Effectiveness of pulse oximetry to detect hypoxaemia in immobilized impala (*Aepyceros melampus*)

by

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Annexure E

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List of Abbreviations

A_{rms}	-	Accuracy root mean squares
CO_2	-	Carbon dioxide
COHb	-	Carboxyhaemoglobin
$cSaO_2$	-	Oxygen haemoglobin saturation calculated from the PaO_2 measured by the blood gas analyser
HbO_2	-	Oxyhaemoglobin
HHb	-	Reduced haemoglobin
LED	-	Light emitting diode
MetHb	-	Methaemoglobin
ODC	-	Oxygen dissociation curve
$PaCO_2$	-	Partial pressure of carbon dioxide in the arterial blood
PaO_2	-	Partial pressure of oxygen in the arterial blood
SaO_2	-	Oxygen haemoglobin saturation measured by co-oximetry
SD	-	Standard deviation
SpO_2	-	Peripheral oxygen haemoglobin saturation measured by pulse oximetry

Abstract

Pulse oximetry is a cost-effective and a simple way to continuously monitor oxygenation of haemoglobin (SpO_2) in humans and animals. Although pulse oximetry has been used extensively in wildlife, its efficacy and accuracy has not been validated. We aimed to establish, in immobilized impala, whether pulse oximetry is a reliable method to measure oxygenation of haemoglobin and aimed to determine which attachment site gives the most accurate measures. Sixteen healthy female impala were immobilized with etorphine or thiafentanil alone, or with a serotonin-agonist drug. Arterial blood samples were collected from the auricular artery at 5 minutes intervals during immobilization. At 40 minutes of immobilization, intranasal oxygen insufflation was provided to the impala at 5 L $minute^{-1}$ flow rate to increase arterial oxygenation. A blood gas analyser was used to measure PaO_2 (partial pressure of arterial oxygen) and calculate $cSaO_2$ (calculated arterial haemoglobin oxygen saturation) and a co-oximeter was used to measure SaO_2 (gold standard measure of arterial haemoglobin oxygen saturation) from arterial blood. Pulse oximeter probes were attached at four sites; namely under the tail, ear, rectum, and cheek. Pulse oximeter readings (SpO_2 and pulse quality) were recorded at each site and compared with SaO_2 and $cSaO_2$ using Bland-Altman and accuracy root mean squares (A_{rms}) methods to determine the efficacy. The pulse quality measured was generally good at each attachment site. Pulse oximetry measured under the tail was accurate and precise but only when SaO_2 values were above 90% (bias = 3.03, precision = 3.15, A_{rms} = 4.32). For the ear probe placement, overall bias and precision were high indicating that pulse oximetry was inaccurate (bias = -3.9) and imprecise (precision = 14.2). The cheek and rectal probe placement failed to give accurate or precise readings (cheek: bias = 11.63, precision = 10.86, A_{rms} = 15.82

and rectum: bias = 4.9, precision = 12.02, A_{rms} = 12.90). In order to get accurate and precise pulse oximetry readings in immobilized impala, probes must be placed under the tail and SaO_2 must be above 90%. Since SaO_2 values are usually low in immobilized impala, pulse oximeter readings should be interpreted with caution.

Keywords: immobilization, impala, pulse oximetry, oxygen saturation.

Chapter 1

Introduction

Chemical immobilization is an essential tool for wildlife conservation and management worldwide. This tool is commonly used in impala, mainly, but not exclusively, for translocation and disease surveillance purposes. To immobilize impala, potent opioids (etorphine or thiafentanil) are primarily used. Impala are particularly sensitive to the adverse effects caused by these drugs and deaths often occur when they are captured (Meyer et al. 2008; Zeiler & Meyer 2017a). These opioids cause respiratory compromise by causing respiratory depression, impedance of oxygen diffusion and functional right-to-left intrapulmonary shunting, ultimately leading to severe hypoxaemia (Meyer et al. 2008; Zeiler & Meyer 2017a; Zeiler & Meyer 2017b). Hypoxaemia, if left untreated can cause tissue hypoxia, organ failure and death (Fahlman 2014). Therefore, it is important that blood oxygenation is continuously and accurately monitored during immobilization to ensure that hypoxaemia is detected and treated early to guarantee the welfare and survival of an animal.

Currently, co-oximeters and blood gas machines are the most effective devices for accurately detecting hypoxaemia. However, these devices do not give continuous readings, they can be costly and the arterial samples they require can be difficult to obtain in some species, including immobilized impala (Proulx 1999). Pulse oximetry, which gives an indirect measure of arterial oxygen haemoglobin saturation (SpO_2) (Pedersen et al. 2009), has become a popular method used for the early detection of hypoxaemia in human and veterinary medicine. It is widely used in the hospital and

field setting because pulse oximetry devices are cheap, portable, easy to apply and use, and give continuous non-invasive measures. Their use and efficacy have been determined and well documented in humans (Lee et al. 2000; van de Louw et al. 2001) and domestic animals (Chaffin et al. 1996; Mathews et al. 2003; Giguere et al. 2014; Grubb & Anderson 2017; Reiners et al. 2017). However, although they are commonly used during the immobilization of wildlife (Martin-Jurado et al. 2011; Haymerle et al. 2016), their efficacy has not been adequately determined. Therefore, the validity of their use for this purpose is still questionable.

Studies in human and domesticated animals show that the efficacy and accuracy of pulse oximetry depends on where the probes are placed (Chaffin et al. 1996; Lee et al. 2000; van de Louw et al. 2001; Mathews et al. 2003; Giguere et al. 2014). In order to simply get a pulse oximetry reading, veterinarians working with immobilized wildlife place probes at many different sites including the ear, lip, intra-rectal, tongue, vulva and the tip of the penis (Proulx 1999). However, whether the readings from these sites are accurate and precise still needs to be elucidated. Although a few studies have already compared SpO_2 readings at different attachment sites in domestic animals (Chaffin et al. 1996; Mathews et al. 2003; Giguere et al. 2014), it appears that no such study has been done in immobilized wildlife. Therefore, this study aimed to:

1. Establish, in immobilized impala, whether pulse oximetry is a reliable method to accurately determine arterial oxygen haemoglobin saturation levels.
2. To determine which attachment site of the pulse oximeter probes gives the most accurate SpO_2 measure.

Chapter 2

Literature Review

Wildlife conservation and management is essential for South Africa's economy as it generates revenue and creates jobs in the tourism sector. Wildlife capture is important for translocation of animals, snare removal, disease surveillance and research purposes (Falhman 2008). It is achieved by restraint of animals, either by physical or chemical (immobilization) capture methods. In order to maintain the biodiversity of the country, wildlife capture methods are essential. Although capture techniques have been improved over the years, morbidity and mortality still occurs. Immobilization drugs such as etorphine and thiafentanil (both potent opioids) are accountable for most of these mortalities (Meyer et al. 2008; Zeiler & Meyer 2017b). These opioids can cause tremors, hypermetabolism and respiratory depression which result in hypoxaemia (Fahlman 2008; De Lange et al. 2017; Zeiler & Meyer 2017b; Buss et al. 2018). Hypoxaemia is associated with tissue hypoxia, organ failure and death (Falhman 2008). Consequently, hypoxaemia is the most life-threatening side-effect during immobilization of wildlife and should be monitored to ensure animal survival.

2.1 Hypoxaemia

Hypoxaemia is defined as an inadequate content of oxygen in the blood (Read 2003). According to the oxygen haemoglobin dissociation curve (ODC) - a sigmoid shaped graph that shows the change in arterial partial pressure of oxygen (PaO_2) in relation to arterial oxygen haemoglobin saturation (SaO_2) in the blood (Collin et al.

2015) - hypoxaemia begins when SaO_2 drops below 95% [(which is equivalent to a PaO_2 of about 80 mmHg in humans) (Read 2003)]. Clinical hypoxaemia is defined as SaO_2 of 90% and PaO_2 of 60mmHg or less (Robertson & Bailey 2002; Read 2003; Fahlman 2008). Below 80% SaO_2 (PaO_2 < 40 mmHg), hypoxaemia is defined as severe hypoxaemia (Humm & Kellett-Gregory 2016). Clinical significant hypoxaemia has been well reported in immobilized impala and white rhinoceros. Values of PaO_2 reaching just below 25 mmHg have been reported in immobilized white rhinoceros (Haw et al. 2014). Causes of hypoxaemia during immobilization or anaesthesia include neurological, cardiovascular and respiratory alterations such as shock, heart failure, decreases in respiratory minute volumes (hypoventilation), anaemia and intra-pulmonary causes (ventilation-perfusion mismatch, shunt and impaired diffusion of oxygen into the lungs). In immobilized wild animals, opioids have been reported to be the main cause of hypoxaemia resulting from respiratory impairment (Read 2003; Boardman et al. 2014; Meyer et al. 2015; Fahlman et al. 2016; van Zijll Langhout et al. 2016; Zeiler & Meyer 2017a).

2.2 Opioid use for chemical immobilization

The main clinical use of potent opioid drugs in wildlife is to induce reversible immobilization and sedation, which is aimed at minimizing stress induced by capture. Etorphine and fentanyl, are mu (μ), kappa (κ), and delta (δ) receptor agonists and they are the main opioids currently used for chemical immobilization of wild animals because they have a high potency and require small doses to immobilize an animal (Haigh 1990; Meyer et al. 2006; Pattinson 2008). Etorphine and fentanyl were among the first groups of opioids used to chemically capture African wild animals (Haigh 1990). Since the first use of these opioids, there has been a vast increase in

the success of wildlife capture, translocations, and research studies (Haigh 1990). Although these opioids are very effective at inducing immobilization and sedation, these effects occur concurrently with side effects like respiratory depression which leads to hypoxaemia, hypercapnia and acidaemia in various species including sheep, goats, cattle, impala, white rhinoceros, and other wildlife species (Read 2003; Meyer et al. 2006; Haw et al. 2014; Meyer et al. 2015; Zeiler et al. 2015).

To understand how these opioids lead to respiratory depression, and therefore hypoxaemia, it is important to know how these opioids control respiration.

2.3 Control of respiration and Opioid-induced respiratory depression

Breathing consists of a series of movements that are regulated by the respiratory neurons located in the brainstem, particularly in the Pre-Bötzinger complex (Butera Jr et al. 1999; Gray et al. 1999). The respiratory neurons are scattered around the narrow column of the ventrolateral medulla and extend from the facial nucleus to the spinal cord (Butera Jr et al. 1999; Gray et al. 1999). The Pre-Bötzinger complex, as part of the respiratory neurons, is responsible for generating respiratory rhythms during the inspiration and expiration phases of breathing (Gray et al. 1999; Marieb & Hoehn 2013). Neurons in the Pre-Bötzinger complex send pacemaker-like action potentials to a complex network of interneurons to generate coordinated rhythms of breathing (Butera Jr et al. 1999). During the inspiration phase of breathing, inspiratory neurons fire and send signals via the release of neurotransmitters to the nerves that activate respiratory muscles in the thorax (external intercoastal muscles) and diaphragm (Marieb & Hoehn 2013). As a result, the thorax expands and allows air to enter the lungs, therefore, enhancing breathing (Marieb & Hoehn 2013). During

the expiration phase, the inspiratory neurons stop firing causing the external intercoastal muscles to relax and less air to enter the lungs (Marieb & Hoehn 2013). The on and off inspiration and expiration phases of breathing generate respiratory rhythms (Marieb & Hoehn 2013).

The respiratory centres in the brain are abundant in μ, κ and δ-opioid receptors. The respiratory depressive effects of opioids, like morphine, etorphine, and thiafentanil, occurs by the depressive actions of these drugs on the control of respiration. These depressive effects are brought about predominantly by the activation of μ-opioid receptors (and to a lesser extent κ and δ-opioid receptors) which are abundant in the respiratory centres of the brainstem, particularly on the respiratory neurons in the Pre-Bötzinger complex (Shook et al. 1990; Pattinson 2008). When these opioids are administered, they inhibit the activity of these neurons by decreasing the production of cAMP through the activation of μ-opioid G-coupled receptors (Read 2003; Pattinson 2008). The reduction of cAMP alters normal respiratory rhythm generation resulting in a decrease in respiratory rate and depth, and an increase in muscle rigidity resulting in decreased chest and abdominal wall compliance, thus decreasing and restricting adequate ventilation and normal gas exchange to the alveoli, causing hypercapnia and hypoxaemia (Read 2003; Pattinson 2008).

Opioids do not only target the respiratory centres in the brain but also target central and peripheral chemoreceptors. Central chemoreceptors are located in the central medulla of the brainstem and peripheral chemoreceptors are located in the aortic arch and in the carotid artery bodies (Marieb & Hoehn 2013). These chemoreceptors play an important role in regulating ventilation by sensing the changes in $PaCO_2$,

PaO_2 and pH in the blood (Pattinson 2008). An increase in $PaCO_2$ or a decrease in PaO_2 and pH excite these receptors to stimulate rapid breathing (hyperventilation) to restore these variables back to their normal ranges (Marieb & Hoehn 2013). However, the administration of opioids blunts the ventilatory responsiveness of central and peripheral chemoreceptors to hypoxaemia, hypercapnia and acidaemia (Kimura & Haji 2013), leading to a poor or no corrective ventilator response.

Pulmonary hypertension is known to cause hypoxaemia in goats (Meyer et al. 2015) and this effect has also been assumed to occur in impala (Zeiler et al. 2015) and white rhinoceros (Haw et al, 2014; van Zijll Langhout et al. 2016). Pulmonary hypertension is believed to cause hydrostatic fluid shifts from the pulmonary vascular system into the interstitial space of the alveoli leading to pulmonary congestion and oedema (Meyer et al. 2015). These effects impede oxygen diffusion by increasing the diffusion distance resulting in less oxygen entering the arterial blood from the alveoli (Read 2003; Meyer et al. 2015; Fahlman et al. 2016). Notwithstanding the fact that the exact mechanisms causing opioid-induced pulmonary hypertension are still unknown, studies in goats and impala suggest that pulmonary hypertension may have resulted from an opioid-induced increase in pulmonary vasoconstriction and vascular resistance due to the activation of the sympathetic nervous system (Meyer et al. 2015; Zeiler et al. 2015). Nevertheless, more research is needed to determine the mechanisms leading to pulmonary hypertension during opioid administration.

Opioids are also believed to cause right-to-left vascular shunts which exacerbate hypoxaemia further (Nyman et al. 1990; Meyer et al. 2015; Buck et al. 2017). Shunting of the blood refers to a process when the blood in the right side of the heart

enters the left side of the heart without taking part in gas exchange in the lungs (Proulx 1999). The relevance of shunting during immobilization is still unclear. This is because in most studies to date, shunt fractions could not be measured (Meyer et al. 2015; Fahlman et al. 2016).

2.4 Other causes of respiratory depression during capture

The recumbent positioning during capture is also associated with the development of hypoxaemia, especially in animals weighing more than 100 kg (Nyman et al. 1990; Read 2003; Morkel et al. 2010; Fahlman et al. 2016). The larger body mass in a recumbent position may put pressure on the peripheral circulation and organs, thereby indirectly reducing blood supply to organs such as the brain, kidneys and the heart (van Zijll Langhout et al. 2016). In addition to that, positioning puts pressure on the diaphragm resulting in atelectasis (compression of the lungs due to abdominal organs putting pressure on the diaphragm), shunting and decreased minute ventilation, thus restricting oxygen from reaching the alveoli and blood (Read 2003). Therefore, when dealing with large animals like rhinoceros, it is ideal to position the animals in sternal recumbency as it has been reported to improve oxygenation by increasing ventilation, blood flow to both lung lobes and reduces dead space (Morkel et al. 2010; Boardman et al. 2014; van Zijll Langhout et al. 2016). This therefore prevent chances of exacerbating hypoxaemia even further (Morkel et al. 2010; Boardman et al. 2014; van Zijll Langhout et al. 2016).

2.5 Ways to counter hypoxaemia induced by respiratory depression

In order to lessen respiratory depression during immobilization, etorphine has to be administered with drugs such as, butorphanol (mixed-mu antagonist and kappa

agonist) and serotonergic receptor agonists (e.g. 5-HT_{1A} and 5-HT_4) which are given in a dose-dependent manner. Butorphanol has been shown to reverse respiratory depression caused by opioids by improving $PaCO_2$, PaO_2, and overall ventilation in goats (Haw et al. 2016) and impala (Zeiler et al. 2015; Buck et al. 2017). In contrast, a recent study done on etorphine-immobilized white rhinoceros reported only mild improvements in $PaCO_2$, PaO_2, and minute ventilation after butorphanol administration (Buss et al. 2018). Therefore, the efficacy of butorphanol administration in different wildlife species needs further investigation.

The use of serotonergic receptor agonists to counter opioid-induced respiratory compromise in immobilized animals has been investigated (Meyer et al. 2005; Meyer et al. 2010). Serotonin is a key neurotransmitter involved in respiratory rhythm generation (Mironov & Richter 2000; Richter et al. 2003; Meyer et al. 2006; Meyer et al. 2010). When serotonin is released, it binds to specific serotonin receptors (5-HT_{1A} and 5-HT_4) and activates rhythm generating respiratory neurons to regulate breathing. It has been reported that serotonin agonists at 5-HT_{1A} and 5-HT_4 receptors improve the activity of neurons involved in regulating respiratory rhythms in cats (Lalley et al. 1994) and rats (Sahibzada et al. 2000). 8-OH-DPAT (5-HT_{1A} agonist) and zacopride (5-HT_4 agonist) also proved to counter opioid-induced respiratory compromise in goats by predominantly correcting ventilation-perfusion mismatching and pulmonary shunting, thereby improving alveoli oxygen gas exchange, without affecting the opioid's catatonic immobilization effects (Meyer et al. 2006). However, in impala injected with the 5-HT_{1A} agonist buspirone, and the 5-HT_4 agonist metoclopramide, it was observed that although effective, the two agents did not completely correct clinical hypoxaemia (Meyer et al. 2010). Therefore, further

studies are needed to determine whether other serotonergic agonists are effective at countering respiratory compromise in wildlife.

Supplementary oxygen administration in hypoxaemic patients overcomes hypoxaemia by improving PaO_2, mucous membrane colour and heart rate (Fahlman 2008; Falhman 2014) thereby improving oxygen delivery to the tissues. However, oxygen therapy may not be effective in some of the cases of hypoxaemia especially if shunting is the cause (Proulx 1999). Additionally, the use of oxygen is not always practical in the field (Proulx 1999).

2.6 Possible consequences of hypoxaemia

Since the body depends on oxygen for survival, lack of oxygen in the blood may lead to inadequate tissue oxygenation (hypoxia) in vital organs of the body including the brain, heart, kidneys and skeletal muscles (Fahlman et al. 2010). The brain is one of the most important organ in the body, and within minutes of poor oxygenation brain damage can occur. Hypoxia can also cause failure in other organs, and if it is severe or prolonged, animals can die (Read 2003; Cambier et al. 2005; Meyer et al. 2006; Fahlman et al. 2010; Risling 2011; Haw et al. 2014). Inadequate tissue oxygenation during immobilization is a common life-threatening side-effect in immobilized wild animals (Read 2003).

In most cases, immobilized animals develop hypoxaemia which is often not monitored for, it is poorly assessed and often left untreated (Fahlman 2008; Fahlman et al. 2010). Therefore, reliable and accurate techniques are important in monitoring hypoxaemia before it leads to organ failure and death in wild animals.

2.7 Assessing and monitoring hypoxaemia

2.7.1 Cyanosis

Cyanosis (bluish discoloration of the mucous membranes) and colour change (bright red to dark red or almost black) in arterial blood are important signs of moderate to severe hypoxaemia (Fahlman 2008; Fahlman et al. 2010). However, cyanosis is difficult to detect in wild animals because of their often-pigmented mucous membranes and frequently collecting arterial blood to monitor hypoxaemia is not always practical in field immobilized animals (Fairman 1992; Chaffin et al. 1996). Furthermore, patients only show signs of cyanosis at later stages of hypoxaemia, therefore it is not always a reliable clinical measure (Gonzalez & Waddell 2016). Moreover, clinical observations are known not to be reliable for the detection of hypoxaemia (Fahlman 2014). Therefore, more suitable, and preferably non-invasive, continuous and reliable techniques are needed to adequately detect and monitor hypoxaemia in clinical settings in the wild.

2.7.2 Co-oximetry and blood gas analysis

Ninety-seven percent of oxygen is carried by the haemoglobin, therefore measuring the amount of oxygen on haemoglobin (SO_2) can give a good estimate of how much oxygen is in the blood (Collins et al. 2015). Currently, co-oximetry and blood gas analysis are considered standard techniques to determine the percentage of oxygen haemoglobin saturation (SO_2) in arterial or venous blood (Hill 2007; Wong et al. 2011; Gonzalez & Waddell 2016). In order to assess hypoxaemia using these methods, an arterial sample is commonly used (Gonzalez & Waddell 2016). SO_2 can be measured directly by using co-oximetry, or if done by a blood gas analyser, it can

be calculated using the oxygen tension measured in arterial blood (PaO_2) and the oxygen-haemoglobin dissociation curve (Fahlman 2014; Collins et al. 2015). When analysing arterial samples, co-oximeters use seven different wavelengths of light (488.4, 520.1, 562.4, 585.2, 597.5, 621.7 and 671.7 nm) to determine the actual fractional SaO_2 directly from the blood (Fahlman 2004; Fujihara et al. 2013; Giguere et al. 2014), taking into account the different haemoglobin species including oxyhaemoglobin (HbO_2), reduced haemoglobin (HHb), methaemoglobin (MetHb) and carboxyhaemoglobin (COHb) (Barker 1993). The fractional SaO_2 is calculated as follows

$$\text{Fractional } SaO_2 = \frac{HbO_2}{HbO_2 + HHb + COHb + MetHb}$$

On the other hand, blood gas analysers do not consider other haemoglobin species when calculating SaO_2; therefore, the readings could be overestimated or underestimated by the machine (Cheng et al. 1988). Co-oximeters and blood gas analysers have been used successfully in humans, however, they have some limitations, especially when they are used in other species like wildlife. They are mostly calibrated for human use, they do not give continuous readings, cassettes are needed to obtain these readings, they can be costly and in some species it is difficult to get arterial samples (Proulx 1999). Therefore, making calculations of SaO_2 from PaO_2 readings (measured by blood gases) or using co-oximetry should be done with caution and if possible, devices that continuously measure SaO_2 should rather be used. Additionally, such devices are not readily available, especially for veterinary use. Due to these limitations, veterinarians tend to use devices like pulse oximeters, which give continuous and indirect measures of SaO_2 through measuring peripheral

oxygen haemoglobin saturation (SpO_2) (Pedersen et al. 2009; Haw et al. 2014; Zeiler et al. 2014; Meyer et al. 2008).

2.7.3 Pulse Oximetry

2.7.3.1 Principles of pulse oximetry

Pulse oximeters are non-invasive, easy-to-use and cost-effective devices used to monitor and detect hypoxaemia (DeMeulenaere 2007; Pedersen et al. 2009; Haymerle et al. 2016). The pulse oximetry method is based on the principle that there must be a presence of pulsatile arterial blood in the tissue bed and that oxygenated-haemoglobin (HbO_2) and deoxygenated-haemoglobin (HHb) have different absorption spectra at the red and infrared light spectrums (Jubran 1999; Proulx 1999). Oxygenated haemoglobin (HbO_2) absorbs more light in the red region and less light in the infrared region whereas deoxygenated-haemoglobin (HHb) absorbs less light in the red region and more light in the infrared region (Jubran 1999; Proulx 1999). The differences in the absorbance between HbO_2 and HHb in the red and infrared regions is shown in graphic form in Figure 2.1 (Chan et al. 2013). Currently, pulse oximeters contain two light-emitting diodes (LED's) that emit light at wavelengths of 660 nm (red) and 940 nm (infrared) (Jubran 1999). The two wavelengths of light are used to calculate SpO_2.

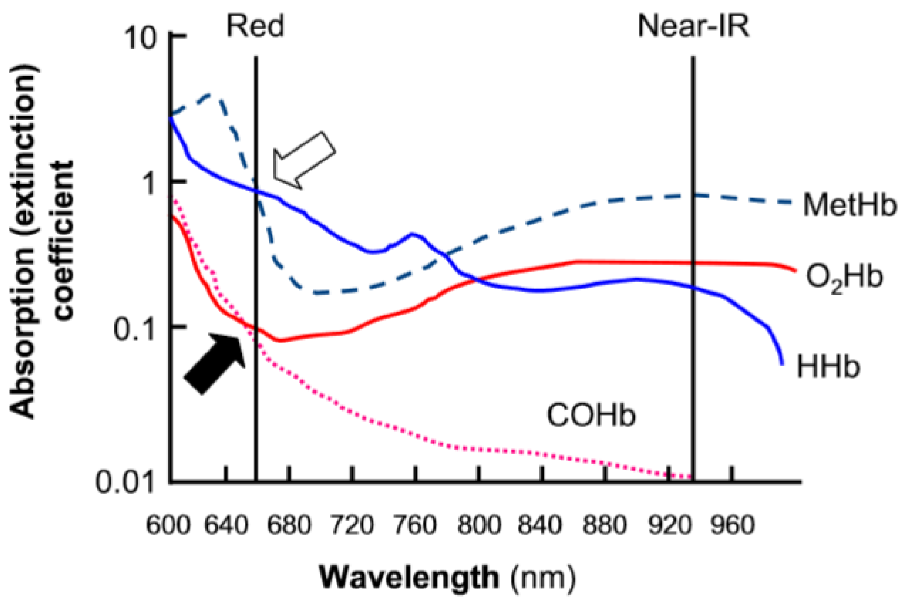

Figure 2.1 The relationship between absorption coefficient and wavelength of four different haemoglobin species [oxyhaemoglobin-HbO_2], reduced haemoglobin [HHb], methaemoglobin [MetHb], carboxyhaemoglobin [COHb]. The black horizontal lines indicate the absorption coefficients of haemoglobin species at the red (660 nm wavelength) and infrared regions (940 nm wavelength) found in Light Emitting Diodes (LED's) of pulse oximeters. (taken from Chan et al. 2013).

Pulse oximetry only calculates SpO_2 from light absorbed by the pulsatile arterial blood (Chan et al. 2013). However, light sent from pulse oximeters is also absorbed by non-pulsatile arterial blood, venous blood, capillary blood, the skin, soft tissue and bone (Chan et al. 2013). Therefore, pulse oximetry distinguishes the light absorbed and divides it into two components; based on the principle that the amount of light (red and infrared light) absorbed by the pulse oximeter differs with the cardiac cycle (Chan et al. 2013). During systole, arterial blood volume increases and during diastole the arterial blood volume decreases, while the volume of blood in the veins, capillaries, skin, soft tissue and bones stay constant (Chan et al. 2013). The light absorbed by the pulsatile arterial blood is detected by the photodetector inside the pulse oximeter as alternating current (AC) (Chan et al. 2013). Whereas the light

absorbed by the constant non-pulsatile blood in the veins, capillaries, skin, soft tissue and bones is detected as direct current (DC).

Pulse oximetry then uses the pulsatile component of red and infrared light absorbance to calculate a ratio (R= $\frac{\text{Ared-AC}/\text{Ared}-\text{DC}}{\text{AIR}-\text{AC}/\text{AIR}-\text{DC}}$) and ignores the non-pulsatile components (see Figure 2.2) (Jubran 1999; Dugdale 2010; Chan et al. 2013). This ratio is then used during calibration of pulse oximeters to determine SpO_2 values using readings obtained from volunteers with SaO_2 values between 70-100% (Jubran 1999; Chan et al. 2013). The calibration algorithms created is then stored inside a micro-processor found in the pulse oximeter (Jubran 1999) and calculates functional SpO_2 as follows

$$\text{Functional } SpO_2 = \frac{HbO_2}{HbO_2 + HHb}$$

Since the 1980s, pulse oximetry technology has vastly improved and can now fairly estimate SaO_2 in humans (Proulx 1999). Pulse oximetry has now become an essential tool of early detection of hypoxaemia in human and veterinary companion animal medicine (Dugdale 2010).

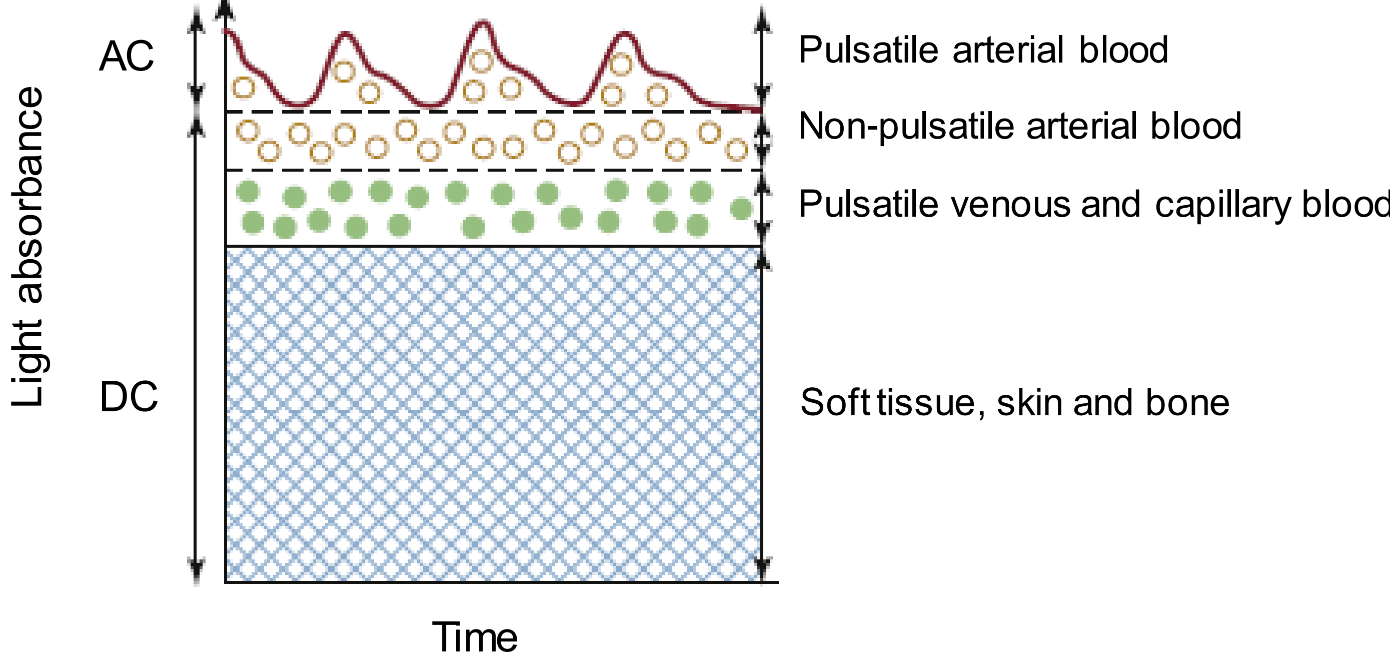

Figure 2.2 Light absorbance over time between pulsatile arterial blood and other light absorbers in the tissue (non-pulsatile arterial blood, venous and capillary blood, soft tissue, skin, and bone). The light absorbers are divided into alternating current (AC) and direct current (DC) and the light absorbance from pulsatile components is used to calculate the R value that is used to determine the SpO_2 value measured by pulse oximeters. (graph was modified from Barker 1993)

The international standard for manufacturers of pulse oximeters requires that SpO_2 above 70% must differ by less than 4% of SaO_2 measured by co-oximetry (Giguere et al. 2014). Studies in humans have shown strong agreement between the SpO_2 measured by pulse oximetry, SaO_2 measured by co-oximetry and $cSaO_2$ calculated from PaO_2 measured by blood gas analysis (Lee et al. 2000; van de Louw et al. 2001; Dubowitz et al. 2015). Therefore, pulse oximeters are accurate in detecting hypoxaemia (between 70 – 95% SaO_2) in humans. However, results from similar studies in domestic animals (cats, dogs and horses) are not as consistent (Sidi et al. 1987). In some animal studies, pulse oximeters either tend to underestimate SpO_2

when SaO_2 were > 90% or overestimate SpO_2 when SaO_2 < 90% (Uysteproyst, Bureau & Lekeux 2000; Koenig et al. 2003; Mathews et al. 2003; DeMeulenaere 2007; Fahlman et al. 2010). However, in a study done on anaesthetised cats, SpO_2 of 88% correlated well with a PaO_2 of 67 mmHg, which is accurate according to the oxygen dissociation curve of cats (Cambier et al. 2004; Zeiler et al. 2014). Moreover, studies done in wildlife, namely antelope and rhinoceros, are highly inconclusive and questionable (Martin-Jurado et al. 2011; Boardman et al. 2014; Haymerle et al. 2016; van Zijll Langhout et al. 2016). In immobilized black rhinoceros, SpO_2 measured by pulse oximetry did not compare well with SaO_2 values calculated from PaO_2 values measured by a blood gas machine (Fahlman et al. 2016). Whereas, SpO_2 readings in immobilized gazelles compared well with $cSaO_2$ values calculated from PaO_2 values measured by blood gas analysis (Martin-Jurado et al. 2011). In contrast, a similar study in immobilized Dama gazelles found that SpO_2 readings were slightly lower than the values of $cSaO_2$ calculated by a blood gas analyser (Schumacher et al. 1997). Many of these inconsistencies are due to the fact that pulse oximetry cannot be calibrated by the user and it does not take into account the other haemoglobin species (MetHb and COHb) (Kelleher 1989; Jubran 1999) or species differences in haemoglobin oxygen binding (species differences in oxygen-haemoglobin dissociation curves). Additionally, carbon monoxide has a high affinity for haemoglobin and competes with oxygen for binding sites of haemoglobin, and has the same absorption coefficient at the 660 nm, therefore pulse oximeter can mistake carboxyhaemoglobin (COHb) for oxy-haemoglobin (HbO_2) (Jubran 1999). For that reason, if an animal breathes in carbon monoxide, and carboxyhaemoglobin is elevated, a pulse oximeter will give falsely elevated SpO_2 values which gives an overestimation of SaO_2 (Figure 1). Motion artefacts, ambient light, darker pigmented

skin, poor perfusion and poor placement of pulse oximeter probes are also responsible for the inconsistencies and errors in pulse oximetry (Chan et al. 2013). Of all these factors, poor placement of pulse oximeter probes causes the most errors in pulse oximetry readings and more studies are needed to determine where these probes should be placed to get the most reliable readings, especially in novel species like wildlife.

2.7.3.2 Pulse oximetry probes and probe placement sites

Pulse oximeters have probes that can be attached at different sites on the body to measure SpO_2 (DeMaeulenaere 2007; Mathews et al. 2003). The accuracy of pulse oximetry is highly dependent on where these probes are placed (Mathews et al. 2003; Shelley et al. 2005). Human studies found that a pulse oximeter probe placed on an earlobe gives better results when compared to a probe placed on the finger (Fanconi 1989; Hamber et al. 1999). A number of studies in animals have used different sites to measure SpO_2 including the pinna, lip, intra-rectal, tongue, vulva and the tip of the prepuce (Proulx 1999). In animal studies, pulse oximetry readings show inconsistency in the measurement of SpO_2 between sites. Mathews et al (2003) showed that probes on the pinna, lip and vulva failed to produce reliable readings in horses. In contrast, Chaffin et al (1996) showed that probes on the pinna and the lip of horses were able to produce reliable SpO_2 readings. Pulse oximetry was accurate when probes were placed on the tail compared to the nasal septum and genital mucosa of cattle (Coghe et al. 1999). Mathews et al (2003) also stated that the accuracy of SpO_2 readings varies when the probes are attached at the same site in different species. However, further studies are needed to compare pulse oximetry readings between species and between sites at which SpO_2 is measured. Although a few studies have scientifically compared SpO_2 readings at different

attachment sites in domestic animals (Chaffin et al. 1996; Coghe et al. 1999; Mathews et al. 2003; Giguere et al. 2014), no similar studies have been done in immobilized wildlife.

Chapter 3

Materials and Methods

3.1 Animals

The study was conducted at Ngongoni Farm, outside Nelspruit in Mpumalanga, South Africa (25^0 31'25.2" S, 31^0 06'50.8" E). Sixteen free-ranging adult female impala (*Aepyceros melampus*), temporarily housed together in a 6 x 8 metres outdoor enclosure (boma), were used in the study. The impala (body weight 34.1 ±5.2 kg - mean ±SD) were habituated to the housing conditions 14 days prior to the study and were fed Lucerne (*Medicago sativa*) *ad libitum* with game pellets supplemented as needed. Water was supplied *ad libitum* from built-in automated troughs.

3.2 Study Design

Data for this study was collected from a different study (data not reported here) which aimed to assess the effects of an experimental serotonin agonist on opioid-induced respiratory depression. This "serotonin study" used a randomised cross-over study design, where the animals were immobilized (one at a time) on six different occasions (six trials - two control, one thiafentanil and three different doses of the serotonergic drug), with a 14 day wash out period between each trial. In each trial the animals were darted with etorphine (0.08 mg kg^{-1}, Captivon® 0.98%; Wildlife Pharmaceuticals (Pty) Ltd., White River, South Africa) or thiafentanil (0.09 mg kg^{-1}, Wildlife Pharmaceuticals (Pty) Ltd., White River, South Africa) alone and etorphine with the experimental agents using a 1.5 ml dart (P-type Pneudarts with ¾" needles)

projected into a muscle mass in the pelvic girdle by a CO_2 gas powered rifle (2.3 bar pressure: X-Caliber dart gun; Wildlife Pharmaceuticals (Pty) Ltd., White River, 1240, South Africa). The dart rifle operator stood on an elevated walkway above the boma wall and darted the impala over a distance ranging from 5 to 12 metres.

3.3 Experimental Procedures

Once the animals were immobilized they were blindfolded and carried to a workstation under a shelter (Gazebo) and placed into sternal recumbency on a table. A peripheral artery (auricular or common palmar digital artery) was aseptically cannulated (20 gauge; Jelco Smiths medical, Lancashire, United Kingdom) for arterial blood sampling. Four pulse oximeters (Nonin PalmSat 2500 A, Kyron laboratories (Pty) Ltd., Benrose, South Africa) were connected, using four probes (Nonin PalmSat 2500 A, Kyron laboratories (Pty) Ltd., Benrose, South Africa) at different locations, to the impala as follows (Figure 3.1):

- Tail connection – reflectance probe (2000T Transflectance probe) was placed and secured gently, using adhesive tape (25mm Elastoplast Tape), on an unpigmented skin on the ventral aspect of the tail base within 2-3 cm from the root of the tail (Figure 3.1A, A_1).
- Rectum connection – reflectance probe (2000T Transflectance probe) was inserted approximately 10 mm into the rectum and secured in position by taping the connecting wire of the probe to the tail's base using adhesive tape (Figure 3.1B, B_1).
- Ear connection – transmission probe (2000SL Lingual probe) was clipped onto a shaved non-pigmented area on the caudal margin of the pinna (Figure 3.1C, C_1).

- Cheek connection – reflectance probe (2000T Transflectance probe) was secured to a holding clip and placed into the mouth and placed against the mucosa of the cheek approximately 30 mm from the commissure of the lip (Figure 3.1D, D_1).

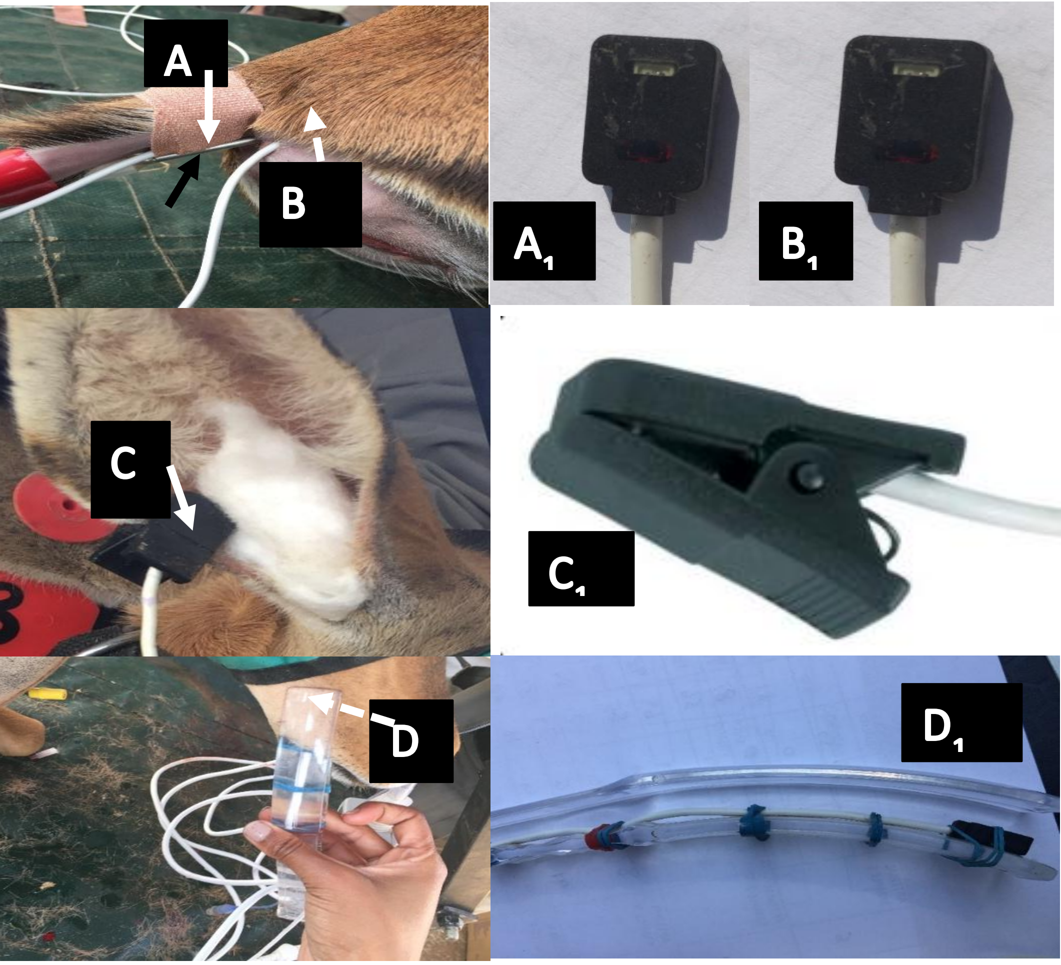

Figure 3.1 Placement of pulse oximeter probe types at four different probe attachment sites. The tail (A) probe was a transflectance probe (A1) that was placed on unpigmented skin at the base of the tail and secured with tape. The probe inserted into the rectum (B) was a transflectance probe (B_1) which was placed 10 mm inside the rectum facing the mucosa next to the rod-like temperature probe (indicated by the black arrow) used for measuring body temperature. The lingual transmission probe (C_1) was clipped on the skin of the shaved margin of the ear (C) of the impala. The probe attached to the cheek (D) was a transflectance probe that was secured on a plastic clip with rubber bands (D_1) and the plastic clip was held so that the probe was held against the mucosa of the cheek 30 mm from the commissure of the lips. White dotted arrows represent internal probes placed on the mucosa and the white solid arrows are probes that are placed externally on the skin.

In all trials, 1 ml of arterial blood was collected at 5, 10, 15, 20 and 30 minutes from the time the animals became recumbent (Figure 3.2). At 40 minutes of immobilization, oxygen supplementation using a high-pressure oxygen cylinder set at 5 L $minute^{-1}$ was administered intranasally via a nasal tube to increase the animal's oxygen haemoglobin saturations. Peripheral oxygen haemoglobin saturation (SpO_2), pulse rate ($beats.minute^{-1}$) and pulse quality data detected by the pulse oximeter and probe were recorded in triplicate, within 30 seconds of collecting each arterial blood sample. Pulse quality (pulse signal strength) was indicated by a flashing coloured light-emitting diode (LED) light on the pulse oximeter; green indicated a good quality; amber an intermediate quality and red a poor quality.

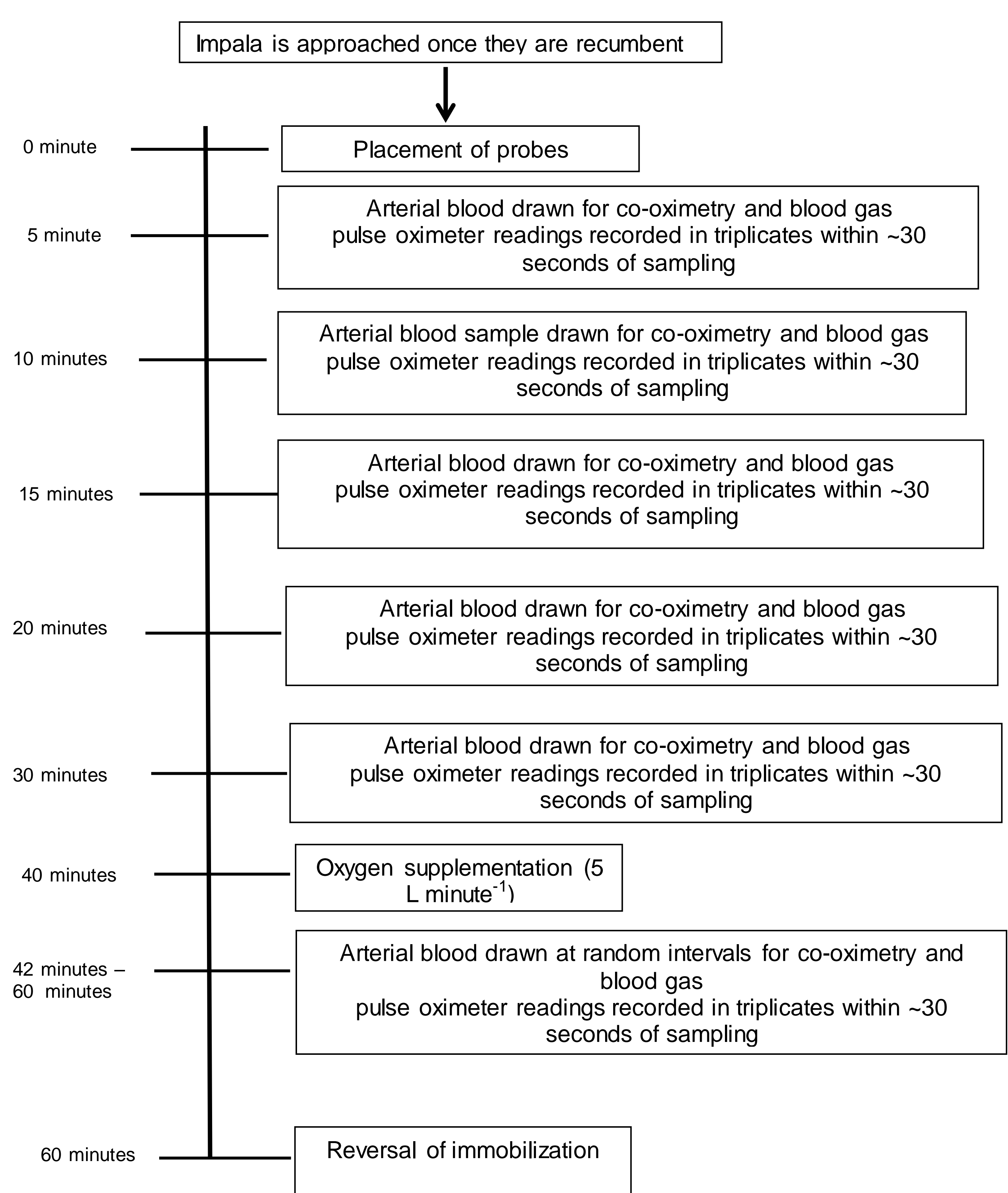

Figure 3.2 Time intervals indicating when SpO_2 readings were obtained using pulse oximetry at four sites in the body, when blood was drawn for arterial blood gas analysis and obtaining co-oximetry readings and the administration of oxygen supplementation during the immobilization period.

3.4 Arterial Blood Analysis

Arterial samples were immediately placed on ice and analysed within 10 min after collection using an EPOC blood gas analysis system (Epocal Inc, Ottawa, Ontario, Canada) using pre-calibrated BGEM3 test cards (Kyron Labs, Johannesburg, South Africa) and an Avoximeter 4000 co-oximeter (Surgical Innovations (Pty) Ltd., Northriding, South Africa). Quality control and calibration were performed daily on the Avoximeter 4000. The blood gas analyser was used to measure partial pressure of oxygen (PaO_2), $cSaO_2$ was calculated from this PaO_2 using an equation programmed in the blood gas analyser. The Avoximeter 4000 was used as a gold-standard machine to measure percentage oxygen haemoglobin saturation (SaO_2).

3.5 Ethical Clearance

The study was approved by the Animal Ethics Committee of the University of Pretoria (Approval number V035-17; Addendum A).

3.6 Data Analysis

Measurements obtained from the Avoximeter 4000 were considered to be the gold-standard measure for the arterial oxygen haemoglobin saturation (SaO_2). The Bland-Altman method for multiple observations (Bland & Altman 2007) was used to determine how accurately and precise the pulse oximeters (SpO_2) measured oxygen haemoglobin saturation at each site compared to the Avoximeter 4000 measure of SaO_2. The Bland-Altman method was further used to determine the accuracy and precision of the oxygen haemoglobin saturation ($cSaO_2$) calculated from PaO_2 by the EPOC Blood gas analyser compared to that measured by the Avoximeter 4000 (SaO_2). Further analysis was made using Bland-Altman method to evaluate the

accuracy and precision of the pulse oximetry's SpO_2 measure, at each site, when compared to the $cSaO_2$ calculated from PaO_2.

The Bland-Altman method calculates the bias (defined as the measure of accuracy - by calculating the mean difference between the two variables (methods) that are compared i.e. SpO_2-SaO_2, SpO_2-$cSaO_2$ and $cSaO_2$-SaO_2, respectively), the precision (defined as a measure of random error - by calculating the standard deviation (SD) of the differences), and limits of agreement (calculated as the Bias ± 1.96 SD).

The combined accuracy and precision of all the variables (methods) that were compared was evaluated using the accuracy root mean square (A_{rms}) (Batchelder & Raley 2007) calculated using the equation (example):

$$Arms = \sqrt{\frac{\sum_{i=n}^{n}(\mathrm{SpO2} - \mathrm{SaO2})^2}{n}}$$

Initially, using these methods, the performance of the pulse oximetry was evaluated using all the SpO_2 data (mean of triplicates) collected ("all-data") i.e. no data were eliminated from this analysis based on its quality. Thereafter, performance was determined only on SpO_2 data (mean of triplicates) that had a good quality ("pass-data") i.e. data that met the exclusion criteria were excluded. Exclusion criteria included the exclusion of any triplicate SpO_2 data where the pulse oximeter pulse quality light indicated red for any reading, or where the light indicated green or amber, but the triplicate data had a standard deviation of more than 3%.

To determine the performance of the pulse oximeters at different arterial oxygen haemoglobin saturation ranges the SpO_2 data were compared at the following different SaO_2 or $cSaO_2$ ranges 0-100%, 70-100% (range claimed to be most

accurate for the pulse oximeter), <70% (the range claimed to be inaccurate for the pulse oximeter), 70-79%, 80-89% and 90-100% for “all-data” and the “pass-data”.

Analyses was performed using GraphPad Prism, version 7 (GraphPad Software, Suite 230 La Jolla, CA, USA).

Chapter 4

Results

4.1 Comparison of the blood gas analyser to the co-oximeter

The calculated oxygen haemoglobin saturation ($cSaO_2$) measured by the blood gas analyser had a good agreement (bias -0.99, limits of agreement 5.26 to -7.25) with the SaO_2 measured by the co-oximeter (Figure 4.1).

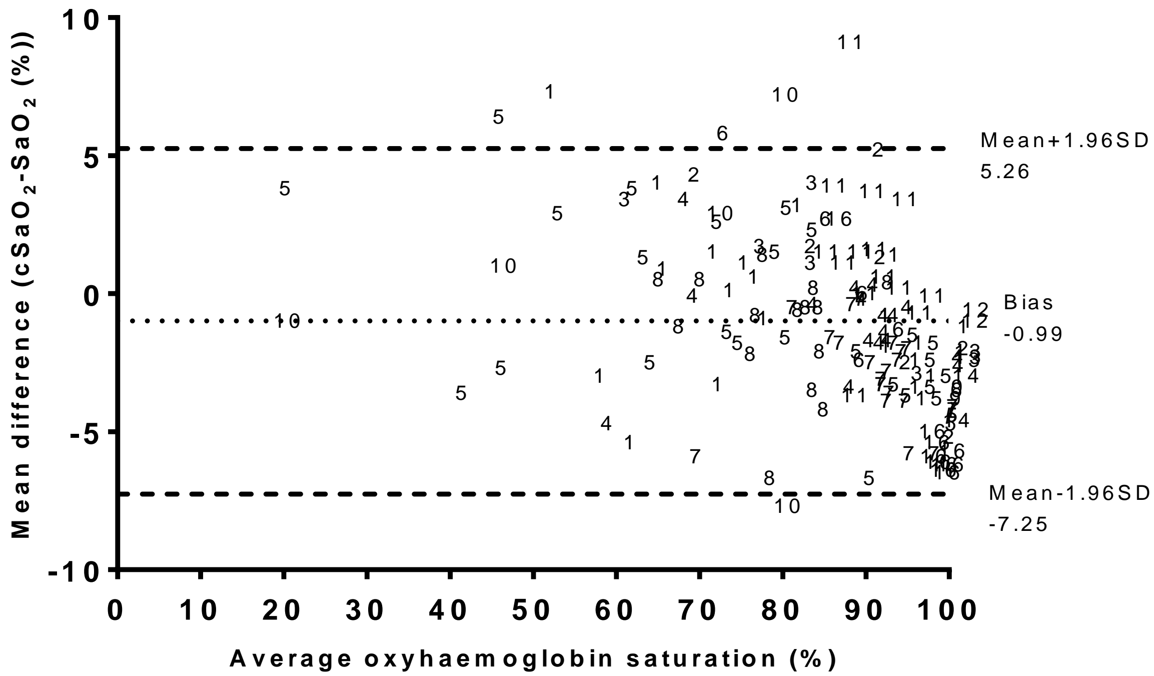

Figure 4.1 Bland-Altman plot showing the agreement between arterial oxygen haemoglobin measured by the co-oximeter (Avoximeter 4000 - SaO_2) and calculated by the blood gas analyser using the measured PaO_2 (EPOC gas analyser - $cSaO_2$). The mean difference between the SaO_2 and the $cSaO_2$ is plotted against the average oxygen haemoglobin saturation ($cSaO_2$ and SaO_2). Each point on the plot represents one paired measurement. Whereas, each number represents the animal ID number of sixteen individual impala from which the arterial samples were taken. The dashed lines represent the limits of agreement (mean ± 1.96SD) and the solid line represents the estimated bias.

4.2 Quality of data collected

4.2.1 Quality of data obtained from the pulse oximeter in comparison to the co-oximeter

Overall, there were 167 SaO_2 readings measured by the co-oximeter and the data ranged from 16.3 to 99.3%.

All probe sites generally gave good pulse quality readings (% readings that had a good pulse quality - green light, Table 4.1), with the highest obtained from the tail and the lowest from the ear. The ear, the cheek and the rectum gave a few SpO_2 readings indicating poor pulse quality (poor signal strength- red light).

Although the tail gave the best quality SpO_2 readings, there was a larger variability in these readings obtained from the tail and the ear (triplicate readings with SD > 3%, Table 1 & 2) compared to the other two sites (rectum and cheek).

Table 4.1 Represents the time matched paired data obtained from the co-oximeter (SaO_2 n = 167) and the pulse oximeters (SpO_2) at the different attachment sites (ear, cheek, tail and rectum). The number of pairs for all the data (all-data) and their pulse quality are presented. Number of data pairs excluded, and the criteria of exclusion and the total number of data pairs not excluded (pass-data) are also presented.

Attachment site	all-data n	Indicator light colour (pulse quality (%))	pass-data n (%)	Exclusion criteria n (%)	
				Triplicate SpO_2 with SD >3 *	Red light (poor pulse quality) #
Tail	167	Green: 98% Amber: 2% Red: 0%	124 (74%)	43 (26%)	0 (0%)
Ear	134	Green: 75% Amber: 21% Red: 4%	91 (68%)	35 (26%)	8 (6%)
Rectum	153	Green: 83% Amber: 16% Red: 1%	115 (75%)	37 (24%)	1 (1%)
Cheek	111	Green: 88% Amber: 11% Red: 1%	88 (79%)	22 (20%)	1 (1%)

(%) percentage of all data, SD standard deviation

*Triplicate SpO_2 data excluded because the SD was more than 3% even though the pulse quality indicated a green or amber light for the readings

#Triplicate SpO_2 data excluded because one or more SpO_2 readings had a poor pulse quality indicated by a red light

4.2.2 Quality of data obtained from the pulse oximeter in comparison to the blood gas analyser

There were 194 $cSaO_2$ readings measured by the blood gas analyser and the data ranged from 15.5 to 99.9%. Although there were more SpO_2 triplicate readings paired with $cSaO_2$ compared to SaO_2 (co-oximeter), the pulse quality from the tail and cheek probes were similar to the SpO_2 - SaO_2 paired data (98% vs 97% tail and 88% vs 87% cheek) and lower from the rectum probe (83% vs 80%) and the ear probe (75% vs 72%) (Table 4.1 vs 4.2). Furthermore, there were more SpO_2 measures excluded from the ear and tail with the lowest excluded from the rectum and cheek. Overall there was more SpO_2 'pass data' paired with the blood gas analyser's $cSaO_2$ compared to the co-oximeter's SaO_2 readings (Table 4.2 vs 4.1) .

Table 4.2 Represents the time matched paired data obtained from the blood gas analyser ($cSaO_2$ n =194) and the pulse oximeters (SpO_2) at the different attachment sites (ear, cheek, tail and rectum). The number of pairs for all the data (all-data) and their pulse quality are presented. Number of data pairs excluded, and the criteria of exclusion and the total number of data pairs not excluded (pass-data) are also presented.

Attachment site	all-data n	Indicator light colour (pulse quality (%))	pass-data n (%)	Exclusion criteria n (%)	
				Triplicate SpO_2 with SD >3 *	Red light (poor pulse quality) #
Tail	194	Green: 97% Amber: 3% Red: 0%	134 (69%)	60 (31%)	0 (0%)
Ear	139	Green: 72% Amber: 24% Red: 4%	80 (58%)	50 (36%)	9 (6%)
Rectum	176	Green: 80% Amber: 19% Red: 1%	129 (73%)	46 (26%)	1 (1%)
Cheek	136	Green: 87% Amber: 12% Red: 1%	105 (77%)	30 (22%)	1 (1%)

(%) percentage of all data, SD standard deviation

*Triplicate SpO_2 data excluded because the SD was more than 3% even though the pulse quality indicated a green or amber light for the readings

#Triplicate SpO_2 data excluded because one or more SpO_2 readings had a poor pulse quality indicated by a red light

4.3 Performance of the pulse oximeter at each probe attachment site

4.3.1 Performance of the pulse oximeter when the probe was placed on the tail compared to the co-oximeter and blood gas readings

Compared to SaO_2 (Table 4.3), the pulse oximeter probe placed on the tail only had an acceptable performance (i.e. it was accurate and precise) in the SaO_2 range of 90-100% for "all-data". In the SaO_2 range of 80-89%, below (70-79% and <70%), and in the whole range of SaO_2 (0-100%) pulse oximetry gave inaccurate and imprecise readings.

In comparison, the "pass-data", at the range of 90-100% pulse oximetry was slightly inaccurate but precise (Table 4.3). Below 90%; however, pulse oximeter was inaccurate and imprecise.

When $cSaO_2$ was compared (Table 4.4.), at the range of 90-100%; the pulse oximeter gave accurate but imprecise readings, however, the A_{rms} indicates that the pulse oximetry was accurate and precise for "all-data" and "pass-data". Below 90%, pulse oximetry was inaccurate and imprecise.

In the manufacturer's claimed performance range (70-100%) the readings were inaccurate and imprecise when compared to the SaO_2 (Table 4.3 & 4.4 and Figure 4.2). However, at the same range (70-100%), pulse oximetry gave accurate but imprecise readings when compared to $cSaO_2$ for "all-data" and "pass-data".

4.3.2 Performance of the pulse oximeter probe placed on the ear compared to the co-oximeter and blood gas analyser readings

When the SpO_2 readings were compared to SaO_2 (Table 4.3), "all-data" readings were accurate but imprecise in all ranges except the ranges of 70-79% and 90-100%, where the readings were both inaccurate and imprecise. For "pass-data", the pulse oximeter readings were accurate but imprecise at the ranges of 70-79% and 80-89%. At the entire range (0-100%) and the range of 70-100%, the pulse oximeter gave accurate but imprecise readings for "pass-data".

When compared to $cSaO_2$ (Table 4.4), the ear pulse oximeter readings were inaccurate and imprecise at all ranges for "all-data" and "pass-data".

4.3.3 Performance of the pulse oximeter probe placed on the rectum compared to the co-oximeter and blood gas analyser readings

At the SaO_2 range of 80-89% and 90-100%, the pulse oximeter gave accurate but imprecise readings (Table 4.3). At ranges below 80% the pulse oximeter was inaccurate and imprecise. For "pass-data", the pulse oximeter gave accurate but imprecise readings at the ranges of 90-100%.
When comparing $cSaO_2$ to pulse oximeter's SpO_2 readings (Table 4.4), the results were similar to the results compared with SaO_2 measured by the co-oximeter.

Furthermore, in the manufacturer's claimed performance range (70-100%), the pulse oximeter gave accurate but imprecise for "all-data" and "pass-data" when compared with both the SaO_2 and $cSaO_2$.

4.3.4 Performance of the pulse oximeter probe placed on the cheek compared to the co-oximeter and blood gas analyser readings

When compared to SaO_2 (Table 4.3), pulse oximeter was inaccurate and imprecise at all ranges except at the range of 90-100% where readings were inaccurate but precise for “all-data” and “pass-data”.

When comparing to $cSaO_2$ (Table 4.4), the cheek probe was also inaccurate but precise at the range of 90-100% for “all-data” and “pass-data”. At all the other ranges analysed, the cheek probe gave inaccurate and imprecise readings.

Table 4.3 The performance of the pulse oximeter readings (SpO_2) compared with the gold standard co-oximetry (SaO_2) at different attachment sites in immobilized impala (n=16)

Attachment site	**SaO_2 Ranges**	**"all-data"**					**"pass-data"**				
		n	**Bias**	**Precision**	**A_{rms}**	**LOA**	**n**	**Bias**	**Precision**	**A_{rms}**	**LOA**
Tail	0-100%	167	6.23	7.36	9.6	20.66 -8.21	124	5.84	6.6	8.75	18.76 -7.09
	70-100%	144	4.31	4.87	6.46	13.86 -5.24	110	4.41	4.57	6.3	13.38 -4.55
	<70%	23	18.25	8.85	20.20	35.60 0.9	14	17.00	9.17	19.14	34.97 -0.97
	70-79%	22	8.89	5.03	10.12	19.94 -2.38	9	10.74	4.50	11.49	19.57 1.91
	80-89%	53	4.09	5.58	6.82	15.02 -6.85	42	4.27	5.56	6.89	15.17 -6.63
	90-100%	69	3.02*	3.15*	4.32*	9.19 -3.15	59	3.55	2.79*	4.47*	9.03 -1.93
Ear	0-100%	134	-3.9	14.20	14.57	23.94 -31.74	91	-2.09*	14.40	14.32	26.14 -30.31
	70-100%	120	-4.38	12.57	13.16	20.24 -29.01	82	-3.16*	12.22	12.33	20.79 -27.12
	<70%	14	0.27*	25.09	23.40	49.45 -48.90	9	7.71	27.43	26.23	61.48 -42.31
	70-79%	14	-4.33	14.81	14.87	24.69 -33.35	5	1.33*	9.33	8.45	19.61 -16.95
	80-89%	44	-1.41*	11.01	13.86	20.17 -23.00	32	-1.22*	11.43	11.06	21.18 -23.62
	90-100%	62	-6.11	12.72	13.86	18.82 -31.03	45	-5.04	12.9	13.50	20.23 -30.32
Rectum	0-100%	153	4.90	12.02	12.90	28.45 -18.66	115	4.78	11.40	12.26	27.12 -17.56
	70-100%	132	1.97*	8.95	9.08	19.51 -15.57	102	2.05*	7.58	7.58	16.91 -12.82
	<70%	21	23.28	12.78	26.38	48.33 -1.78	13	23.95	18.03	26.39	59.3 -11.4
	70-79%	20	7.67	13.22	14.98	33.59 -18.25	11	8.64	8.86	11.89	26 -8.72
	80-89%	50	2.83*	8.19	8.59	18.88 -13.23	34	3.78	6.37	7.41	16.26 -8.71
	90-100%	62	-0.56*	6.72	6.61	12.62 -13.74	57	-0.43*	6.98	6.83	13.26 -14.12
Cheek	0-100%	111	11.63	10.86	15.82	32.92 -9.66	88	12.09	10.75	16.05	33.16 -8.98
	70-100%	97	8.91	7.48	11.57	23.58 -5.75	78	9.57	7.25	11.93	23.78 -4.64
	<70%	14	30.44	12.11	32.52	54.18 6.7	10	31.74	13.36	34.01	57.93 5.55
	70-79%	18	17.05	11.28	19.76	39.15 -5.05	11	22.43	5.12	22.94	32.47 12.39
	80-89%	37	9.87	5.45	11.21	20.55 -0.88	28	11.21	4.41	11.99	19.86 2.55

SpO_2, oxygen haemoglobin saturation measured by the pulse oximeters; SaO_2, oxygen haemoglobin saturation measured by co-oximetry (AVOXimeter 4000); *Results that are acceptable according to manufacturer guidelines and DIN EN ISO 80601-2-61 (Bias ≤ ±3%, Precision ≤ 3% and A_{rms} ≤ 4%).

Table 4.4 The performance of the pulse oximeter readings (SpO_2) compared with the blood gas analyser ($cSaO_2$ calculated from PaO_2) at different attachment sites in immobilized impala (n=16)

Attachment site	$cSaO_2$ Ranges	"all-data"					"pass-data"				
		n	Bias	Precision	A_{rms}	LOA	n	Bias	Precision	A_{rms}	LOA
Tail	0-100%	194	5.88	9.11	10.79	23.74 -11.98	134	4.96	7.65	9.05	19.96 -10.04
	70-100%	157	3.19*	6.36	7.07	15.65 -9.27	113	2.9*	5.24	5.94	13.18 -7.38
	<70%	37	17.29	10.16	19.96	37.19 -2.61	21	16.02	9.09	18.24	33.84 -184
	70-79%	27	8.65	7.12	10.86	22.61 -5.30	10	9.44	5.68	10.79	20.59 -1.70
	80-89%	56	3.84	7.37	8.03	18.28 -10.6	36	4.94	4.84	6.84	14.43 -4.54
	90-100%	74	0.4*	3.8	3.76*	7.85 -7.06	67	0.83*	4.1	4.11*	8.88 -7.22
Ear	0-100%	139	-8.94	14.59	17.00	19.65 -37.53	80	-7.03	14.70	16.12	21.79 -35.85
	70-100%	119	-8.72	14.74	16.94	20.16 -37.61	72	-6.95	14.57	15.88	21.61 -35.51
	<70%	20	-10.26	13.92	17.25	17.01 -37.54	8	-7.74	17.51	18.11	26.57 -42.05
	70-79%	17	7.82	15.64	17.89	25.16 -40.80	8	-5.93	18.21	17.76	29.76 -41.62
	80-89%	42	-8.28	16.59	18.18	24.2 -40.80	22	-5.2	15.82	15.96	25.81 -36.20
	90-100%	60	-9.29	13.09	15.78	16.36 -34.94	42	-8.07	13.64	15.45	18.68 -34.80
Rectum	0-100%	176	5.22	13.27	14.18	31.23 -20.78	129	4.33	13.13	13.71	30.06 -21.40
	70-100%	144	1.54*	8.99	9.04	19.16 -16.07	115	1.33*	8.91	8.84	18.79 -16.13
	<70%	32	21.78	16.29	27.15	53.70 -10.14	14	29.03	16.03	33.01	60.44 -2.38
	70-79%	26	9.46	7.51	12.88	24.18 -5.26	18	10.05	8.09	12.68	25.91 -5.81
	80-89%	50	3.38*	7.4	7.35	17.88 -11.12	37	3.66	5.67	6.60	14.77 -7.45
	90-100%	68	-2.87*	7.99	8.37	12.83 -18.5	60	-2.74*	8.36	8.65	13.65 -19.12
Cheek	0-100%	136	12.01	11.22	16.21	33.99 -9.98	105	12.39	10.99	16.47	33.93 -9.16
	70-100%	115	9.06	7.62	11.79	23.99 -5.87	90	9.72	7.71	12.34	24.84 -5.39
	<70%	21	28.14	12.73	30.65	53.09 3.19	15	28.38	14.05	31.37	55.91 0.85
	70-79%	24	16.17	9.96	18.82	35.69 -3.35	16	21.01	6.07	21.79	32.92 9.11
	80-89%	44	10.97	4.87	11.97	20.52 -1.43	30	11.97	4.68	12.81	21.15 2.80
	90-100%	48	3.68	3.46*	4.95	10.46 -3.10	44	4.08	3.31*	5.20	10.56 -2.40

SpO_2, oxygen haemoglobin saturation measured by the pulse oximeters; $cSaO_2$, oxygen haemoglobin saturation calculated from PaO_2 measured by EPOC Blood gas analyser; *Results that are acceptable according to manufacturer guidelines and DIN EN ISO 80601-2-61 (Bias ≤ ±3%, Precision ≤ 3% and A_{rms} ≤ 4%).

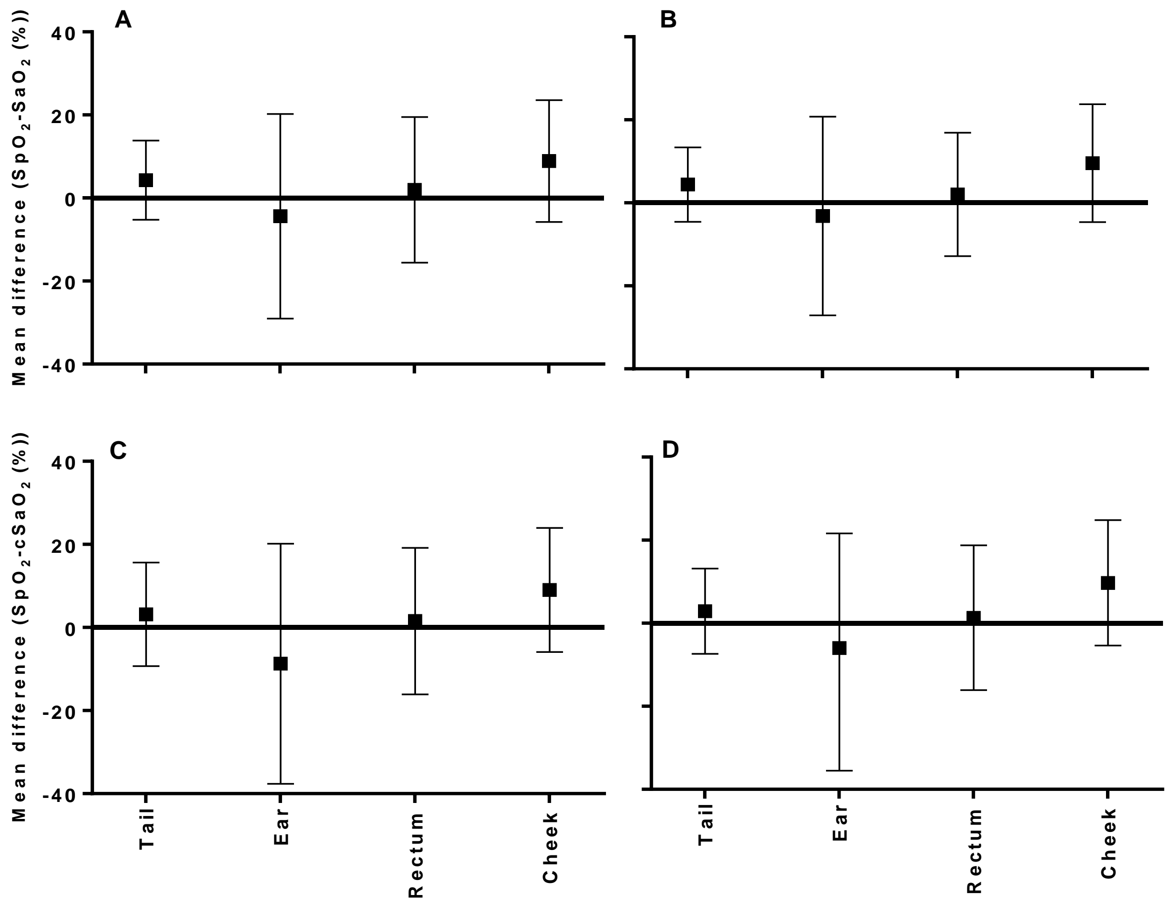

Figure 4.2 The performance of the pulse oximeters readings compared to SaO_2 (co-oximeter) (A and B) and $cSaO_2$ (Blood gas analyser) (C and D) in the range of 70-100% (manufacturer's claimed performance range of the pulse oximeters) at four probe attachment sites in sixteen impala. A and C indicate "all-data" sets (i.e. when exclusion criteria were not applied) and B and D indicate "pass-data" sets (i.e. when exclusion criteria were applied). The data were analysed using the Bland-Altman method for multiple comparisons per individual. The mean differences (Bias – black squares) (SpO_2-SaO_2; SpO_2-$cSaO_2$) and the limits of agreement (mean ± 1.96SD – error bars) are plotted for the four probe attachment sites. The black line at 0 indicates a bias of 0, the closer the mean differences are to this line the more accurate the pulse oximeter measures are, and the smaller the limits of agreement the more precise they are.

Chapter 5

Discussion and Conclusion

5.1 Discussion

In the immobilized impala, peripheral oxygen haemoglobin saturation measured by a commercial veterinary pulse oximeter device was a good measure of oxygenation, however, it was only acceptable (accurate and precise) at high arterial oxygen haemoglobin saturations and was influenced by where the probes were placed on the impala.

Pulse oximetry was most accurate and precise when the probe was placed on the base of the tail when the saturation levels were above 90%. The tail probe obtained high pulse quality readings, however, the variability of these readings was high.

The efficacy of pulse oximetry was poor when the probes were placed on the ear, inside the rectum and against the cheek. However, in some instances, when the probes were placed on the ear and inside the rectum the data was accurate but imprecise in various ranges (< 70%, 80-89% and 90-100%). The differences in the performance of the pulse oximeter at different attachment sites highlights the importance of proper probe placement when monitoring and assessing oxygen levels in free-ranging wild ungulates during immobilization.

Unfortunately, the co-oximeter was not available on all sampling days which resulted in a smaller sample size of SaO_2 compared to $cSaO_2$ readings. However,

this sample size was still adequate for the comparisons made, and similar findings occurred with the comparisons made with the $cSaO_2$ readings. To further validate pulse oximetry, many studies also compare the heart rate measured by the pulse oximeter to that of the animal; if the numbers are similar then this indicates that the pulse oximeter is measuring a pulse and not movement artefacts (Bohnhorst et al. 2002; Young et al. 2002; Reiners et al. 2017). Often the agreement between these heart rates is used to include or exclude data before comparisons between measured oxygen haemoglobin saturations. However, normal variability between heart rate measured from ECG and pulse rate measured by pulse oximetry at different sites have been observed in cats, dogs and horses (Mathews et al. 2003). Therefore, using heart rate alone can have its limitations. Most studies that validated their readings using pulse rate do not take pulse quality into consideration. This practice may limit the ability to make good comparisons and therefore both pulse rate and pulse quality should be considered. Unfortunately, we could not record the heart rate at the exact time of our data collection (ECG was not measured, and arterial samples were drawn from the catheter), therefore our pulse oximeter performance was based solely on the pulse quality determined by the amount of light absorbed (internal LED function). Furthermore, to ensure that data were comparable we excluded any SpO_2 data that was highly variable (triplicate data with SD > 3%). In some instances, more than 30% of this excluded highly variable data was data that had a good pulse quality, possibly indicating an error in the measure of pulse quality by the pulse oximeter or acute changes in SaO_2 of the animal at the time of measurement.

The accuracy of pulse oximetry has been assessed by making comparisons with calculated arterial oxygen haemoglobin saturation from blood gas results (Cheng et al. 1988; Chaffin et al. 1996; Coghe et al. 1999; Uystepruyst et al. 2000; Wong et al. 2011) or by measured arterial oxygen haemoglobin saturation by co-oximetry (Young et al 2002; Mathews et al 2003; Quinn et al. 2013; Giguere et al. 2014; Dawson et al. 2014; Grubb & Anderson 2017). All these studies have been performed in anaesthetised domesticated animals and humans. This is the first study designed to formally validate the accuracy of pulse oximetry in an immobilized wildlife species using both calculated and measured arterial oxygen haemoglobin saturation.

Although the co-oximeter and the blood gas analyser were designed and tested for human use, the paired SaO_2 and $cSaO_2$ values measured by both machines showed good agreement with each other, indicating that impala may have a similar oxygen dissociation curve, with similar light absorption characteristics to that of humans. This finding may be unique as the human oxygen dissociation curve differs from the oxygen dissociation curve of other species such as sheep and goats (Whitehair et al. 1990; Clerbaux et al. 1993).

Pulse oximetry is commonly used in wildlife and many studies claim its efficacy by doing simple correlations or comparisons of SpO_2 measures with $cSaO_2$ or SaO_2 values (Pye et al. 2001; Kreeger et al. 2011; Lian et al. 2017). Basing efficacy on simple correlations or comparisons may be erroneous and more studies are needed to properly assess agreement between measures. It has been well documented that bias, precision and A_{rms} are the best methods for properly assessing the efficacy of

pulse oximetry, and that simple linear regression is not adequate (Bland & Altman 1986; Uystepruyst et al. 2000; Batchelder & Raley 2007). Linear regression only shows linearity and the direction of the relationship between the methods but not the agreement. Studies have shown that data that has good correlation does not always have good agreement (Serfontein & Jaroszewicz 1978; Oldham et al. 1979) demonstrating that using correlation is not sufficient enough to properly assess efficacy of any method. Therefore, in this study we used statistical approaches to adequately determine agreement and hence the accuracy and precision of pulse oximetry.

In the impala pulse oximetry was only accurate and precise at the SaO_2 range higher than 90% and only when the probe was placed at the base of the tail. Inaccuracy and imprecision of pulse oximetry at ranges below 90% have been widely reported in a number of animal species including foals (Chaffin et al. 1996; Giguere et al. 2014), calves (Uystepruyst et al. 2000), cats (Mathews et al. 2003), dogs (Burns et al. 2006), and man (DeMeulenaere 2007). Pulse oximeters are normally calibrated by their manufacturers using algorithms determined from healthy human patients that only have SaO_2 values >80% (Uystepruyst et al. 2000). This methodology may in part account for why the pulse oximeters were mostly inaccurate at low SaO_2 levels.

Many factors should be taken into consideration when determining the efficacy of pulse oximetry in different species. These factors include the effects of ambient light, thickness of tissue and its perfusion, skin pigmentation, artefacts created by motion and site of probe placement (Young et al. 2002).

Site of probe placement arguably plays the most important role in the accuracy and precision of pulse oximetry (Chaffin et al. 1996; Mathews et al. 2003; Grubb & Anderson 2017) and is species dependent (Mathews et al. 2003). According to ISO 80601-2-61:2011 (International Organisation for Standardisation) guidelines, pulse oximetry is regarded as accurate and precise when the bias, precision and A_{rms} are ≤ ±3%, ≤ 3% and ≤ 4% respectively. In the impala, the pulse oximeters only met these guidelines when measurements were taken from the base of the tail when blood oxygenation was high (SaO_2 90-100%). Similar findings were observed from pulse oximetry measured from the base of calves' tails, where the bias was low (0.6%) and precision high (3.2%) mainly when SaO_2 was above 90% (Uystepruyst et al. 2000). In contrast, Chaffin et al. (1996) found that pulse oximetry measured from the tail base performed poorly at all SaO_2 ranges compared to other sites (ear, lip and tongue) in anesthetized foals. Indeed, the efficacy of pulse oximetry will differ between species because anatomical sites will have species specific factors that influence measures. In the impala, the better efficacy in the tail compared to other sites could be explained by 1) the thin skin under the tail having a well perfused vascular bed with little pigment, 2) little movement of the tail during immobilization and 3) protection of the probe from ambient light by the tape used to secure the probe to the tail.

The ear has been used widely in humans because it is known to have the least vasoactivity compared to other sites (Grap 2002). Furthermore, it is also a useful site in animals with a larger body size (> 50 kg) as they tend to have large arteries within the tissue bed. In the impala pulse oximetry measured from the ear was accurate at most SaO_2 ranges, mostly with a slight underestimation of SaO_2, but

measures were imprecise. These findings were similar to those in horses where pulse oximetry measures from the ear were accurate but imprecise (Chaffin et al. 1996). During immobilization there was a lot of movement in the impala's ears, it was difficult to find areas without pigmentation and the curvature and thickness of the cartilage hindered good probe placement; these factors could account for the imprecision in the measures.

In cynomolgus monkeys, good agreement occurred between measures of pulse oximetry from the cheek mucosa and SaO_2 measured by co-oximetry (bias = 2.7) and $cSaO_2$ calculated from the blood gas analyser (bias of 1.8). From the cheek of the impala this agreement was poor, possibly because poor probe contact was caused by 1) the buccal papillae on the cheek mucosa, 2) saliva from hypersalivation and 3) movement from chewing.

The SpO_2 readings from the impala's rectum were accurate above 90% SaO_2 but imprecise; readings below 90% were mostly inaccurate and imprecise. In contrast, in critically ill dogs, the rectal mucosa is considered to be a useful and effective site to measure SpO_2 when there is limited access to other peripheral sites (Barton et al. 1996). Unlike our rectal probe placement, Barton et al. (1996) used a sensor specifically designed to place in the rectum and secured it using a soft silicone plug, factors that may account for their better findings. A similar study done by Giguere et al (2014) indicated larger bias (23%) at saturations below 85% when the probe was placed inside the rectal mucosa of anaesthetised neonatal foals.

Etorphine is known to cause severe hypoxaemia in immobilized impala (Meyer et al. 2010; Zeiler et al. 2015). During immobilization overestimation from a pulse oximeter is arguably worse than underestimation, as it can give an impression that an animal with hypoxaemia has normal oxygen levels when it in fact does not. In this study, probes that were placed in the rectum and on the cheek overestimated SaO_2, therefore these sites should be avoided. This fact is particularly important especially in clinically ill patients.

It is common that during immobilization impala become hypoxaemic with saturation values reaching below 90%. Therefore, even if the pulse oximeter used in this study is placed in the best site (under the tail), it would still have limited use in accurately detecting hypoxaemia, especially clinically relevant hypoxaemia. Therefore, in order to make this pulse oximeter of clinical value in wildlife immobilization it should be calibrated or adjusted to better detect low SaO_2 in impala and other wildlife species.

Furthermore, future studies should also include other drug combinations, especially those using alpha2-adrenoreceptor agonist, that may possibly have an effect on pulse oximetry accuracy. We used the Nonin PalmSat pulse oximeter because of its robust design and anecdotal evidence of its sensitivity and efficacy in wildlife. However, assessing different pulse oximeter models and devices will also be of great value to find devices with the greatest efficacy.

5.2 Conclusion

We have shown that pulse oximetry, using a commercial veterinary device, is effective in immobilized impala but only when oxygen haemoglobin saturations are

high (>90%) and when a transflectance probe is used on the base of the tail. Since chemical immobilization often results in severe hypoxaemia in impala the use of pulse oximetry to measure blood oxygenation using this device should be done with caution and additional monitoring, possibly using blood gasses or co-oximetry, should rather be considered. However, pulse oximetry use in wildlife is still important as it helps show trends in oxygenation during monitoring, but improvement of the pulse oximetry devices and adaptation to wildlife would be desirable in the future. Identifying that the tail base is an optimal site for measuring pulse oximetry in impala may help with the refinement and development of future and more sensitive pulse oximeters that can be used effectively in wild antelope species.

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Addendum

Addendum A

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Animal Ethics Committee

PROJECT TITLE	**Assessment of hypoxaemia during chemical immobilization of wildlife**
PROJECT NUMBER	**V035-17 (Amendment 1)**
RESEARCHER/PRINCIPAL INVESTIGATOR	**Prof. L Meyer**

STUDENT NUMBER (where applicable)	----------
DISSERTATION/THESIS SUBMITTED FOR	**Academic**

ANIMAL SPECIES	**Blesbok**	**Impala**
NUMBER OF SAMPLES	**12**	**12**
Approval period to use animals for research/testing purposes		**July 2017 - July2018**
SUPERVISOR	**Prof. L Meyer**	

KINDLY NOTE:

Should there be a change in the species or number of animal/s required, or the experimental procedure/s - please submit an amendment form to the UP Animal Ethics Committee for approval before commencing with the experiment

APPROVED	Date 31 July 2017
CHAIRMAN: UP Animal Ethics Committee	Signature

S4285-15