernardis et al. (2020) suggested that hypertension occurs in humans when MAP is greater than 100 mmHg, while Strong et al. (2018) suggested that hypotension in chimpanzees occur when MAP drops below 70 mmHg. Any decreases in MAP below 60 mmHg may be too low to maintain perfusion of vital
organs and is considered as hypotension (Ashley et al., 2021; Murphy et al., 2012). The chimpanzees in the currently study was therefore neither hyper- nor hypotensive with MAP ranges falling within acceptable limits.

In addition to a decrease in HR, the inclusion of medetomidine in a combination may also result in higher BP readings as has been reported in various great ape species (Rodriguez Lopez del Rio et al., 2014). The use of dissociative agents as the sole anaesthetic agents may result in a higher than normal HR and BP (Grimm and Lamont, 2007; Strong et al., 2018). The action of alpha-2 agonists on the peripheral adrenoreceptors in nonhuman primates may lead to an increase in systemic vascular resistance which will result in a biphasic blood pressure response where an initial increase in blood pressure is observed, followed by a period prolonged of hypotension or normotension (Ashley et al., 2021; Murphy et al., 2012; Sinclair, 2003; Unwin et al., 2009; Wenger, 2004; Williams et al., 2003; Young et al., 1999). In the present study, general blood pressure trends for the mean SBP, DBP and MAP in both anaesthetic protocols were all highest shortly after the induction of anaesthesia, but gradually decreased over the period of monitoring. Although, the initial SBP readings recorded for the MedZol treatment fell within the pre-hypertensive range, subsequent readings recorded were all within the normal described range. In a study by Young et al. (1999), it was noted that initial transient hypertension was not observed when anaesthesia was induced in cynomolgus monkeys with a MedKet combination. Using Zoletil® alone may also produce a biphasic BP response that is attributed to the tiletamine portion of the combination (Lin et al., 1992).

Invasive blood pressure readings could only be obtained for a handful of individuals in this study (n = 11, continuous readings = 7 and partial readings = 5). Monitoring invasive blood pressure (IBP) requires arterial catheterisation and appropriate monitoring equipment (Horne, 2001). The former was not always possible in all the animals because of the difficulty in locating arteries that were suitable for catheterization. To our knowledge, no literature has been published on techniques and anatomical locations used for the placement of arterial catheters in chimpanzees. Ashley et al. (2021), however, suggested that to ensure that arterial catheterisation is successful, it should be done soon after anaesthesia was induced as a decrease in BP during anaesthesia will decrease the ability to palpate the arteries. Generally, there is a good correlation between invasive and non-invasive blood pressure (NIBP), but
observed variations between the two BP collection techniques could challenge the
validity of using NIBP methods for monitoring blood pressure (Lee et al., 2010; Murphy
et al., 2012; Murray and Gorven, 1991). The recorded IBP readings in the current study
corroborated the overall decrease in NIBP over time.

Administering any anaesthetic agents may affect respiratory function to some
degree (Fahlman, 2005; Horne, 2001). Unfortunately, individual respiratory
parameters will not give an accurate indication of respiratory status, and the different
respiratory parameters - RR, SpO₂, EtCO₂, PaO₂ and PaCO₂ - should be combined to
assess ventilation in individuals under anaesthesia. As the normal described RR for
large primates falls within the 10 - 15 breaths/min range (Murphy et al., 2012), the RR
observed for both anaesthetic protocols (MedZol = 26.46 ± 14.17 breaths/min; MedKet
= 20.73 ± 10.12 breaths/min) was higher than the normal ranges described. Ketamine
administration may be associated with a mild to moderate respiratory depression of
short duration (Grimm and Lamont, 2007; Horne, 2001; Kohrs and Durieux, 1998;
Lewis, 1993; Unwin et al., 2009; Young et al., 1999; Zanos et al., 2018) and could also
produce a characteristic apneustic breathing pattern (Capuano III et al., 1999; Grimm
and Lamont, 2007; Jalanka and Roeken, 1990; Lin et al., 1992). Although, this study
indicated that MedKet produced a lower RR compared to MedZol, respiratory
depression was not observed, and irregular breathing was not detected when
ketamine was included in the combination. The administration of Zoletil® to primates
has been reported to cause a decrease in respiration and ventilation that varies in
severity and lead to the occurrence of hypoxemia when administered to primates
(Kearns et al., 2000; Lin et al., 1992; Wilson et al., 1993). Additionally, in some cases
alpha-2 agonists (like medetomidine) have been reported to cause a mild depression
on the respiratory system in rhesus macaques and cynomolgus monkeys (Authier et
al., 2010; Capuano III et al., 1999; Winterborn et al., 2008). Anaesthesia induced with
alpha-2 agonists may cause a decreased responsiveness to changes in CO₂ (Grimm
and Lamont, 2007).

Pulse oximetry is considered the most suitable means of assessing blood oxygen
saturation levels (Horne, 2001; Romagnoli et al., 2016), but may result in the
occurrence of inaccuracies when obtaining continuous SpO₂ readings. Inaccuracies
may arise from difficulties in placing the oximeter probe in chimpanzees (Melis et al.,
2012), and the vasoconstrictive properties of anaesthetic agents like medetomidine
Analysis of arterial blood gasses acts as a sensitive indicator and could assist in reducing the inaccuracies that may occur with measuring SpO₂ through pulse oximetry (Horne, 2001; Lee et al., 2010; Romagnoli et al., 2016). In this study, the oxygen saturation of blood did not differ significantly ($p = 0.119$) over time for both treatments (MedKet: $87.85 \pm 5.56\%$; MedZol: $85.68 \pm 8.41\%$). The observed SpO₂ for both these anaesthetic protocols was below the saturation level of 90% that is described as ideal, indicating that some degree of hypoxemia occurred in both protocols (Bakker et al., 2013; Fahlman, 2005; Mosley and Gunket, 2007). It was also noted that the mean SpO₂ for the MedZol group in this study was initially low but increased over time. The low SpO₂ observed for both anaesthetic protocols in this study may be related to the vasoconstrictive properties of medetomidine or may occur due to impaired pulmonary gas exchange (Fahlman, 2005). Ideally, oxygen should be supplied to assist in returning low SpO₂ values to normal levels (Ely et al., 2011; Melis et al., 2012; Unwin, 2005; Unwin et al., 2009).

The mean EtCO₂ in this study was observed to be higher when anaesthesia was induced with MedZol ($43.92 \pm 10.53\text{ mmHg}$) when compared to the EtCO₂ observed in animals that received MedKet ($37.74 \pm 11.21\text{ mmHg}$) combination. A study conducted by Horne et al. (1998) reported that the EtCO₂ in chimpanzees ranged between 37 - 44 mmHg for both MedZol and MedKet anaesthesia. Observed EtCO₂ in the current study fell within the ranges described by other authors. In cynomolgus monkeys, the observed increase in EtCO₂ over time was significantly different when individuals were treated with MedKet (0.05 mg/kg medetomidine and 2 mg/kg ketamine) compared to ketamine (10 mg/kg) alone (Young et al., 1999). The increase in EtCO₂ within the MedKet group described by Young et al. (1992), indicates that the inclusion medetomidine into anaesthetic protocols may be beneficial to ensure good ventilation. The dose of MedKet administered by Young et al. (1992) to cynomolgus monkeys contained a lower ketamine dose compared to the MedKet (0.05 mg/kg medetomidine and 5 mg/kg ketamine) dose used in the current study.

Values for arterial blood gas analysis have not been extensively studied in chimpanzees, but are considered to be similar to or slightly higher compared to reference values described for humans (Howell et al., 2003). For this study, a single arterial blood sample was collected and analysed for each individual. Blood gas analysis values (including PaO₂ and PaCO₂) was observed to be similar to one another.
between the two anaesthetic protocols and no statistical differences between the two anaesthetic protocols were observed \((p > 0.05)\). The partial pressure of \(O_2\) \((PaO_2)\) in arterial blood can be used to assess oxygenation efficacy, while \(PaCO_2\) assesses the ventilation status of an individual (Horne, 2001). Moderate hypoxemia is described as a \(PaO_2\) less than 80 mmHg but greater than 60 mmHg (Horne, 2001), severe hypoxemia when \(PaO_2\) is less than 60 mmHg (Horne, 2001; Mosley and Gunket, 2007), while \(PaO_2\) less than 40 mmHg indicates that the individual is severely hypoxemic (Horne, 2001). Considering the data obtained in this study, the \(PaO_2\) obtained for MedZol and MedKet \((59.79 \pm 11.43 \text{ mmHg and } 60.95 \pm 7.68 \text{ mmHg, respectively; } p = 0.872)\) measured at approximately 1100 m above sea level fell into the ranges that are considered to indicate moderate to severe hypoxemia – indicating that the administration of these anaesthetic combinations did result in some degree of hypoxemia but no significant difference \((p > 0.05)\) was observed between the two treatments. Hypoxemia is a common respiratory complication observed when primates are under anaesthesia (Fahlman, 2005; Ferreira, 2016; Horne, 2001) – it may develop from ventilation-perfusion mismatching, but may also develop from a reduction in inspired \(O_2\) at altitude, hypoventilation or a diffusion deficit of \(O_2\) into the blood (Fahlman, 2008; Horne, 2001; Ølberg, 2007; Read, 2003; Sleeman, 2007). Altitude plays an important role in the development of hypoxemia as higher altitudes or low barometric pressures will impair the amount of oxygen that is inspired (Fahlman, 2008; Mosley and Gunket, 2007; Sleeman, 2007). Results from the current study indicate that hypoxemia may occur with MedZol and MedKet induced anaesthesia, and therefore oxygen supplementation to individuals should be considered (Fahlman, 2005; Read, 2003). This is in agreement to when anaesthetic combinations containing medetomidine were administered to macaques - mild hypoxemia \((PaO_2 = 60 – 70 \text{ mmHg})\) and a high \(PaCO_2\) were also reported (Lee et al., 2010).

The mean partial pressure of \(CO_2\) \((PaCO_2)\) for both anaesthetic protocols in this study \((\text{MedZol } = 43.23 \pm 4.89 \text{ mmHg and MedKet } = 43.85 \pm 2.59 \text{ mmHg; } p = 0.645)\) were similar, but the observed range when anaesthesia was induced with MedZol was wider when compared with MedKet. The normal \(PaCO_2\) range described for most species (including primates) is 35 - 45 mmHg (Horne, 2001; Lee et al., 2010). Values below 35 mmHg may indicate that the individual is hyperventilating, while hypoventilation may be occurring when \(PaCO_2\) is greater than 45 mmHg (Horne,
During hypoventilation, the amount of CO₂ that is retained in the blood is increased since CO₂ is not adequately being exhaled (Mosley and Gunket, 2007). Hypoxemia caused by hypoventilation will correspond to the amount of O₂ that is inspired, while the PaCO₂ remains elevated (Arnemo and Caulkett, 2007). Even though most individuals had PaCO₂ concentrations that fell within acceptable ranges, there was an indication that some individuals included (n = 4) into the MedKet anaesthetic protocol suffered from hypoventilation (PaCO₂ > 45 mmHg). The effects of hypoventilation and ventilation-perfusion mismatching can be mitigated by providing individuals with oxygen supplementation while under anaesthesia (Fahlman, 2005; Mosley and Gunket, 2007; Ølberg, 2007; Read, 2003; Sarkar et al., 2017).

When combining a low PaO₂ and a higher than normal PaCO₂, it may suggest that respiratory depression could have occurred (Young et al., 1999). Even though, PaO₂ results from the current study were lower than normal, except for the four individuals suffering from hypoventilation, PaCO₂ remained within normal ranges suggesting that respiratory depression did not occur when anaesthesia was induced with either of the protocols. EtCO₂ measurements provide a means of estimating PaCO₂ without repeated arterial blood analysis (Razi et al., 2012). Generally, in healthy humans, there would be a good correlation between PaCO₂ and EtCO₂, with the PaCO₂ usually 2 – 5 mmHg higher compared to EtCO₂ (Horne, 2001; Razi et al., 2012). Statistical analysis indicated that there was a good correlation (R² = 0.56, P = 0.04) between PaCO₂ and EtCO₂ in two thirds of the individuals (n = 13/22). However, some individuals (n = 10) from both anaesthetic combinations exhibited a difference (PaCO₂-EtCO₂) greater than the 5 mmHg, which could indicate that a ventilation-perfusion mismatch occurred in both of the anaesthetic treatments (Hedenstierna, 2014; Mosley and Gunket, 2007; Read, 2003; Sarkar et al., 2017).

The normal A-a gradient observed in a healthy human adult ranges between 10 – 12 mmHg, but will increase with age (Sarkar et al., 2017; Stubbs, 2020). There was no significant difference in A-a gradient between the treatments (p = 0.90) and both the MedKet group (35.67 ± 4.34 mmHg) and the MedZol group (31.85 ± 5.20 mmHg), had A-a gradients that were higher than the values described as normal. This supports the theory that a ventilation-perfusion mismatch occurred when anaesthesia was induced with both treatments. Since PaCO₂ for both the anaesthetic protocols remained within normal ranges, this suggests that the hypoxemia was not caused by
hypoventilation but rather by other intrinsic factors (Horne, 2001; Pfitzer et al., 2021; Young et al., 1999), such as the impaired transport of oxygen across the alveolar membrane and shunts caused by inadequate oxygenation or CO₂ removal (Hedenstierna, 2014; Mosley and Gunket, 2007; Read, 2003; Sarkar et al., 2017; Stubbs, 2020). Both dissociative and alpha-2 agonists are known to affect ventilation-perfusion mismatching (Hedenstierna, 2014; Read, 2003).

From the data, it is evident that anaesthesia induced with MedZol produced higher RR and EtCO₂. The higher EtCO₂ observed in the MedZol treated group could indicate that overall gas exchange was better compared to the MedKet group. However, the rate of respiration was slightly higher than normal in both anaesthetic protocols which, when combined with the A-a gradients, further substantiates the theory that that there may have been a ventilation perfusion mismatch in both treatment groups. The higher RR combined with the low PaO₂ and SpO₂ may indicate that the breathing was shallow with inadequate perfusion.

Capillary refill time (CRT) assists in assessing peripheral perfusion and adequacy of tissue oxygenation (Murphy et al., 2012). Certain alpha-2 agonists cause peripheral vasoconstriction that will alter the colour of the mucous membranes (Horne, 2001). CRT for all individuals in the present study was less than 2 seconds and no changes in mucous membrane colour was observed over time (Horne, 2001; Jacquier et al., 2006), indicating that tissues were adequately oxygenated throughout the anaesthetic period.

Monitoring the rectal temperature during anaesthesia is important as it will allow for the identification of certain anaesthetic complications before they become life threatening (Cunningham et al., 2015; Lee et al., 2003; Murphy et al., 2012). From this study, the mean observed temperature remained stable and within the normal described range for both the anaesthetic protocols and did not differ significantly between treatments (MedZol = 36.58 ± 0.89 °C; MedKet = 36.51 ± 0.68 °C; p = 0.157). The normal body temperature for chimpanzees ranges between 35.5 °C and 37.8 °C (Adami et al., 2013; Johnson-Delaney, 1994; Melis et al., 2012). The stable temperature observed over time for both anaesthetic protocols is probably associated with the provisioning of an external heat source (in the form of blankets) during anaesthesia when a drop in rectal temperature was noted. Most anaesthetic agents - such as Zoletil®, ketamine and medetomidine - are known to depress the
thermoregulatory ability of individuals (Capuano III et al., 1999; Grimm and Lamont, 2007; Jalanka and Roeken, 1990; Klein and Klide, 1989; Lee et al., 2003; Melis et al., 2012; Murphy et al., 2012; Sinclair, 2003; Tribe and Spielman, 1996; Unwin et al., 2009; Wenger, 2004), but this could not be assessed as additional heat was provided. In captive orangutans, MedZol (0.018 – 0.023 mg/kg medetomidine and 0.9 – 1.3 mg/kg Zoletil®) has been reported to decrease thermoregulation ability, which might be related to the inclusion of medetomidine in the combination (Capuano III et al., 1999; Fahlman et al., 2006; Grimm and Lamont, 2007; Melis et al., 2012; Sleeman, 2007; Wenger, 2004). Significant decreases in temperature could prolong the recovery of the individual but this was not observed in the current study (Bakker et al., 2013; Murphy et al., 2012; Young et al., 1999).

The overall recovery time observed in the Med Ket group (22.57 ± 9.63 min) was not significantly longer ($p = 0.555$) than the recovery period observed for MedZol (20.29 ± 7.83 min). First sign of recovery ranged between 1 - 27 min (mean 15.12 ± 7.51 min) for MedZol induced anaesthesia and 0 - 25 min (mean 13.11 ± 8.37 min) for MedKet induced anaesthesia after atipamezole was administered ($p = 0.565$). The time between showing signs of recovery and the time to when the individual lifted its head also did not show any marked differences ($p = 0.152$) between treatments (MedKet: 18.87 ± 10.67, range: 7.0 – 35.0 min; MedZol: 13.14 ± 6.78, range: 4.0 – 23.5 min). The administration of incremental ketamine doses to maintain anaesthesia have been suggested to prolong the recovery period (Lewis, 1993; Tribe and Spielman, 1996; Unwin et al., 2009). However, in this study a single IV ketamine dose was administered to individuals in both the anaesthetic protocols and was therefore not expected to result in significant differences in recovery times between treatments. The administration of a MedKet top-up after the monitoring period could have an effect on the recovery time. Unfortunately, the effect of the MedKet top-up on recovery between the two anaesthetic protocols could not be assessed as only one animal received this top-up. Generally, it has been reported that Zoletil® based anaesthesia may result in a prolonged recovery period when compared to ketamine-based anaesthesia protocols in primates (Fahlman, 2005; Horne, 2001; Melis et al., 2012; Murphy, 2008; Tribe and Spielman, 1996; Unwin et al., 2009; Wenger, 2004; Wilson et al., 1993). MedKet induced anaesthesia in chimpanzees has also been reported to result in rapid emergence from anaesthesia and faster return to normal behaviour.
The overall anaesthesia time observed in the current study appeared to be longer when anaesthesia was induced with MedKet (80.60 ± 42.84 min) compared to MedZol (56.75 ± 13.12 min) although this difference was not statistically significant ($p = 0.081$). The length of anaesthesia in the MedKet group showed greater variation when compared to the MedZol group which, when combined with the relatively small sample size of each treatment group, could have contributed to the lack of significant difference between the two anaesthetic protocols. Should the sample size have been greater, a significant difference may have been observed. Interestingly, Adams et al. (2015) also reported considerable variability in recoveries following atipamezole administration in chimpanzees that were anaesthetised with medetomidine and ketamine maintained with isoflurane. The authors reported recovery periods (defined as time from atipamezole administration to extubation) ranging from 2 – 29 min with total anaesthetic times ranging from 28 - 74 min. Although their samples size was also very small ($n = 6$), the two individuals with the longest recovery times (25 and 29 min, respectively), were also anaesthetised for longer than most of the other individuals (48 and 74 min, respectively). This is a contradiction to previous reports that Zoletil® combinations result in longer recovery periods compared to ketamine combinations in primates (Fahlman, 2005; Horne, 2001; Horne et al., 1998; Melis et al., 2012; Murphy, 2008; Tribe and Spielman, 1996; Unwin et al., 2009; Wenger, 2004; Wilson et al., 1993). It is unclear why the prolonging of the anaesthetic period with the administration of isoflurane would result in prolonged recoveries since inhalant anaesthetics like isoflurane are generally rapidly eliminated through respiration, resulting in rapid recoveries once their administration is ceased (Chinnadurai et al., 2016; Grimm and Lamont, 2007; Murphy et al., 2012). However, the delayed recovery observed may be related to the solubility of inhalant anaesthetic agents, the duration of tissues exposure to the agent or if the agent is administered close to the end of the procedure (Adams et al., 2003; Beaussier et al., 1998; Mishal et al., 2016). Further investigation is required to elucidate this.

The quality of the recovery was for the most part smooth and uneventful in both anaesthetic protocols. One individual from the MedKet group exhibited extremely light anaesthesia and reacted to external stimuli once placed in the night rooms, which poses a severe threat to human safety as critical bite injuries and exposure to zoonotic disease during the response period may occur (Adami et al., 2013; Adams et al., 2003;
Carter and Story, 2013; Murphy et al., 2012; Sleeman, 2007; Unwin, 2005; Unwin et al., 2009; Young et al., 1999). The atipamezole dose could be successfully administered and recovery was smooth after the external stimuli was ceased. The anaesthetic dose administered was similar to the doses that other individuals received, and it seems unlikely that recovery of this individual was related to the induction dose administered. This suggest that there might have been an individual variation in response to the anaesthetic combination used (Puri, 2012). In humans, genetic variations can greatly affect the effectiveness and tolerability of agents, which may result in the occurrence of side effects (Puri, 2012).

The anaesthetic effect produced was successfully antagonised by administering atipamezole at 4 – 5 times the original dose. The mean atipamezole dose that was administered to partially reverse the anaesthetic effect of medetomidine in individuals treated with MedKet was 0.24 (± 0.03) mg/kg, and MedZol was 0.16 (± 0.03) mg/kg. Administering atipamezole, reverses the effect that is produced by medetomidine and effectively shortens the recovery period when medetomidine is used in the anaesthetic combination (Cunningham et al., 2015; Fahlman, 2005; Jalanka and Roeken, 1990; Melis et al., 2012; Murphy et al., 2012; Naples et al., 2010; Sinclair, 2003; Tribe and Spielman, 1996; Young et al., 1999). In chimpanzees, spontaneous recovery using MedKet may occur around 60 – 120 min without the administration of an antagonist and the animal may also remain slightly sedated and show limited activity for a prolonged period (Jalanka and Roeken, 1990; Lewis, 1993). Zoletil® on its own and Zoletil® combinations have the potential to induce longer anaesthetic periods (Chinnadurai et al., 2016; Cunningham et al., 2015; Fahlman, 2008, 2005; Grimm and Lamont, 2007; Lee et al., 2003; Lin et al., 1992; Naples et al., 2010; Unwin et al., 2009), but are also associated with longer periods of recovery (Fahlman, 2005).

Ideally, atipamezole should not be administered earlier than 30 min after the administration of medetomidine in order to prevent re-sedation and reduce the occurrence of unstable recoveries and rapid changes in cardiovascular function (Baker et al., 2011; Ranheim et al., 1997; Unwin et al., 2009; Williams et al., 2003). No side effects or re-sedations were observed after the administration of atipamezole in individuals included in either one of the anaesthetic protocols that were compared in this study.
There were several limitations to this study. One of the main limitations was that invasive monitoring (both blood pressure and collection of arterial blood sample) is not routinely performed in captive primates as monitoring equipment is expensive and the risk of zoonotic disease transmission is high. There is therefore limited literature to refer to on how these techniques can be applied effectively and as a result, we experienced many difficulties with this type of monitoring and sampling in many of the individuals. The dataset would have been much improved had it been possible to place arterial catheters easily in all the individuals at the beginning of anaesthesia so that more arterial blood samples could be taken over the monitoring period and IBP could also be monitored continuously in all the individuals. In this study, anaesthetic protocols were not randomly assigned to chimpanzees in the sanctuary which created some bias. Individuals included into this study were scheduled for their yearly health assessments and if there were concerns regarding a particular individuals health, then a specific protocol was selected to reduce the risk of anaesthetic complications. Since data was also collected over a two-year period, some individuals were anaesthetised on more than one occasion. This may also have resulted in some bias. Due to logistical reasons, inconsistencies in the timing and type of top-up occurred. It should be noted that these inconsistencies may impact the outcome and accuracy of the measured physiological parameters. The small sample size included into this study may have increased the margin of error that could have occurred when measuring and comparing physiological parameters between the two anaesthetic protocols.
13. Conclusion

An ideal anaesthetic protocol should provide simple, effective, reversible, and predictable anaesthesia, which ensures the safety for the animals and the staff taking care of them. The results from this study indicated that both MedKet and MedZol provide smooth and rapid inductions while good muscle relaxation and anaesthetic depth during anaesthesia was also observed. Our data indicated that the anaesthetic protocols that were compared resulted in changes in cardiovascular and respiratory function. The observed HR was significantly higher when anaesthesia was induced with the MedKet combination, while BP readings over the monitoring period were higher when anaesthesia was induced with MedZol. Hypoxemia and changes in respiration were observed in both anaesthetic protocols and oxygen supplementation is advised regardless of which protocol is used. The changes that occurred in the physiological parameters were seemingly well tolerated by all individuals or could at least be effectively managed. The changes in physiological parameters may be related to the vasoconstrictive properties of medetomidine, but the dissociative agents (ketamine and tiletamine) may also produce respiratory depressions to varying degrees. The inclusion of medetomidine into both the anaesthetic protocols allowed for the anaesthetic effect to be reversed and the dose requirements of the dissociative anaesthetics to be reduced, which effectively reduced the recovery time. However, the duration of anaesthesia did have an impact on the overall recovery period. Even though changes in physiological parameters did occur, we concluded that both anaesthetic combinations (MedKet and MedZol) are suitable for inducing short-term anaesthesia in chimpanzees.
13. References


Agricultural Science, Uppsala.


### 14. Annexures

**Annexure A** Scoring to assess various characteristics associated with anaesthesia (adapted from Strong *et al.* (2018)).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-anaesthetic activity level</td>
<td>1</td>
<td>None; individual remained very still.</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Low; individual exhibits low levels of activity which is limited to moving elsewhere in the enclosure to avoid the administration of anaesthetic agents or the aim of the dart.</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Moderate; individual moves to various areas around the enclosure to avoid the administration of anaesthetic agents.</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>High; individual constantly move around the enclosure to numerous locations which resulted in an extended time before induction agents are administered.</td>
</tr>
<tr>
<td>Pre-anaesthetic demeanor</td>
<td>1</td>
<td>Individual depressed; reduced awareness to external stimuli, individual collapsed, sick or sedated.</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Alert; individual aware of stimuli but remain relaxed. A reduced response observed compared with the anticipated level of response</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Apprehensive; individual responsive to stimuli, taking action to evade administration of induction agents - individual hiding in the corners of the enclosure or behind another individuals.</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Aggressive; individual is hyperresponsive to stimuli, displaying aggressive behaviour to the person administering the induction agent.</td>
</tr>
<tr>
<td>Quality of induction</td>
<td>1</td>
<td>Excellent; rapid, calm, and smooth induction, adequate and safe level of anaesthesia achieved upon recumbency.</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Good; induction slightly prolonged but induction was smooth, a safe level of anaesthesia achieved but some response to stimuli is observed. Individual can be safely moved from the enclosure, but successful intubation required additional anaesthetic agents.</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Fair; individual becomes recumbent, but a safe level of anaesthesia not achieved – a lethargic response to stimuli is observed, and the administration of additional anaesthetic agents must be administered before the individual can be safely handled and moved.</td>
</tr>
</tbody>
</table>
4 Poor; inadequate anaesthetic effect achieved, remained very responsive to stimuli. Additional anaesthetic agents must be administered before the enclosure can be entered.

| Degree of muscle relaxation (muscle tone) | 1 | Excellent; trunk and limb muscles are relaxed, no twitching of muscles of excessive muscle tone observed |
| 2 | Good; muscles relaxed with the occasional mild twitching of the muscles of the eyes, lips, hands, and feet. |
| 3 | Fair; small amount of muscle tone, muscle twitching of larger muscle bodies (i.e., the movement of entire limbs) observed. |
| 4 | Poor; large muscle groups (trunk and limb muscles) remained rigid with severe muscle twitching occurring in these muscle groups. |

| Depth of anaesthesia | 1 | Mild sedation; signs of sedation observed, individual was still able to move around without ataxia but at reduced speed. |
| 2 | Heavy sedation occurred; purposeful movements occur in response to external stimuli like sound and touch. Safe level of anaesthesia for handling non-dangerous animals. |
| 3 | Light anaesthesia; considered to be a safe level of anaesthesia for handling dangerous animals. Individuals are non-responsive to stimuli (touch and sound), but responded when painful stimuli occurred, most reflexes still present. |
| 4 | Surgical anaesthesia achieved; an appropriate level of anaesthesia for surgical intervention and individual is non-responsive to painful stimuli. Most reflexes are absent. |
| 5 | Excessively deep anaesthesia; CNS excessively depressed resulting in the inability to maintain normal function. Individual is at risk of death during anaesthesia. |

| Ease of intubation | 1 | Easy; coughing or gagging during intubation is absent or minimal. |
| 2 | Moderate; small to moderate swallow reflex retained, some coughing or gagging is present, but individual can be successfully intubation without the administration of additional anaesthetic agents |
| 3 | Difficult; marked coughing or gagging observed, successful intubation required the administration of additional anaesthetic agents. |
4 Extremely difficult; severe swallowing, coughing, or gagging present, individual required the administration of additional anaesthetic agents for intubation or intubation is unsuccessful.

<table>
<thead>
<tr>
<th>Anaesthetic Recovery</th>
<th>1</th>
<th>Excellent; recovery is smooth, no vocalization or uncoordinated movements observed.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
<td>Good; minor paddling and short duration excitation, but no vocalization or uncoordinated movement was observed.</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Fair; some vocalization, paddling or uncoordinated movement occurred but was of short duration and easily calmed</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Poor; vocalization, moderate to severe paddling or uncoordinated movement occurring over a longer period of time.</td>
</tr>
</tbody>
</table>
Annexure B: Data sheets for recording doses for anaesthetic agent, time data, scoring data and physiological parameters

**ANESTHETIC DATA SHEET**

Animal name: ________________ Treatment: ________________

Date ________________ Place Chimp Eden
Altitude ________________ Humidity ________________ Outside temperature ________________ °C
Animal species Chimp Age ________________ yrs
Body weight: Estimated ________________ kg Actual ________________ kg

**Anesthesia**

- Pre-anaesthetic activity level: ________________
- Pre-anaesthetic demeanour: ________________
- Pre-anaesthetic drugs: ________________

<table>
<thead>
<tr>
<th>Drugs in dart</th>
<th>Estimated dosage (mg/kg)</th>
<th>Total mg</th>
<th>Dose in ml</th>
<th>Actual dosage (mg/kg)</th>
<th>Time</th>
<th>Comments</th>
</tr>
</thead>
</table>

Darting equipment: ________________

Dart volume ________________ ml Darting site ________________

Induction time:

- 1st sign of sedation ________________ min
- Initial recumbency ________________ min
- Approached ________________ min

Quality of induction: ________________

**Reversal and recovery**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dosage (mg/kg)</th>
<th>Total mg</th>
<th>Time and route of administration</th>
<th>1st sign of recovery</th>
<th>Head up and sternal</th>
<th>Standing</th>
<th>Walking</th>
</tr>
</thead>
</table>

Quality of recovery: ________________

**Other drugs**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dosage (mg/kg)</th>
<th>Total mg</th>
<th>Time of administration</th>
<th>Route</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time after recumbency (min)</td>
<td>HR</td>
<td>RR</td>
<td>SpO₂</td>
<td>ETCO₂</td>
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<td>10 min</td>
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<td>20 min</td>
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<td>25 min</td>
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<td>30 min</td>
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<td>35 min</td>
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<td>65 min</td>
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<tr>
<td>70 min</td>
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</tbody>
</table>
Annexure C Ethical Clearance certificate Ext 1

10 June 2021

Approval Certificate
Annual Renewal
(EXT1)

AEC Reference No.: REC228-19
Title: A study to compare the physiological effects of two different anaesthetic protocols following oral pre-medication with midazolam for immobilization of healthy chimpanzees (*Pan troglodytes*)

Researcher: Katja Koeppel

Student's Supervisor: 

Dear Katja Koeppel,

The Amendment as supported by documents received between 2021-05-13 and 2021-06-04 for your research, was approved by the Animal Ethics Committee on its quorate meeting of 2021-06-04.

Please note the following about your ethics approval:

The use of species is approved:

<table>
<thead>
<tr>
<th>Species and Samples</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chimpanzee (<em>Pan troglodytes</em>)</td>
<td>18</td>
</tr>
<tr>
<td>Blood</td>
<td>60 (1 ml each)</td>
</tr>
</tbody>
</table>

1. Ethics Approval is valid for 1 year and needs to be renewed annually by 2022-06-10.

2. Please remember to use your protocol number (REC228-19) on any documents or correspondence with

3. the AEC regarding your research.
4. Please note that the AEC may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.

5. **All incidents** must be reported by the PI by email to Ms Marleze Rheeder (AEC Coordinator) within 3 days and must be subsequently submitted electronically on the application system within 14 days.

6. The committee also requests that you record major procedures undertaken during your study for own-archiving, using any available digital recording system that captures in adequate quality, as it may be required if the committee needs to evaluate a complaint. However, if the committee has monitored the procedure previously or if it is generally can be considered routine, such recording will not be required.

**Ethics approval is subject to the following:**

- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.

Yours sincerely

Prof V Naidoo

CHAIRMAN:  UP-Animal Ethics Committee
Annexure D – Ethical Clearance certificate

Faculty of Veterinary Science
Animal Ethics Committee

4 August 2020
Approval Certificate
New Application

AEC Reference No.: REC228-19
Title: A study to compare the physiological effects of two different anaesthetic protocols following oral pre-medication with midazolam for immobilization of healthy chimpanzees (Pan troglodytes)

Researcher: Katja Koeppel

Dear Katja Koeppel,

The New Application as supported by documents received between 2019-11-07 and 2020-07-27 for your research, was approved by the Animal Ethics Committee on its quorate meeting of 2020-07-27.

Please note the following about your ethics approval:

1. The use of species is approved:

<table>
<thead>
<tr>
<th>Species and Samples</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chimpanzee (Pan troglodytes)</td>
<td>18</td>
</tr>
<tr>
<td>Blood</td>
<td>60 (1 ml each)</td>
</tr>
</tbody>
</table>

2. Ethics Approval is valid for 1 year and needs to be renewed annually by 2021-08-04.

3. Please remember to use your protocol number (REC228-19) on any documents or correspondence with the AEC regarding your research.

4. Please note that the AEC may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.

Ethics approval is subject to the following:

- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the
methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research. Yours sincerely

Prof V Naidoo
CHAIRMAN: UP-Animal Ethics Committee
Annexure E Section 20 exemption as granted by the Department of Agriculture, Forestry and Fisheries

agriculture, forestry & fisheries
Department:
Agriculture, Forestry and Fisheries
REPUBLIC OF SOUTH AFRICA
Directorate Animal Health, Department of Agriculture, Forestry and Fisheries
Private Bag Pretoria 0001
Enquiries: Mr Herry Gololo • Tel: +27 12 319 7532 • Fax: +27 12 319 7470 • E-mail: HerryG@daff.gov.za Reference: 12/11/P

Responsible person(s): Dr Laubscher, Dr Raath, Dr Koeppel
Chimp Eden Chimpanzee Sanctuary
Email: katia.koeppel@up.ac.za; katiakoeppel@gmx.net

Dear Drs Laubscher, Raath and Koeppel,

NO OBJECTION IN TERMS OF SECTION 20 OF THE ANIMAL DISEASES ACT, 1984 (ACT NO 35 OF 1984) FOR RESEARCH PROJECT

Title of research project I study: "To compare the physiological effects of two different anaesthetic protocols following oral pre-medication with midazolam for immobilization of healthy chimpanzees (Pan troglodytes)

Your application, requesting permission under Section 20 of the Animal Diseases Act, 1984 (Act no 35 of 1984) to perform the research project or study stipulated above, refers.
Based on the information provided in your enquiry, your study does not fall under the scope of Section 20 of the Animal Diseases Act, 1984 (Act no 35 of 1984) provided the statements 1 to 6 hereunder are, and remain, accurate in relation to your research project. Should the accuracy of any of the statements 1 to 6 hereunder change in any way in relation to your project, you are required to inform the Section 20 Secretariat. You may not proceed with any activities until written permission to do so have been granted by the National Director: Animal Health.

1. No work will be done with controlled and notifiable animal diseases (see attached list), which includes any animal diseases which do not occur in South Africa;

2. No imported material of animal origin or imported animal pathogens will be utilized in the study;

3. No samples that originate from a biobank will be used in the study;

4. No clinical studies will be performed in the target species, either in a laboratory or in the field;

5. The areas where the samples are to be collected are not under restriction for controlled or notifiable diseases to which the species of animal, from which the samples are obtained, is susceptible. For this research project / study no samples will be removed from the facility;

6. No samples or products will be obtained from an abattoir.

Written permission from the Director: Animal Health must be obtained prior to any deviation from the conditions and / or information as supplied to DAFF. Application must be sent in writing to HerryGOdaff.gov.za.

Failure to obtain written permission as above may be considered a contravention of the Animal Diseases Act, 1984 (Act no 35 of 1984).

Kind regards,

[Signature]

DIRECTOR OF ANIMAL HEALTH

Date: 2021-03-25
SUBJECT: NO OBJECTION IN TERMS OF SECTION 20 OF THE ANIMAL DISEASES ACT, 1984 (ACT NO 35 OF 1984) FOR RESEARCH PROJECT