Canine blood oxygenation using ultra-low blood flow in an *in vitro*, single-circuit, extracorporeal membrane oxygenator model

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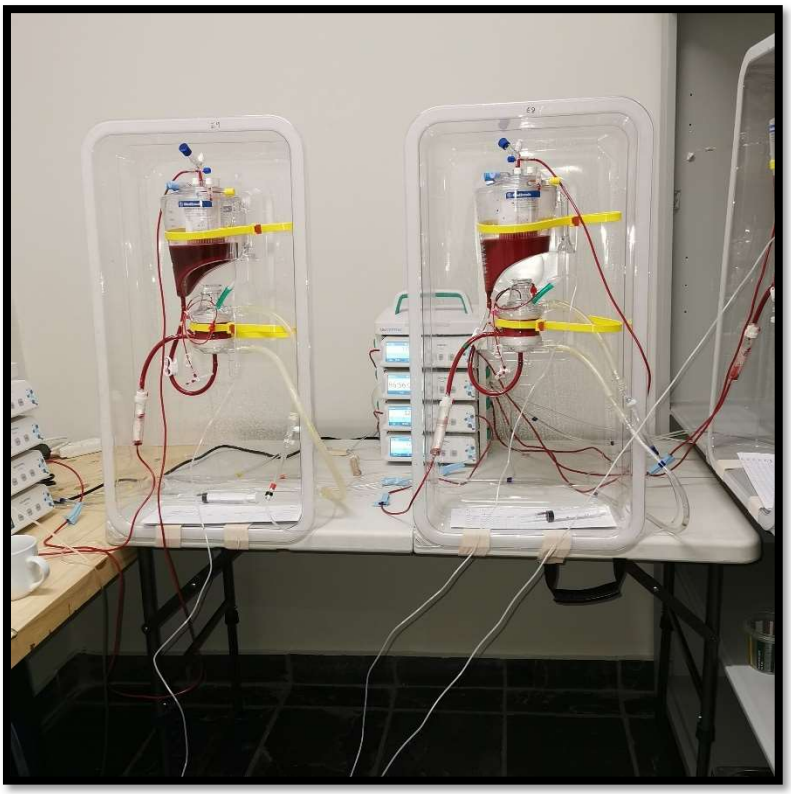

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In the name of the Almighty, the most Gracious, the most Merciful

Recite in the name of your Lord who created – Created man from a clinging substance. Recite, and your Lord is the most Generous – Who taught by the pen – Taught man that which he knew not.

(The Holy Qur'an, 96:1 – 5)

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Abstract

Objective To ascertain the efficacy of a single circuit, *in vitro* extracorporeal membrane model at oxygenating blood and removing carbon dioxide (CO_2) with ultra-low blood flow, in replicates either exposed to room air (fraction inspired oxygen 0.21) (n = 6) or ultra-low 100% oxygen flow (n = 6) driven by a linear peristaltic volumetric infusion pump. The effects of both replicates on free plasma haemoglobin (fHb) concentration was also determined. Furthermore, the ability of the oxygen replicates to maintain global oxygen delivery (DO_2) was predicted in a theoretical model.

Study Design *In vitro*, experimental study.

Methods Twelve units of canine stored whole blood were used, with a median (minimum - maximum) volume of 465 (440 – 500) mL, packed cell volume of 0.5 (0.36 – 0.56) L L^{-1} and storage time of 3 (1 – 41) days. The blood circuit was constructed in the following order, assembled in series; blood reservoir, oxygenator, linear peristaltic infusion pump and tubing to complete the circuit by returning the blood to the reservoir. The water circuit was constructed by connecting a bath warmed to 44°C containing lactated Ringer's solution to the water inlet of the oxygenator by tubing and a linear peristaltic infusion pump. Tubing connected to the water outlet of the oxygenator completed the water circuit by returning the water to the bath. Gas supply to the room air replicates was ensured by removing the gas inlet safety protection cap during assembly. For the oxygen replicates, an oxygen supply rig was constructed to split the oxygen flow to supply an ultra-low flow of oxygen (0.03 L $minute^{-1}$) to each oxygen replicate. Before the sampling commenced, the blood and water phases of the oxygenators were primed. After 2 minutes of the blood and water circulation (both flows at 0.02 L $minute^{-1}$), the first samples were collected (T0). Samples were collected for blood gas analysis post oxygenator (PaO_2 and $PaCO_2$) and pre-oxygenator (PvO_2 and $PvCO_2$). In the room air replicates, samples were collected hourly for the first 8 hours (T1, T2, T3, T4, T5, T6, T7 and T8) then at 24, 32, 48 and 56 hours (T24, T32, T48 and T56). In the oxygen replicates, the oxygen supply was connected after T0 and samples were then collected at 15-minute intervals for the first hour (T0.25, T0.5, T0.75 and

T1) and then hourly for 8 hours (T2, T3, T4, T5, T6, T7 and T8) and then at 24 hours (T24). All the post-oxygenator samples in both replicates were centrifuged and analysed for fHb concentrations, except at T0.25, T0.5 and T0.75 in the oxygen replicates. Data was compared using a linear mixed model (fixed effect: time; random effect: replicates) and *post-hoc* analysis using Dunnet's method within each replicate where each time point was compared to T0. Statistical significance was set at $p < 0.05$. A theoretical model predicting the effect on DO_2 over a range of weights was constructed assuming that the oxygenator was augmenting mixed venous oxygen content. The effects on DO_2 were extrapolated using PaO_2, arterial oxyhaemoglobin saturation (SaO_2) and haemoglobin concentrations (Hb) from the study as well as 2 hypoxaemia scenarios: hypoxic hypoxaemia (PaO_2 40 mmHg; SaO_2 75%) and anaemic hypoxaemia (Hb 6.7 g dL^{-1}). These theoretical DO_2 values were compared to a critical DO_2 of dogs which is reported to be 9.8 mL kg^{-1} $minute^{-1}$.

Results All replicates were operational for the duration of the study period, except 2 of the room air replicates which failed due to thrombosis between T32 and T48. In the room air replicates, the PaO_2 significantly increased from T0 during T1 to T8; the $PaCO_2$ significantly decreased from T0, during T2 to T56. In the oxygen replicates, the PaO_2 significantly increased from T0 for the entire study duration; the $PaCO_2$ significantly decreased from T0 at all time points. In the room air replicates, the rate of change of fHb concentration did not change from T0 for the study duration. However, in the oxygen replicates, the rate of change of fHb concentration was increased from T0 at T3, T4 and T6. In the theoretical model, the predicted DO_2 was maintained above the critical DO_2 when calculated from study variables and the hypoxic hypoxaemia scenario. However, in the anaemic scenario, the predicted DO_2 fell below the critical DO_2.

Conclusion and clinical relevance The extracorporeal membrane oxygenator configured for ultra-low blood and oxygen flow significantly increased the PaO_2 and decreased the $PaCO_2$ for 24 hours. Furthermore, the rate of change of fHb concentrations within the replicates indicate acceptable blood handling characteristics by the circuit components. An increase in DO_2 was identified using the

theoretical model and may clinically improve myocardial oxygenation in pathological conditions characterised by an oxygen debt. Further studies are needed to ascertain whether this study can be translated into clinical setting.

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Abbreviations

°C	Degrees Celsius
µL	Microlitre
ACT	Activated clotting time
ARDS	Acute respiratory distress syndrome
AV-ECMO	Arteriovenous extracorporeal membrane oxygenation
CaO_2	Oxygen content of blood
CI	Confidence interval
CO	Cardiac output
CO_2	Carbon dioxide
CPB	Cardiopulmonary bypass
CvO_2	Oxygen content of mixed venous blood in a theoretical DO_2 model
dL	Decilitre
DO_2	Global oxygen delivery
$ECCO_2R$	Extracorporeal carbon dioxide removal
ECLS	Extracorporeal life support
ECMO	Extracorporeal membrane oxygenation
fHb	Free plasma haemoglobin
FiO_2	Fraction inspired oxygen
G	Gram
G	Birmingham gauge
g dL^{-1}	Grams per decilitre
Hb	Haemoglobin
IU	International unit
Kg	Kilogram
L	Litre
L $minute^{-1}$	Litres per minute
LRS	Lactated Ringer's solution
mL	Millilitre
mL $hour^{-1}$	Millilitres per hour
Mm	Millimetre
mmHg	Millimetres mercury
P	Statistical p-value
$PaCO_2$	Tension of carbon dioxide in blood post-oxygenator
PaO_2	Tension of oxygen in blood post-oxygenator/arterial system
PCO_2	Tension of carbon dioxide in blood
PO_2	Tension of oxygen in blood
PVC	Polyvinylchloride
$PvCO_2$	Tension of carbon dioxide in blood pre-oxygenator
PvO_2	Tension of oxygen in blood pre-oxygenator
Q-Q	Quantile-quantile
RRT	Renal replacement therapy
SaO_2	Percentage arterial oxyhaemoglobin saturation
SIRS	Systemic inflammatory response syndrome

T0	First study time point
T0.25, T0.5, T0.75	Study time points of 15-minute intervals during the first hour of the study
T1, T2, T3, T4, T5, T6, T7, T8, T24, T32, T48, T56	Study time points of hourly intervals
tHb	Total haemoglobin
UFH	Unfractionated heparin
VA-ECMO	Venoarterial extracorporeal membrane oxygenation
VO_2	Global oxygen consumption
VV-ECMO	Venovenous extracorporeal membrane oxygenation

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Chapter one

1.1 Background physiological concepts

1.1.1 Oxygen supply to tissues

The cardiovascular and respiratory systems work in concert to fulfil their essential functions of supplying oxygen to metabolically active cells whilst removing carbon dioxide (CO_2) and other metabolites. Oxygen is an essential component of aerobic respiration, where it acts as a terminal electron acceptor in the mitochondrial membrane during oxidative phosphorylation. This process ultimately yields adenosine triphosphate, a source of energy for metabolic processes. Oxygen transport within the mammalian body can be divided into 2 processes, convection and diffusion. Convection relates to the bulk transport of oxygen via the cardiovascular system, whereas diffusion relates to oxygen moving down a gas tension gradient at the level of blood vessel capillary networks. The principal capillary networks are the pulmonary capillaries and the systemic capillaries to the cells of tissues, where oxygen will eventually diffuse into the mitochondria. Convection is an active process requiring energy. Conversely, diffusion is a passive process (Dunn et al. 2016).

Oxygen diffuses across the alveolar membrane into the pulmonary capillaries, is transported to systemic capillary networks in the blood by convection within the cardiovascular system, then enters the interstitial space and finally the cell, by diffusion. Thus, the global oxygen delivery (DO_2), the amount of oxygen delivered to tissues per unit of time, is dependent upon the ability of the cardiovascular system to transport blood around the body, as determined by the cardiac output (CO). Furthermore, the DO_2 is also dependent upon the oxygen content of arterial blood (CaO_2). This relationship between DO_2, cardiac output and CaO_2 is arithmetically demonstrated as follows:

$DO_2 = CO \times CaCO_2$

Cardiac output is defined as the amount of blood that the heart pumps per unit time (Vincent 2008). The determinants of CO are heart rate, cardiac contractility, preload and afterload (Vincent 2008;

Peterson & Moses 2011). In addition to DO_2, the concept of cardiac output is important in terms of oxygen uptake into the bloodstream. The right heart receives deoxygenated blood from the systemic circulation and pumps it into the pulmonary circulation where oxygenation of the blood takes place. Oxygenation transfer into the blood is dependent upon adequate perfusion of the pulmonary capillaries. The left heart subsequently receives oxygenated blood and pumps it through the systemic circulation. Consequently, DO_2 relies on an adequate volume status and cardiovascular function.

The arterial oxygen content per 100 mL (decilitre; dL) of blood (CaO_2) is dictated by the haemoglobin content of blood and the ability of oxygen to dissolve within the plasma at physiological temperature as follows (Peterson & Moses 2011; Dunn et al. 2016):

$$CaO_2 = (1.34 \times Hb\ (g\ dL^{-1}) \times SaO_2/100) + (0.003 \times PaO_2)$$

Where 1.34 is the Hüfner's constant for canine haemoglobin, every gram of canine haemoglobin (Hb) is able to bind 1.34 mL of oxygen per dL of blood; 0.003 is the solubility coefficient for oxygen at body temperature (37°C) according to Henry's Law, 0.003 mL of oxygen dissolved per dL of blood for every 1 mmHg oxygen tension in arterial blood (PaO_2); SaO_2 is the percentage oxyhaemoglobin saturation and PaO_2 is the oxygen tension within arterial blood (in mmHg) (Peterson & Moses 2011; Dunn et al. 2016). Haskins et al. (2005) compiled reference cardiopulmonary values for normovolaemic, unsedated, clinically healthy dogs, breathing room air. The mean ± standard deviation (95% confidence interval) values reported for CaO_2, Hb, SaO_2 and PaO_2 are 17.8 ± 2.3 mL dL^{-1} (17.4; 18.3), 13.6 ± 1.8 g dL^{-1} (13.3; 14), 96.3 ± 0.9% (96.1; 96.5) and 99.5 ± 6.8 mmHg (98.1; 100.8), respectively (Haskins et al. 2005).

Although the major determinant of the oxygen carrying capacity of the blood is related to its haemoglobin concentration and the PaO_2 ultimately only contributes approximately 0.3 mL oxygen per dL of blood under normal circumstances, the PaO_2 dictates the affinity of haemoglobin for oxygen (Peterson & Moses 2011; Dunn et al. 2016). Each molecule of haemoglobin can reversibly bind up to 4 molecules of oxygen, with each subsequent oxygen molecule binding to haemoglobin with greater

affinity. This phenomenon of subsequent molecules binding with increased affinity is known as positive cooperativity (Patel et al. 2021).

The tertiary structure of haemoglobin changes according to its environment. In low pH circumstances in the tissues, where carbon dioxide predominates, haemoglobin exists in its *taut* form, which has a low oxygen affinity, promoting oxygen release. Conversely, in high pH environments such as the lungs, where oxygen predominates and the carbon dioxide tension (PCO_2) is low, haemoglobin exists in its *relaxed* form, which has a high oxygen affinity, promoting oxygen binding (Peterson & Moses 2011; Dunn et al. 2016). This interplay of pH, oxygen and CO_2 is known as the Bohr effect (Dunn et al. 2016).

Carbon dioxide is primarily transported within the plasma, either dissolved in a gaseous state or as bicarbonate. It is also carried within erythrocytes as carbaminohaemoglobin. In comparison to oxygenated blood, deoxygenated blood has a greater capacity for carrying CO_2, a phenomenon known as the Haldane effect. The mean ± standard deviation (95% confidence interval) value reported for $PaCO_2$ by Haskins et al. (2005) is 40.2 ± 3.4 mmHg (39.5; 41). The combination of the Bohr and Haldane effects facilitates oxygen uptake and CO_2 release in the pulmonary capillaries and the converse within capillaries at the tissues (Dunn et al. 2016).

Ultimately, factors affecting cardiopulmonary performance will hinder the uptake of oxygen into the blood and therefore DO_2. A reduction in CaO_2, also known as hypoxaemia, will also lead to a reduction in DO_2. Since the oxyhaemoglobin concentration and PaO_2 are the determinants of CaO_2, hypoxaemia can be subclassified as anaemic hypoxaemia (packed cell volume of < 0.37 L L^{-1} (Paltrinieri 2014)) and hypoxic hypoxaemia (PaO_2 < 60 mmHg (Hopper & Powell 2003)) (Pittman 2011).

The objective of ensuring an adequate DO_2 is to maintain optimal tissue oxygenation. Tissue oxygenation may be estimated from the global oxygen consumption (VO_2), oxygen extraction ratio (OER) and oxygen consumption per unit tissue weight (Crystal et al. 1991; Giordano 2005; Peterson & Moses 2011). The VO_2 is the product of CO and the arteriovenous oxygen content difference and the OER is the quotient of VO_2 and DO_2 (Peterson & Moses 2011). Oxygen consumption per unit tissue

weight is usually calculated experimentally (Crystal et al. 1991). The systemic oxygen extraction ratio and oxygen consumption is 20 – 30% and 4.1 mL kg^{-1} $minute^{-1}$, respectively (Chen et al. 1984; Peterson & Moses 2011).

1.1.2 Oxygen supply to the myocardium and the consequences of myocardial hypoxia

In stark comparison to systemic oxygen utilisation, the oxygen extraction ratio and oxygen consumption of the resting myocardium is approximately 70% and 8 – 15 mL $minute^{-1}$ per 100 g tissue, respectively (Crystal et al. 1991; Giordano 2005). Thus, it is clear that the myocardium is dependent on a constant supply of oxygen and cannot produce sufficient energy under sustained anaerobic conditions to maintain vital cellular processes (Giordano 2005). Under conditions of severe hypoxic hypoxaemia (PaO_2 < 40 mmHg), cardiac contractility and thus cardiac output is significantly diminished (Walley et al. 1988). The reasons behind this diminished contractility have not been completely elucidated, however, they are explained in part by excessive intracellular sodium and calcium concentrations within myocytes. These ions accumulate intracellularly as a result of a cascade of events triggered by a hypoxic hypoxaemia episode. Ultimately, ventricular function is hampered by the development of arrythmias, a reduction in ventricular compliance and eventual ventricular wall necrosis (Salameh et al. 2020).

1.1.3 Therapeutic options in the face of a reduction in DO_2

The conventional treatment for pathological states resulting in a diminished DO_2 is using fluid therapy, inotropes and vasopressors to optimise the haemodynamic status of the patient and thus DO_2. Additionally, ventilatory support may be instituted to improve the oxygen content of the blood. However, these therapies may increase myocardial workload and increase myocardial oxygen consumption (Peterson & Moses 2011). In circumstances where pharmacological and ventilatory interventions have failed, the institution of an extracorporeal life support (ECLS) system, namely, extracorporeal membrane oxygenation (ECMO) may be warranted.

1.2 Literature review of ECMO and associated equipment

1.2.1 The history and background of extracorporeal systems

The role of ECLS systems has dramatically increased over the last 4 decades within the scope of treatment of cardiovascular and pulmonary conditions. Broadly, ECLS systems are indicated for reversible conditions where traditional pharmacological and ventilatory interventions have failed or are worsening the underlying pathology (Ali & Vuylsteke 2019). These systems oxygenate blood and remove CO_2, either alone or incorporating haemodynamic support, depending on the configuration. Extracorporeal membrane oxygenation remains the most common ECLS modality (McRae & de Perrot 2018; Ali & Vuylsteke 2019). The basic premise behind ECMO is the removal of blood from the systemic circulatory system and pumping it through a membrane oxygenator which oxygenates the blood, removes CO_2 and warms the blood before returning it to the systemic circulation (Ali & Vuylsteke 2019; Lequier et al. 2013).

In 1972, ECMO was successfully used for the first time in an adult patient suffering respiratory failure secondary to trauma (Ali & Vuylsteke 2019; Hill et al. 1972; Kawahito et al. 2002). By 1976, ECMO became an accepted therapeutic option for moribund infants suffering hyaline membrane disease. However, ECMO was reluctantly used in adults due to the high mortality and morbidity rate (>40%) associated with its use. In 1979, the first randomised controlled study on the use of ECMO in 90 adults suffering respiratory failure confirmed these morbidity and mortality rates (Zapol et al. 1979). Initial ECMO units consisted of cardiopulmonary bypass (CPB) units adapted to intensive care unit use. The oxygenators within these CPB circuits were not designed to tolerate more than several hours usage. Consequently, the oxygenation membrane within these early units would rapidly deteriorate leading to a large air-blood interface and consequent mortalities. In addition, injurious ventilatory practices (excessive airway pressures and inspired oxygen concentrations) also contributed to morbidity and mortality in these patients (Bartlett et al. 1986; McRae & de Perrot 2018; Ali & Vuylsteke 2019; Lequier et al. 2013).

1.2.2 ECMO in a veterinary context

Extracorporeal membrane oxygenation has been used successfully in human medicine over the last 4 decades to treat a variety of conditions associated with cardiopulmonary compromise. Its application in animals has largely been in dogs, pigs and sheep experimental models for translation to human therapies (Millar et al. 2019). The practice of ECMO in human medicine was borne out of modification of CPB circuits, when they were repurposed for longer use in an intensive care unit setting (Ali & Vuylsteke 2019). However, unlike human medicine, the evolution of the clinical application of ECMO in veterinary patients is not following on from the clinical application of CPB. The challenges associated with applying ECMO clinically in veterinary patients, however, may be very similar to the application of CPB. The extracorporeal circuits and associated equipment available for the application of CPB and ECMO are specifically designed for use in human patients and thus may not cater for the greater variation in size and anatomy of veterinary patients, especially very small patients. Furthermore, the small blood volumes associated with these patients heightens the risks of severe haemorrhage, which may be further compounded by limited availability of veterinary blood products (Pelosi et al. 2013).

Regardless, traditional ECMO may not be practically feasible in most veterinary clinical setting due to the intensive monitoring required for the various potential complications of ECMO, especially the risk for catastrophic, fatal haemorrhage associated with cannulation of large central vessels (Allen et al. 2011). Furthermore, the vast costs, complicated equipment and intense monitoring associated with ECMO may have not allowed it to progress further than use as experimental models in animals.

Extracorporeal membrane oxygenation may have a role to fulfil in veterinary medicine, considering that a retrospective study concluded that the mortality rate from acute respiratory distress syndrome (ARDS) was 84% and 100% in dogs and cats respectively (Boiron et al. 2019). The exact incidence of ARDS in veterinary patients is unknown due to the limitations in the veterinary diagnostic criteria for the syndrome. However, the most common inciting cause for ARDS in dogs was identified to be aspiration pneumonia (Boiron et al. 2019).

1.2.3 Traditional ECMO configurations

Depending on the degree of support and underlying patient pathology, 3 different ECMO configurations exist. Venovenous ECMO (VV-ECMO) is indicated in patients suffering reversible respiratory failure with refractory hypoxaemia or hypercapnia despite mechanical ventilation. Instituting VV-ECMO allows lessening the intensity of ventilation, decreasing the degree of ventilator induced lung injury. Lung perfusion is maintained allowing normal pulmonary endocrine functions to continue. Blood is drained from the *vena cava* and returned to the right atrium (McRae & de Perrot 2018; Lequier et al. 2013; Richards & Joubert 2013). Venoarterial ECMO (VA-ECMO) is applied in cases of reversible cardiogenic shock non-responsive to conventional therapy (with or without respiratory failure), as a bridge to heart transplant or implantation of cardiac assist devices. Blood is usually drained from the *vena cava* and returned via one of the femoral arteries. The pulmonary circulation is unloaded, however, there may be increased left ventricular afterload, compromise to coronary and cerebral perfusion and limb ischaemia (McRae & de Perrot 2018; Ali & Vuylsteke 2019; Lequier et al. 2013). Arteriovenous ECMO (AV-ECMO) also known as extracorporeal CO_2 removal ($ECCO_2R$) is a pumpless configuration driven by the patient's cardiac output. It allows complete removal of CO_2 but only partial oxygenation; thus, the indication for this configuration is usually hypercapnic respiratory failure (Richards & Joubert 2013; McRae & de Perrot 2018; Ficial et al. 2021).

1.2.4 ECMO circuit components

All ECMO circuits, in their most basic form, consists of a pump, oxygenator and heat exchanger arranged in series. These are linked by tubing originating at the blood outflow catheter and returning via the infusion catheter. More complex circuits may contain saturation monitors, sampling ports and bridges bypassing circuit components (Lequier et al. 2013; Tulman et al. 2014).

Arguably, the crux ECMO circuit is the oxygenator. The development of portable, efficient and durable oxygenators has spanned the last 60 years (Kawahito et al. 2002). It was during the 1970's that membrane oxygenators were first used (McRae & de Perrot 2018). The most prolific of these was the

Kolobow oxygenator. It was manufactured out of flat reinforced sheet of silicone rubber membrane which was wrapped around a wire mesh in a spiral. Blood flowed in a counter current manner to the gas and exchange occurred by diffusion across the membrane (Kolobow & Bowman 1963). The Kolobow oxygenator was efficient, however, patient-specific size oxygenations were required, necessitating a variety of oxygenators to be available. In addition, the Kolobow oxygenator required a large priming volume, posed a high resistance to blood flow, was technically challenging to use and was also relatively expensive (Lequier et al. 2013; Kawahito et al. 2002). The development of hollow fibre oxygenators improved blood flow characteristics but were immensely prone to plasma leakage and mechanical damage (Funakubo et al. 1996). Recent refinements to the hollow fibre design resulted in the development of silicone and polymethylpentene membranes which are more resistant to plasma leakage than their predecessors thus, limiting air-blood interfaces. Furthermore, these newer membranes are more resistant to mechanical damage, provoke less inflammation and thrombosis and are suited to long term use (Funakubo et al. 1996; Kawahito et al. 2002; McRae & de Perrot 2018;).

The oxygenator allows the flow of blood, oxygen and water through various pathways within the construct and these are referred to the blood, gaseous and water phases, respectively. The oxygenation membrane within an oxygenator creates an interface between the gas and blood phases of the oxygenator in order for gaseous exchange to take place. Gas exchange across a membrane is related to the difference of gas tensions across the membrane. The arrangement of the gas and blood phases within the oxygenator housing for counter current exchange further optimises the gas tension gradient across the membrane. Carbon dioxide diffuses across the membrane more readily than oxygen and can accumulate within the gaseous phase. Thus, oxygenation is related to blood flow whilst CO_2 removal is related to gas flow (sweep speed) (Allan et al. 2011; Lequier et al. 2013; Finney et al. 2014; Ficial et al. 2021). The rated flow of a membrane is defined as the amount of blood with 75% oxyhaemoglobin saturation, that increased to 95% in 1 minute (Lequier et al. 2013). The rated flow represents the maximal oxygenation capability of that oxygenator and is related to the surface

area of the oxygenation membrane in contact with the blood phase (Lequier et al. 2013). The recommendation for the ratio of gas flow to blood flow for optimal oxyhaemoglobin saturation and CO_2 removal, ranges from 2:1 to 4:1 (Funakubo et al. 1996; Kawahito et al. 2002;). Blood flow through the oxygenator should be approximately 60% of the patient's cardiac output to achieve a haemoglobin saturation of > 90% (McRae & de Perrot 2018). Considering that the cardiac output of a dog is 0.868 L kg^{-1} $minute^{-1}$ (Adachi et al. 1976), a minimum blood flow of 0.521 L kg^{-1} $minute^{-1}$ would be required to fulfil the traditional ECMO requirement. Carbon dioxide removal from the oxygenator housing requires the flow of gas (sweep gas) to drive it out the gas outlet. Thus, the basis of the recommendation for the higher sweep gas flow in relation to blood flow (Kawahito et al. 2002; Funakubo et al. 1996).

In adult ECMO systems, a blood flow of < 1.5 L $minute^{-1}$ is considered to be low-flow system while a blood flow of > 4 L $minute^{-1}$ is considered to be a high-flow system (Ki et al. 2019; Chan et al. 2021).

The removal of large volumes of blood and passing it through an oxygenator cools down the blood, predisposing the ECMO patient to hypothermia. This heat loss is counteracted by means of a water-based heat exchanger. Water is passed through sealed hollow fibres counter to the blood flow, maximising heat exchange (Allan et al. 2011). Older ECMO systems used a heat exchange unit within the ECMO circuit. However, newer oxygenators integrate the heat exchange system, also known as the water phase, into the oxygenator housing (Allan et al. 2011; Lequier et al. 2013). It is recommended the temperature of a water-based heat exchanger be set at 37°C or as required to maintain the patient's temperature as close to 37°C as possible (Extracorporeal Life Support Organization 2017).

Traditionally, semi-occlusive roller pumps provided the siphon and flow within ECMO systems. These pumps are prone to wear and tear, the motors are bulky, tubing can rupture within the pump head and infusion pressures can rapidly increase in case of line occlusion leading to blowout. Semi-occlusive pumps have mostly been replaced by centrifugal pumps (Lequier et al. 2013). These centrifugal pumps

generate flow by means of a spinning rotor which generates a pressure differential across the pump. Centrifugal pumps are lighter and are assembled from components more tolerant to wear, thus, allowing prolonged use (Lequier et al. 2013). The likelihood of erythrocyte damage is lower with centrifugal pumps but thrombus formation and heat generation within the pump head remains an issue (McRae & de Perrot 2018; Lequier et al. 2013; Tulman et al. 2014).

The costs of the ECMO equipment contributes substantially to the overall costs and thus alternatives must be sought. Considering that volumetric infusion pumps are ubiquitous in most referral veterinary centres in South Africa, these pumps may be effective in driving ECMO circuits.

1.2.5 Volumetric infusion pumps

Volumetric infusion pumps use various mechanisms to drive fluid flow, which have varying effects on the degree of haemolysis. Manual roller pumps use rollers attached to a wheel which repeatedly compresses infusion tubing, moving fluids through it (Hansen et al. 1988). In linear peristaltic infusion pumps, flow is achieved by completely compressing infusion tubing in a wave like fashion. A variation on linear peristaltic pumps are shuttle-type pumps which mechanically deforms infusion tubing for forward flow whilst closing an upstream valve and simultaneously opening a downstream valve. The tubing is reshaped during filling by opening an upstream valve and closing a downstream valve (Frey et al. 2003; Poder et al. 2017). With respect to the drive mechanism, manual roller pumps cause the most haemolysis and shuttle-type pumps the least (Hansen et al. 1988; Frey et al. 2003; Poder et al. 2017). However, the speed of infusion, as well as the age of the blood also influences haemolysis, with higher flow rates and older blood resulting in highest degree of haemolysis (Stiles & Raffe 1991; Wilson et al. 2016; Poder et al. 2017). Contrary to these findings Kusahara et al. (2015), no significant haemolysis was detected during blood transfusions with flow rates ranging between 10 mL $hour^{-1}$ to 300 mL $hour^{-1}$ (0.00017 to 0.005 L $minute^{-1}$) administered via a linear peristaltic infusion pump.

In contrast to the monitoring intensity and technical expertise required for ventilating a patient, the relative ease of placing a central line and setting up an ECMO circuit using equipment and consumables

readily found in veterinary referral centres should be investigated. However, considering that the maximum flow rate of most volumetric infusion pump (1200 mL $hour^{-1}$ which is 0.02 L $minute^{-1}$) is much less than an ECMO pump (low-flow 1.5 L $minute^{-1}$), an ECMO circuit driven by a volumetric infusion pump would have to function at an ultra-low blood flow and gas flow rate.

1.2.6 Complications as a result of ECMO

The reported incidence of haemolysis as a consequence of ECMO is between 5 and 18%. Erythrocytes may be damaged within the ECMO circuit by exposure to sheer stress, high flow rates and being subject to pressure changes within the oxygenator. Haemolysis releases haemoglobin into plasma which then binds nitric oxide resulting in undesirable systemic vasoconstriction, endothelial dysfunction, and platelet aggregation. Consequently, acute renal injury and multiple organ dysfunction may occur (Lehle et al. 2015; Omar et al. 2015). Markers of haemolysis include free plasma haemoglobin (fHb) and lactate dehydrogenase concentrations. Haemolysis, as a complication of ECMO, is defined as fHb concentration > 0.05 g dL^{-1}, with normal concentrations being < 0.01 g dL^{-1} (Lehle et al. 2015; Omar et al. 2015). In a retrospective study, Lehle et al. (2015) reported that survivors of VV-ECMO experienced lower peak fHb concentration versus non-survivors. Omar et al. (2015) found that a fHb concentration > 0.05 g dL^{-1} after 24 hours of instituting ECMO resulted in a 3.4 fold increase in the odds of patient mortality.

Thrombosis, thromboembolic events and haemorrhage are common complications encountered during the application of ECMO in humans (Mulder, et al.2017; Cho et al. 2017). These coagulopathies can result from the disease necessitating ECMO and the blood-circuit interface. Shortly after initiation of ECMO, albumin and globulins adhere to the circuit, providing a surface for platelets to activate and initiate the coagulation cascade, causing thrombosis and culminating in a consumptive coagulopathy (Mulder et al. 2017; Wong et al. 2018). Hence, anticoagulant therapy is indicated for patients undergoing ECMO (McRae & de Perrot 2018; Ali & Vuylsteke 2019; Cho et al. 2017; Mulder et al. 2017).

In addition to thrombosis, the blood-circuit interface also evokes an inflammatory cascade, similar to that seen during in systemic inflammatory response syndrome (SIRS) (Millar et al. 2016). This inflammatory cascade rapidly causes a rise in pro-inflammatory cytokines which in turn activates the complement and contact systems. Unchecked, this inflammatory response may lead to endothelial damage with a resultant disruption in the microcirculation and eventual end-organ dysfunction (Millar et al. 2016). Currently, ECMO induced SIRS is primarily mitigated by the initiation of anticoagulant therapy and anticoagulant bonded circuits due to the initiation of the inflammatory cascade as a result of circuit contact. Future therapies are focussing on mesenchymal stromal cell therapy as a possible modulator of the ECMO induced inflammatory cascade (Millar et al. 2016).

1.2.7 Anticoagulation during ECMO

Unfractionated heparin (UFH) functions by amplifying the effects of antithrombin, which primarily inhibits factors IIa and Xa; factors IXa and XIIa are inhibited to a lesser extent (Mulder et al.2017). Unfractionated heparin forms the cornerstone of anticoagulant therapy during ECMO, due to it being inexpensive, familiar to physicians, having a rapid onset and offset and is antagonised by protamine (Cho et al. 2017; Mulder et al.2017; Wong et al. 2018). However, UFH has unpredictable clinical effects due to ineffective inhibition of platelet bound factor Xa, phospholipid-bound factor Va–Xa complex and fibrin-bound thrombin. Furthermore, UFH induces a reduction in antithrombin concentrations leading to heparin resistance, a phenomenon where high doses of UFH are required for clinical effect. Moreover, UFH can cause heparin induced thrombocytopaenia, where immune mediated destruction of platelets occurs after forming complexes with UFH (Baroletti & Goldhaber 2006; Wong et al. 2018). Therefore, numerous alternatives have been investigated, such as low molecular weight heparin, citrate, bivalirudin and argatroban (Mulder, et al.2017; Cho et al. 2017; Wong et al. 2018; Doymaz 2018).

Due to the critical nature of human patients requiring ECMO, multi-organ failure is not uncommon and renal replacement therapy (RRT) often must be instituted simultaneously in almost 50% of

patients (Seczyńska et al. 2014; Mitra et al. 2021). Nonetheless, ECMO has only been applied experimentally in dogs as a pre-clinical model (van der Hulst et al. 1991; Miyasaka et al. 1996; Millar et al 2019), whilst RRT has been clinically applied in dogs and cats for approximately 25 years (Cowgill & Langston 1996; Diehl & Seshadri 2008). Although citrate is the local anticoagulant of choice in RRT systems, it is not required in patients requiring both ECMO and RRT because of the systemic anticoagulation instituted during the initiation of ECMO (Seczyńska et al. 2014). Moreover, citrate has never been clinically applied as the sole anticoagulant in human ECMO systems, although Cardenas et al. (2006) successfully used local (within the extracorporeal circuit only) citrate anticoagulation in an experimental ovine ECMO model (Mulder, et al.2017; Cardenas et al. 2006). Barring the effects of citrate on ionised calcium and bicarbonate concentrations, it has been variably demonstrated to increase PaO_2 or to have no effect on PaO_2 (Suki et al. 1988).

1.2.8 Summary and problem statement

In veterinary medicine, conditions causing severe hypoxic hypoxaemia and hypercapnia, such as acute ARDS, pulmonary contusions secondary to trauma, aspiration pneumonia, cardiac failure are traditionally treated by instituting invasive mechanical ventilation (Hopper & Powell 2003). Furthermore, catecholamine therapy may be instituted in cases of haemodynamic instability non-responsive to fluid therapy (Peterson & Moses 2011). However, these therapies may worsen the underlying pathology and consequently impair DO_2 (Peterson & Moses 2011; Ali & Vuylsteke 2019). These cases may benefit from the application of ultra-low flow ECMO, which could maintain DO_2; the intensity of mechanical ventilation and haemodynamic support may be reduced, allowing the cardiopulmonary system time to recover. Furthermore, ECMO may be applied in conditions where a relative blood oxygen content deficit exists, such as anaemia or hypermetabolic states.

Extracorporeal membrane oxygenation has been used successfully in human medicine over the last 4 decades to treat a variety of conditions associated with cardiopulmonary compromise. Its application in animals has largely been in dogs, pigs and sheep experimental models for translation to human therapies (Millar et al. 2019). Regardless, traditional ECMO may not be practically feasible in most veterinary clinical setting due to the intensive monitoring required for the various potential complications of ECMO, especially the risk for catastrophic, fatal haemorrhage associated with cannulation of large central vessels (Allen et al. 2011). Furthermore, the vast costs, complicated equipment and intense monitoring associated with ECMO has not allowed it to progress further than use as experimental models in animals. In addition to the economic advantages, an ultra-low flow ECMO configuration could mitigate the risk of catastrophic haemorrhage during ECMO therapy.

1.3 Aims and objectives

The aim of the study is to evaluate the efficacy of blood oxygenation and CO_2 removal, as well as the propensity for haemolysis of an *in vitro* ECMO circuit configured for ultra-low flow. This circuit will be assembled out of equipment readily available in an *in vitro* veterinary referral hospital setting. The secondary aim of the study is to predict the ability of an ultra-low flow ECMO system to maintain an adequate DO_2 over a range of weights of dogs. The aims were fulfilled by the following objectives:

1. The longevity of the circuits running in an ultra-low flow configuration will be ascertained by running the room air replicate group for a total of 56 hours.
2. The efficacy of the blood oxygenation and CO_2 removal capacity of the ultra-low flow ECMO system will be determined by measuring arterial and venous (pre- and post-oxygenator respectively) blood oxygen (PO_2) and carbon dioxide (PCO_2) tensions.
3. The propensity of the ultra-low flow ECMO system to cause haemolysis will be determined by centrifuging arterial (post-oxygenator) samples and determining the fHb concentration of the supernatant.

4. A theoretical DO_2 model will be conceptulised in dogs over a range of weights based on the contribution of oxygenated blood from an ultra-low flow ECMO system to CO.

1.4 Hypotheses

1.4.1 Primary hypotheses

- H_0: There will not be a significant increase in PaO_2, nor a significant decrease in $PaCO_2$ and fHb concentration will not significantly increase over the duration of the experiment.
- H_1: There will be a significant increase in PaO_2, a significant decrease in $PaCO_2$ and fHb concentration will significantly increase over the duration of the experiment.

1.4.2 Secondary hypotheses

- H_0: The CaO_2 achieved by the oxygenators will not be sufficient to improve DO_2 (> 9.8 mL kg^{-1} $minute^{-1}$) in a theoretical dog model in which DO_2 is supplemented by ultra-low flow ECMO.
- H_1: The CaO_2 achieved by the oxygenators will be sufficient to improve DO_2 (> 9.8 mL kg^{-1} $minute^{-1}$) in a theoretical dog model in which DO_2 is supplemented by ultra-low flow ECMO.

Chapter two

2.1 Materials and methods

2.1.1 Source of canine stored whole blood

This study was approved by the Animal Ethics and Research Committees of the University of Pretoria (REC200-19). Canine stored whole blood was used, sourced from the Onderstepoort Animal Blood Bank from client owned and in-house donor dogs. Routine consent forms for donation were completed by the owners. This form does indicate that the blood donated may be used for research rather than clinical use, due to the blood bank being part of an academic institution. Donors are blood typed at their first donation and are health screened annually by means of a clinical examination, a full blood count (which routinely includes a blood smear to check for the presence of blood parasites) and a comprehensive blood chemistry panel.

A total of 12 units of canine whole blood, stored in citrate phosphate dextrose with adenine (JMS North America Corporation, USA) were used, with a median (minimum - maximum) volume of 465 (440 – 500) mL, packed cell volume of 0.5 (0.36 – 0.56) L L^{-1} and storage time of 3 (1 – 41) days. It must be clarified however, that only a one unit of blood was 41 days old. The rest of the units used were between 1 and 3 days old. This single older unit of blood widely skews the age of the blood. The blood units used in each replicate were not randomised – the quantity of units needed per experimental day were supplied the day before by the blood bank and therefore used as they became available.

2.1.2 Study design and model

An *in vitro*, experimental study was performed to evaluate the effects of ultra-low blood flow through an extracorporeal membrane oxygenator on blood gases and free plasma haemoglobin. The sample size was calculated to be 6 replicates based on the assumption that there would be a maximum difference of 40 mmHg between the mean PO_2 measured from the first blood sample to one obtained 1 hour later (assumed standard deviation of 20 mmHg; target power 0.8; alpha 0.05; power and

sample size calculator for a one-way ANOVA). A sample size of 6 replicates was also deemed to be sufficient when a maximum difference of 20 mmHg between the mean PCO_2 and a maximum difference of 0.4 g dL^{-1} between the mean fHb concentration measured from the first blood sample to one obtained 1 hour later (assumed standard deviation of 10 mmHg and 0.2 g dL^{-1} respectively; target power 0.8; alpha 0.05; power and sample size calculator for a one-way ANOVA). Furthermore, the sample size was comparable to other *in vitro* ECMO studies (Graulich et al. 2000; Graulich et al. 2002). A total of 12 replicates were performed, 6 with room air (fraction inspired oxygen: FiO_2 of 0.21) and 6 with 100% oxygen (FiO_2 of 1.0).

Blood and warmed lactated Ringer's solution (LRS) (Lactated Ringer's Solution, Fresenius-Kabi, South Africa) were circulated through the blood and water phases, respectively, in both replicate groups. However, in the room air replicates, the gaseous phase was exposed to ambient air whilst medical oxygen (Air Products (Pty) Ltd, South Africa) was supplied to the gaseous phase of the oxygen replicates.

First the room air replicates were completed followed by the oxygen replicates in order to first gain familiarity with the circuit setup without the addition of the gaseous phase. The study took place at the Valley Farm Animal Hospital, Pretoria, South Africa, between the 17th and 22nd of May 2021. Median (minimum – maximum) barometric pressure was determined to be 651 (647 - 658) mmHg as detected by a hand-held, point-of-care blood gas analyser (epoc Blood Analysis System, Siemens Healthineers, Germany), in the morning (07:00) and afternoon (12:00).

2.1.3 Assembly of *in vitro* ECMO circuit

Each replicate was constructed from the Cardiac Surgery Division Europe Custom Pack (Medtronic Cardiac Surgery Division Europe, the Netherlands) within the clear plastic packaging tray (760 mm x 405 mm x 160 mm; length x width x depth) supplied. The polypropylene microporous fibre oxygenator (Affinity Pixie Oxygenator, Medtronic Cardiac Surgery Division Europe, the Netherlands) and blood reservoir (Affinity Pixie Cardiotomy/Venous Reservoir, Medtronic Cardiac Surgery Division Europe, the

Netherlands) were supplied within. This oxygenator is designed for paediatric use (neonates, infants and small children), with a priming volume of 48 mL (Medtronic Affinity Pixie Oxygenation System 2012). The oxygenator and reservoir were secured to the packaging tray by releasable cable ties. When the packaging tray was stood upright, the oxygenator was positioned in the middle of the plastic packaging with the reservoir positioned immediately above it, both correctly spatially oriented for use (**Photo 1**).

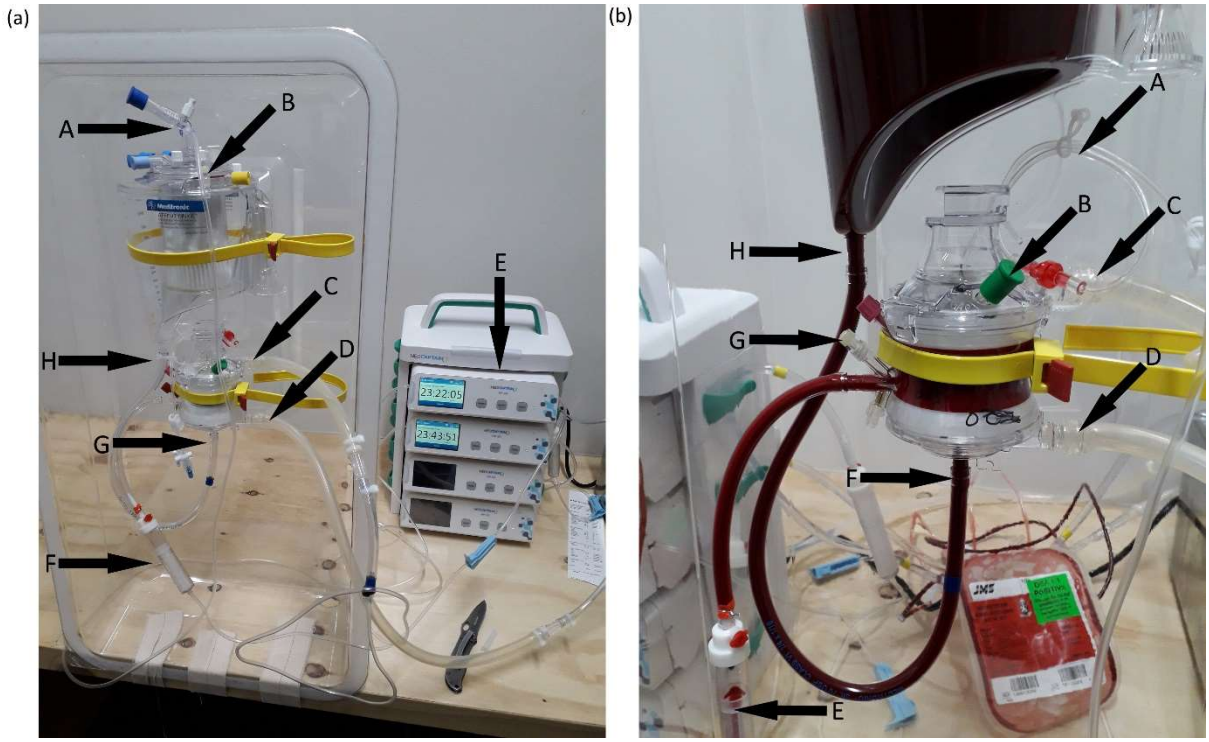

Photo 1 *An assembled in vitro extracorporeal membrane oxygenation circuit ready for operation using ultra-low blood and oxygen flow rates. Various components are indicated (a), An assembled in vitro extracorporeal membrane oxygenation circuit in operationusing ultra-low blood and oxygen flow rates. This photo was taken immediately before the safety protection caps from the gas inlet was removed to connect the oxygen supply (b).*

(a) A: central reservoir inlet; B: peripheral reservoir inlet; C: oxygenator water inlet D: oxygenator water outlet; E: linear peristaltic infusion pump; F: drip chamber of blood circuit; G: reservoir blood outlet; H: reservoir blood outlet.

(b) A: pre-membrane purge port line; B: gas inlet safety protection cap; C: oxygenator water inlet D: oxygenator water outlet; E: drip chamber of blood circuit; F: oxygenator blood inlet; G: post-oxygenator arterial sampling port; H: reservoir blood outlet.

For the blood phase, the single-circuit was constructed using tubing provided in the pack. For the venous section of the single-circuit, a 200 mm section of polyvinylchloride (PVC) tubing (5 mm internal

diameter; 0.625 mm wall thickness) was cut and then attached to the outlet of the reservoir (**Photo 1a H and Photo 1b H**) and blood inlet of the oxygenator (**Photo 1a G and Photo 1b F**). An atraumatic surgical clamp was positioned midway along this tube before priming the reservoir and oxygenator with blood. For the arterial section of the single-circuit, a 100 mm length of the same PVC tubing used for the venous section, was attached to the blood outlet of the oxygenator. The free-end of this 100 mm length of tubing was secured to the bag spike of the drip chamber (**Photo 1a F and Photo 1b E**) of a blood administration set (Pump Blood Transfusion Set, MedCaptain, Amsino International Inc, USA) by means of a stainless-steel wire tie (3 mm wire). The free-end of the blood administration set was then secured to the central inlet at the top of the reservoir (**Photo 1a A**) incorporating a screen filter and defoamer, thus completing the single-circuit of the blood phase.

For the water phase, a single-circuit was constructed also using various tubing that was provided in the pack. A length of silicone tubing (7 mm internal diameter; 0,625 mm wall thickness) connected to PVC tubing (25 mm internal diameter; 0.625 mm wall thickness) by a reducing connector, was cut such that 100 mm of silicone tubing remained on each side of the connector. To construct the inlet section of the single-circuit, the silicone tubing from the previously cut length of tubing was attached to the water inlet of the oxygenator (**Photo 1a C and Photo 1b C**). The PVC tubing on the on the other side of the connector was cut to 100 mm in length and connected to the intravenous catheter connector side (luer lock) of a blood administration set (Pump Blood Transfusion Set, MedCaptain, Amsino International Inc, USA) and secured with a stainless-steel wire tie. The bag spike of the drip chamber was attached to a 300 mm length of PVC tubing (5 mm internal diameter; 0.625 mm wall thickness), secured with a stainless-steel wire tie. The free-end of the PVC tubing was immersed in a water bath (Medi-Bath, Medi-Vet, Australia). To construct the outlet section of the single-circuit, a similar tubing configuration as connected to the water inlet (silicone tubing connected to PVC tubing by means of a reducing connector) was connected to the water outlet of the oxygenator (**Photo 1a D and Photo 1b D**). The PVC part of the tubing for this segment was cut to 300 mm in length. The free-end of the PVC tubing was then immersed in the water bath to complete the single-circuit.

For the gaseous phase, there were two configurations used, one for the room air replicates and another for the oxygen replicates. The gaseous phase configuration for the room air replicates was simply removing the safety protection caps from the gas inlet (**Photo 1b B**) and outlet ports of the oxygenator during assembly. The configuration of the gaseous phase for the oxygen replicates included a number of components to rig a high-pressure oxygen cylinder to deliver an ultra-low oxygen flow, in parallel. A bull-nose medical oxygen regulator (Afrox Saffire Oxygen Regulator, Afrox, South Africa) was attached to a size H oxygen cylinder (Air Products (Pty) Ltd, South Africa). The regulator outlet was attached to 1 m length of intermediate-pressure hosing, the other end of which was connected to low-flow oxygen flowmeter scavenged from a redundant anaesthetic delivery device (Humphrey Free-Ox, Anaequip, UK). The flowmeter outlet was connected to a 1 m length of PVC tubing (5 mm internal diameter; 0.625 mm wall thickness) terminating in a y-connector (supplied in the pack). The arms of the 'Y' were attached to 25 mm lengths of PVC tubing of the same internal diameter, respectively. The free-ends of the 25 mm lengths of PVC tubing were each connected to a 3-gang, 4-way manifold (supplied in the pack). The open ends of the manifolds (6 open ends in total) were each connected to low volume infusion line (Injectomat Line, Fresenius-Kabi, Germany). The open ends of the low-volume infusion lines were then attached to 50 mm lengths of PVC tubing (25 mm internal diameter; 0.625 mm wall thickness). The free-ends of the PVC tubing were connected to the gas inlet of the oxygenator, when required.

Heparin lock stoppers (Triple-D Heparin Cap, Shanghai International Holding Corp. GmbH, Germany) were placed on a peripheral inlet (**Photo 1a B**) at the top of the reservoir, the pre-oxygenator venous sampling port of the pre-membrane purge port line (**Photo 1b A**) and the post-oxygenator arterial sampling port (**Photo 1b G**).

2.1.4 Study procedure

Four litres of LRS (Lactated Ringer's Solution, Fresenius-Kabi, South Africa) were added to the water bath and the fluid was heated to the pre-set temperature of 44°C. The higher than conventional

temperature for the water bath was used to compensate for the assumed heat loss to the environment, in an ultra-low flow configuration. Thereafter, the blood administration set forming part of the single-circuit of the water phase was inserted into a linear, peristaltic infusion pump (HP-60, MedCaptain Medical Technology Co. Ltd, PRC) (**Photo 1a E**). The pump was switched on and set to the 'purge' function in order to prime the line after which it was run at 0.02 L minute^{-1} (1200 mL hour^{-1}).

Once the water phase of the oxygenator was completely primed and the water circuit completed, the blood administration set forming part of the blood phase single-circuit was inserted into an infusion pump (HP-60). The tubing between the reservoir and oxygenator was clamped after which canine stored whole blood was added to the reservoir via a gravity dependant, blood administration set (Adult Non-Vented Blood Set, Amsino International Inc, USA). The blood was infused via an inlet of the reservoir, continuous with the screen filter and defoamer. Once the entire unit of blood had been decanted into the reservoir, 500 IU of sodium heparin (0.5 mL; Heparin Sodium Fresenius 1000 IU mL^{-1}, Fresenius Kabi, South Africa) was added to the reservoir via the heparin lock stopper at the peripheral inlet at the top of the reservoir. The UFH dose was based on an assumption of the blood volume of a dog being 90 mL kg^{-1} (Hopper et al. 2012) and an expected volume of 450 mL per unit of blood. Thus, these values were extrapolated to assume a dog of 5 kg receiving 100 IU kg^{-1} of unfractionated heparin (Plumb 2018). This dose of UFH should also increase the activated clotting time to 160 – 220 seconds, which is what is targeted for ECMO (Green 1980; Allen et al. 2011; McRae & de Perrot 2018).

Once the stored whole blood was infused, the atraumatic surgical clamp on the tube between the reservoir and oxygenator was then released. Then the electronic infusion pump set to the 'purge' function to prime the line, after which it was run at 0.02 L minute^{-1} (1200 mL hour^{-1}).

For the oxygen replicates, before starting the infusion pumps, the handwheel of the oxygen cylinder was opened half a turn and the regulator set to a flow of 5 L minute^{-1}. The oxygen flowmeter was set to flow of 0.2 L minute^{-1} resulting in an estimated flow of 0.03 L minute^{-1} at the distal end of the oxygen

supply rig. Oxygen flow out of the distal end of the oxygen rig was confirmed by simultaneously submerging all the outlets in 50 mm of water and observing for equal sized bubbles out of each outlet. When oxygen flow was required, then the gas inlet and outlet safety protection caps were removed and the distal end of the oxygen tree was connected to the gas inlet of the oxygenator.

2.1.5 Sampling procedure

Syringes used for blood sampling were prepared by first attaching a needle (21 G x 1''; Surgi Plus, China) to a syringe (3 mL; Sterile 3-part syringe, Surgi Plus, China). The syringes were then heparinised by inserting the needle into a heparin bottle (Heparin Sodium Fresenius 1000 IU mL^{-1}, Fresenius Kabi, South Africa), drawing heparin through the length of the syringe barrel and injecting it back into the bottle three times. This method of heparinising syringes introduces no error in blood gas measurement secondary to dilutional effects of heparin (Hopper et al. 2005).

Blood was anaerobically aspirated from the venous and arterial sampling ports for blood gas analysis. Venous blood samples were obtained by first removing a 10 mL of blood from the pre-membrane purge port line using a 20 mL syringe (Sterile 3-part syringe, Surgi Plus, China) and then aspirating a 2 mL blood sample for analysis into a pre-heparinised syringe over 30 seconds. The pre-sampling blood was returned to the reservoir via the peripheral heparin lock stopper at the top of the reservoir. Arterial blood samples were obtained by aspirating 2 mL of blood over 30 seconds from the low-volume arterial sampling port of the oxygenator. Any air bubbles entrained into the samples were expelled before capping the syringes with needle that was folded over.

Arterial and venous blood samples were obtained from the room air replicates at 2 minutes of blood flow within the circuit (T0) and hourly for 8 hours (T1, T2, T3, T4, T5, T6, T7 and T8, respectively) (Graulich et al. 2000) and thereafter at 24, 32, 48 and 56 hours (T24, T32, T48 and T56, respectively).

Arterial and venous blood samples were obtained from the oxygen replicates at T0, immediately after which the oxygen flow used for the gaseous phase, was supplied to the oxygenator. Then, arterial and

venous blood samples were obtained at 15-minute intervals (T0.25, T0.50, T0.75) for the first hour (T1), then hourly for 8 hours (T2 to T8) and lastly at 24 hours (T24).

2.1.6 Sample analysis and processing

Once the blood samples were collected, they were stored at room temperature and analysed within 10 minutes using a self-calibrating, bench-top analyser (RAPIDPoint 500, Siemens Healthineers, Germany) at 37°C. Before the syringe was inserted into the analyser the sample was gently agitated to ensure complete mixing of the sample; a few drops of blood was also injected onto a cotton swab, from each syringe, to ensure that the sample had not clotted. When blood samples were collected from more than 1 replicate at a time then all the arterial blood samples were analysed first, followed by all the venous blood samples. Analytes of interest were the PO_2, PCO_2 and total haemoglobin (tHb).

Once the blood gas analysis had taken place, the sample was transferred to a 1.5 mL Eppendorf tube (Sample Transfer Tube, IDEXX BioAnalytics, USA). The Eppendorf tube was then placed into a centrifuge (PrO-Vet, Centurion Scientific Ltd, UK) and spun down to separate the cellular from fluid components using the 'Serum 1' setting (6000 revolutions per minute for 10 minutes). The resulting supernatant (plasma) was siphoned using a pipette and transferred into a second Eppendorf tube. The pellet was discarded according to the facility's biohazard waste protocol. The plasma was then refrigerated (< 8°C) for 1 week. Then, fHb concentration was measured, within a day, using a manufacturer calibrated analyser (HemoCue Plasma Low/Hb Photometer, HemoCue, Sweden). Prior to analysis, the plasma samples were removed from the refrigerator and allowed to stand for 2 hours in order to come to room temperature. For each sample, a plasma volume of 10 µL was drawn into a cuvette (HemoCue Plasma Low/Hb Microcuvette, HemoCue Sweden) by capillary action after which the cuvette was inserted into the photospectrometric analyser for measurement. The fHb concentration from the replicate in which the 41 day old blood was used was omitted from analysis as the results exceeded analyser detection limit at all timepoints.

2.1.7 Decommissioning procedure

The endpoint of the room air replicates was 56 hours or until failure due to clotting or air entrainment or any other reason for failure. The endpoint of the oxygen replicates was 24 hours or until failure. Once the endpoint for the replicate was reached it was decommissioned by removing the oxygen connection and then clamping the water single-circuit at the oxygenator inlet and outlet. The water tubing was then removed from the oxygenator. The oxygenator and reservoir were then removed from the packaging tray and the blood administration set part of the blood single-circuit removed from the infusion pump. The entire blood single-circuit was then discarded of, according to the biohazard disposal protocol of the facility, as a single unit, to limit spillage. The blood administration set part of the water single-circuit was removed from the infusion pump after which both the clamps from the water single-circuit were removed and the fluid was allowed to drain back into the water bath. The remainder of the plastic tubing was disposed of according to the non-hazardous material protocol of the facility. The fluid from the water bath was disposed of into a hand washing sink which drained into the municipal sewer system.

2.1.8 Data analysis

The data was assessed for normality by inspection of histograms, quantile-quantile (Q-Q) plots, descriptive statistics and the Anderson-Darling test for normality. The distribution of the data was non-parametric; therefore, all variables were reported as median (1^{st} quartile; 3^{rd} quartile). Variables of interest were the PaO_2 (oxygen tension in blood post-oxygenator), PvO_2 (oxygen tension in blood pre-oxygenator), $PaCO_2$ (carbon dioxide tension in blood post-oxygenator), $PvCO_2$ (carbon dioxide tension in blood pre-oxygenator), tHb and fHb concentrations.

The magnitude in change for the gas variables was determined by subtracting the arterial values from the venous values for each time point for all gases ($PaO_2 – PvO_2$ and $PaCO_2 – PvCO_2$, respectively). Then, the percentage change was calculated by dividing the magnitude in change by the arterial values and converting the solution to a percentage for each time point for all gases. Additionally, the rate of

change of fHb concentration was calculated by subtracting the fHb concentration at that time point from the fHb concentration at the preceding time point and then dividing the solution by the time interval (hours).

The variables, measured or calculated, within the room air and oxygen replicates were analysed separately by comparing the values over time using a general linear mixed model (fixed effect: time; random effect: replicates). Model fits were assessed by inspecting residual plots to assess linearity, homogeneity of variances, normality, and outliers. Then, *post-hoc* analysis was applied using the Dunnett method where T0 within each replicate type (room air or oxygen) was used as the control variable. The data were analysed using commercially available software (MiniTab 18; Minitab; USA) and statistical significance interpreted at $p < 0.05$.

The expected PaO_2 value for the room air replicates was calculated by multiplying FiO_2 of room air (0.21) by barometric pressure. Whereas the expected PaO_2 value for the oxygen replicates was equivalent to the median barometric pressure where the FiO_2 was assumed to be 1.0. The arterial and venous oxygen content of the blood (CaO_2 and CvO_2) at each time point was calculated by as follows:

$$CO_2 \text{ (mL dL}^{-1}) = (1.34 \text{ x tHb x } SaO_2/100) + (0.003 \text{ x } PO_2)$$

where 1.34 is the carrying capacity of haemoglobin for oxygen (mL g^{-1}); SaO_2 is the percentage saturation of haemoglobin with oxygen (obtained from blood gas analysis).

A theoretical model for determining the DO_2 in dogs was constructed over a range of weights ([1 – 50 kg]; interval between classes: 5 kg) where DO_2 = blood oxygen content x CO where CO in a dog was taken as 0.868 L kg^{-1} $minute^{-1}$ (Adachi et al. 1976). Thus, CO for each weight class was calculated as body weight (kg) x 0.868 (L kg^{-1} $minute^{-1}$). The following was assumed, cardiac output is equal to venous return at steady state (Magder 2016) and that the lungs did not participate at all in the oxygenation of blood.

The mean CvO_2 at T0 for the oxygen replicates was taken as a theoretical mixed venous oxygen content and thus an unaugmented DO_2, calculated as follows:

Unaugmented DO_2 = cardiac output calculated for weight class x mean CvO_2 at T0

An augmented DO_2 was calculated when 20 mL of blood passing through an oxygenator was substituted into the venous return for each weight class. The mean CaO_2 over all time points in the oxygen replicates was used to calculate the augmented DO_2, when factoring in the venous return and cardiac output for each weight class as follows:

Augmented DO_2 = [mean CvO_2 at T0 x (cardiac output calculated for weight class – 20 mL)] + [mean CaO_2 over all time points x 20 mL]

The impact on DO_2 was also calculated by manipulating the variables contributing to CaO_2 to simulate two theoretical hypoxaemia scenarios, a low PaO_2 and anaemia. For the low PO_2 scenario, the variables used in the augmented DO_2 equation to calculate the mean CvO_2 at T0 were changed to a PvO_2 of 40 mmHg and a resultant SaO_2 of 75% (Hafen & Sharma 2021). For the anaemic scenario, the tHb of the CvO2 calculation at T0 was changed to 6.7 g dL^{-1} (packed cell volume 0.2 L L^{-1}). A critical DO_2 value of 9.8 mL kg^{-1} $minute^{-1}$ (Cain 1977) was calculated for each weight class and used as a comparison point. Lastly, the percentage change in DO_2 for each weight class and scenarios was calculated by first obtaining the difference in DO_2 (augmented DO_2 – unaugmented DO_2), which was then divided by the unaugmented DO_2 and converting to a percentage.

Chapter three

3.1 Results

The assembly of the oxygenator to provide flow through the blood, water and gaseous phases operated efficiently for the duration of the study period in all replicates with the exception of 2 room air replicates which failed. The failure occurred between T32 and T48 time periods and was related to blood clotting in the reservoir and drip chamber in both, but one also had air entrainment. It is uncertain, but we speculate that the electronic linear peristaltic pumps stopped operating during an unobserved period overnight and this led to blood stasis, gravitation of cellular components to the bottom of the reservoir which subsequently developed a large, diffusely spread, loose clot. The study objectives were achieved, and the *null* hypothesis was rejected because there were significant differences in gas tensions and fHb concentrations over time. Furthermore, the secondary objective of describing a theoretical model to predict the effects of DO_2 in dogs weighing from 1 kg to 50 kg was also met.

The PaO_2 and $PaCO_2$ over time for both the room air and oxygen replicates are presented in **Table 1**. In the room air replicates, the PaO_2 significantly increased from T0 and this increase plateaued and was sustained from T1 to T8 (**Figure 1a**). However, there was no difference in PaO_2 from T24 to T56 compared to T0. The PaO_2 – PvO_2 (**Figure 1b**) and percent change in PO_2 (**Figure 1c**) in the room air replicates were no different over time from T0. The $PaCO_2$ was not different from T0 at T1. However, the $PaCO_2$ subsequently significantly decreased from T0 over time, from T2 to T56 (**Figure 1d**). The $PaCO_2$ – $PvCO_2$ (**Figure 1e**) and percent change in PCO_2 (**Figure 1f**) at all time points, were no different from T0.

Table 1 *Oxygen (PaO_2) and carbon dioxide ($PaCO_2$) tensions over time (in mmHg) in an in vitro extracorporeal membrane oxygenation model using canine stored blood at an ultra-low flow (0.02 L minute^{-1}) from the first sample taken soon after inducing blood, water and gaseous phase flow (T0) to the study endpoint (T56 for room air replicates; T24 for oxygen replicates). The gaseous phase of the oxygenators was either room air (n = 6; fraction inspired oxygen 0.21) or ultra-low flow oxygen (n = 6; fraction inspired oxygen 1.0), respectively.*

Variable	Room air replicates			Oxygen replicates		
	Time point (number of replicates)	Median	(95% CI; *p*-value)	Time point (number of replicates)	Median	(95% CI; *p*-value)
PaO_2 (arterial/post oxygenator)	T0 (*n* = 6)	154	-	T0 (*n* = 6)	114	-
				T0.25 (*n* = 6)	650*	(442; 585; < 0.001)
				T0.5 (*n* = 6)	628*	(439; 582; < 0.001)
				T0.75 (*n* = 6)	645*	(455; 599; < 0.001)
	T1 (*n* = 6)	192*	(8; 84; 0.01)	T1 (*n* = 6)	599*	(427; 570; < 0.001)
	T2 (*n* = 6)	202*	(13; 89; 0.003)	T2 (*n* = 6)	595*	(392; 548; < 0.001)
	T3 (*n* = 6)	205*	(18; 94; 0.001)	T3 (*n* = 6)	639*	(443; 599; < 0.001)
	T4 (*n* = 6)	211*	(20; 95,9; 0.001)	T4 (*n* = 6)	624*	(423; 579; < 0.001)
	T5 (*n* = 6)	214*	(20; 96,5; < 0.001)	T5 (*n* = 6)	624*	(434; 590; < 0.001)
	T 6 (*n* = 6)	218*	(22; 98,1; < 0.001)	T6 (*n* = 6)	640*	(449; 605; < 0.001)
	T7 (*n* = 6)	215*	(19; 94,7; 0.001)	T7 (*n* = 6)	694*	(482; 638; < 0.001)
	T8 (*n* = 6)	212*	(16; 92; 0.002)	T8 (*n* = 6)	650*	(445; 601; < 0.001)
	T24 (*n* = 6)	196	(-9; 67; 0.2)	T24 (*n* = 6)	590*	(398; 553; < 0.001)
	T32 (*n* = 4)	188	(1; 86; 0.05)	-	-	-
	T48 (*n* = 4)	191	(-3; 82; 0.09)			
	T56 (*n* = 4)	179	(-11; 74; 0.3)			
$PaCO_2$ (arterial/post oxygenator)	T0 (*n* = 6)	74	-	T0 (*n* = 6)	78	-
				T0.25 (*n* = 6)	20*	(-83; -51; < 0.001)
				T0.5 (*n* = 6)	11*	(-92; -60; < 0.001)
				T0.75 (*n* = 6)	8*	(-95; -62; < 0.001)
	T1 (*n* = 6)	62	(-32; 7; 0.4)	T1 (*n* = 6)	7*	(-96; -63; < 0.001)
	T2 (*n* = 6)	52*	(-3; 8; 0.02)	T2 (*n* = 6)	6*	(-93; -69; < 0.001)
	T3 (*n* = 6)	46*	(-3; 9; 0.003)	T3 (*n* = 6)	6*	(-93; -69; < 0.001)
	T4 (*n* = 6)	41*	(-4; 7; < 0.001)	T4 (*n* = 6)	5*	(-93; -70; < 0.001)

T5 (*n* = 6)	40*	(-1; 10; < 0.001)	T5 (*n* = 6)	5*	(-93; -70; < 0.001)
T6 (*n* = 6)	37*	(-1; 10; < 0.001)	T6 (*n* = 6)	5*	(-93; 70; < 0.001)
T7 (*n* = 6)	34*	(-61; -22; < 0.001)	T7 (*n* = 6)	5*	(-93; -69; < 0.001)
T8 (*n* = 6)	30*	(-64; -25; < 0.001)	T8 (*n* = 6)	5*	(-93; -70; < 0.001)
T24 (*n* = 6)	27*	(-66; -28; < 0.001)	T24 (*n* = 6)	7*	(-92; -69; < 0.001)
T32 (*n* = 4)	15*	(-84; -36; < 0.001)	-	-	-
T48 (*n* = 4)	14*	(-92; -31; < 0.001)			
T56 (*n* = 4)	15*	(-91; -30; < 0.001)			

PaO_2: Oxygen tension in blood post-oxygenator/arterial; $PaCO_2$: Carbon dioxide tension in blood post-oxygenator/arterial; 95% CI: 95% confidence interval of the difference of means between the time point and the control (T0); *Denotes statistical significance according to Dunnett's Test ($p < 0,05$)

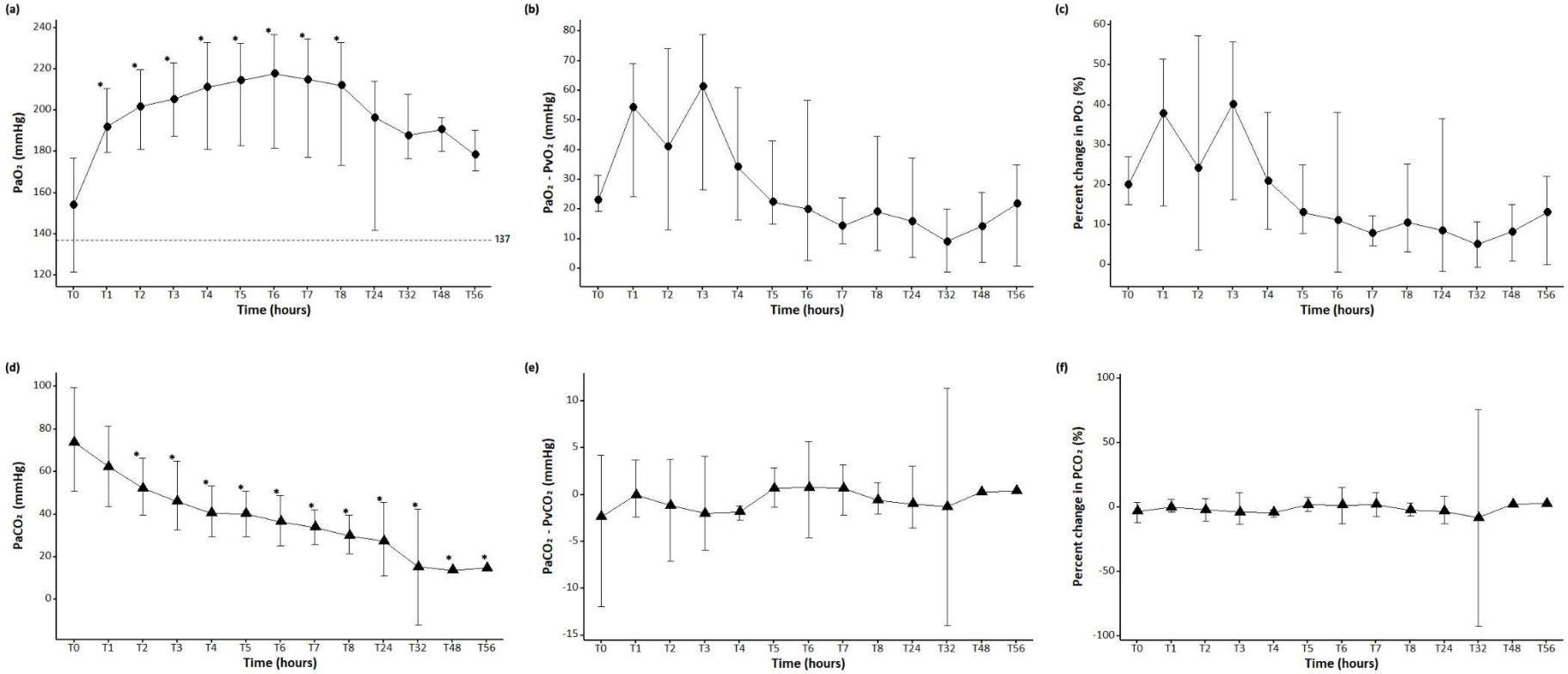

Figure 1 *Confidence interval plots (median ± 95% confidence interval) over 56 hours, indicating the (a) arterial/post-oxygenator blood oxygen tension (PaO_2), (b) change in PO_2 across the oxygenator membrane, (c) percentage change in PO_2 across the oxygenator membrane, (d) carbon dioxide tension $PaCO_2$ (arterial/post-oxygenator), (e) change in PCO_2 across the oxygenator membrane and (f) percentage change in PCO_2 across the oxygenator membrane, in a group of 6 oxygenators open to room air (fraction inspired oxygen 0.21) and during ultra-low flow of stored canine whole blood at 0.02 L minute^{-1}. *Denotes statistical significance according to Dunnett's Test ($p < 0,05$).*

In the oxygen replicates, the actual values for the PaO_2 significantly increased from T0 and this increase was sustained for the entire study duration (**Figure 2a** and **Figure 3a**). The PaO_2 – PvO_2 were significantly different from T0 at T0.25, T0.5, T0.75 and T4 (**Figure 2b** and **Figure 3b**); however, the PaO_2 – PvO_2 was not different from T0 at the remainder of the time points. The percent change in PO_2 was significantly different from T0 at T0.25, T2, T3 and then from T5 to T24 (**Figure 2c** and **Figure 3c**). The $PaCO_2$ significantly decreased from T0 at all time points (**Figure 2d** and **Figure 3d**). The $PaCO_2$ – $PvCO_2$ was significantly different from T0 at T0.25 and then from T2 to T24 (**Figure 2e** and **Figure 3e**). The percentage change in PCO_2 was significantly different from T0 only at T0.25, T0.5 and T0.75 (**Figure 2f** and **Figure 3f**).

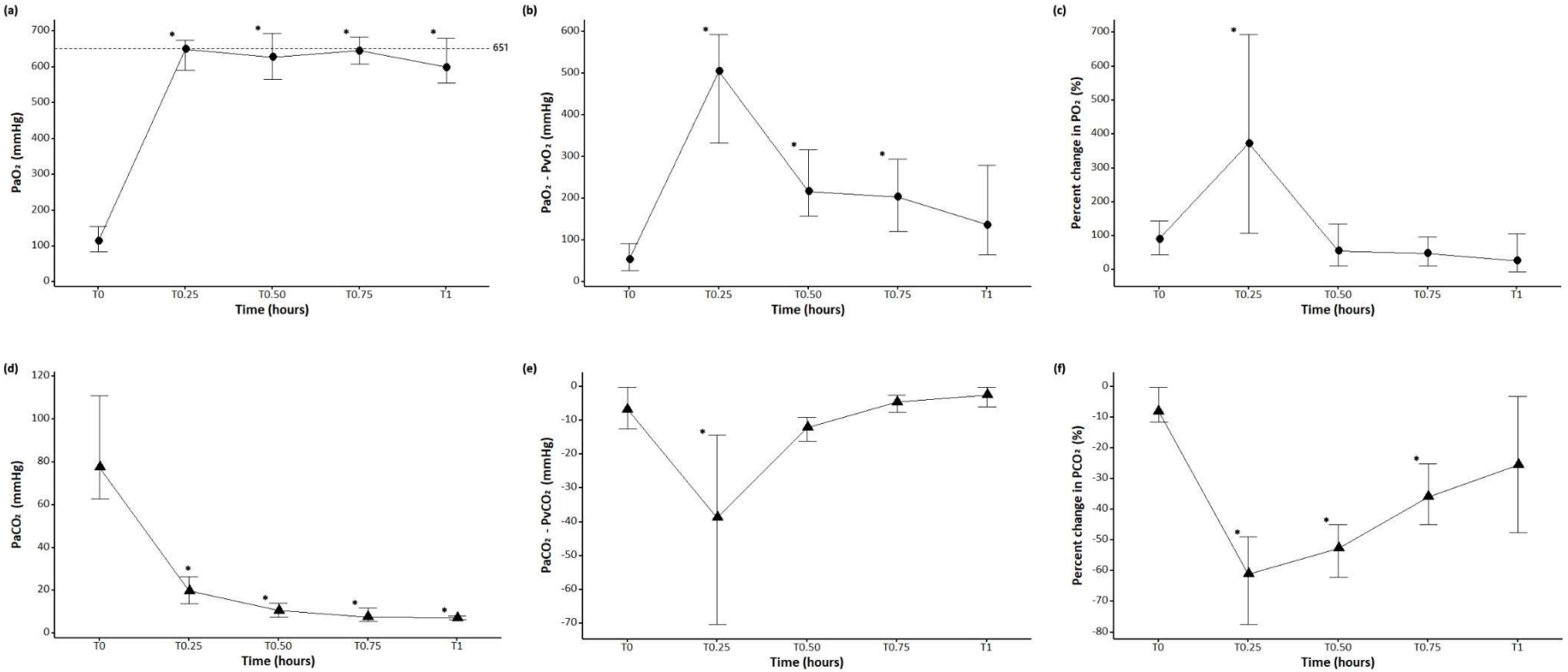

Figure 2 *Confidence interval plots (median ± 95% confidence interval) over 1 hour, indicating the (a) arterial/post-oxygenator blood oxygen tension (PaO_2), (b) change in PO_2 across the oxygenator membrane, (c) percentage change in PO_2 across the oxygenator membrane, (d) carbon dioxide tension $PaCO_2$ (arterial/post-oxygenator), (e) change in PCO_2 across the oxygenator membrane and (f) percentage change in PCO_2 across the oxygenator membrane, in a group of 6 extracorporeal oxygenators during ultra-low flows of oxygen (fraction inspired oxygen 1.0) at 0.03 L minute^{-1} and stored canine whole blood flowing at 0.02 L minute^{-1}. *Denotes statistical significance according to Dunnett's Test ($p < 0,05$).*

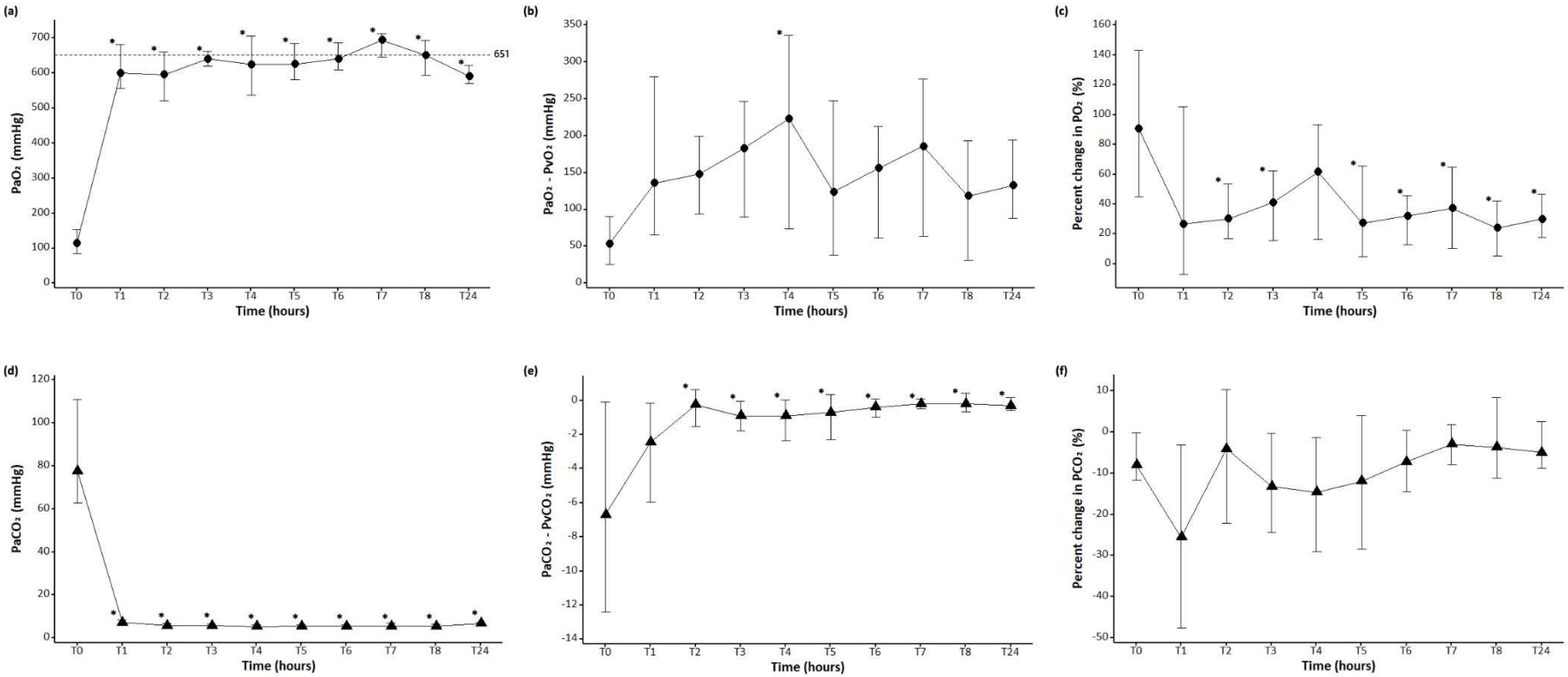

Figure 3 *Confidence interval plots (median ± 95% confidence interval) over 24 hours, indicating the (a) arterial/post-oxygenator blood oxygen tension (PaO_2), (b) change in PO_2 across the oxygenator membrane, (c) percentage change in PO_2 across the oxygenator membrane, (d) carbon dioxide tension $PaCO_2$ (arterial/post-oxygenator), (e) change in PCO_2 across the oxygenator membrane and (f) percentage change in PCO_2 across the oxygenator membrane, in a group of 6 extracorporeal oxygenators during ultra-low flows of oxygen (fraction inspired oxygen 1.0) at 0.03 L minute^{-1} and stored canine whole blood flowing at 0.02 L minute^{-1}. *Denotes statistical significance according to Dunnett's Test ($p < 0,05$).*

The tHb concentration in both the room air and oxygen replicates did not change throughout the study duration, when compared to T0 (**Figure 4a** and **Figure 4d**). The fHb concentration in the room air replicates increased significantly from T0 at T8 to T56 (**Figure 4b**). However, in the oxygen replicates, the fHb concentration significantly increased from T0 earlier than the room air replicates, from T5 to T24 (**Figure 4e**). In the room air replicates, the rate of change of fHb concentration did not change from T0 for the study duration (**Figure 4c**). However, in the oxygen replicates, the rate of change of fHb concentration was significantly increased from T0 at T3, T4 and T6 (**Figure 4f**).

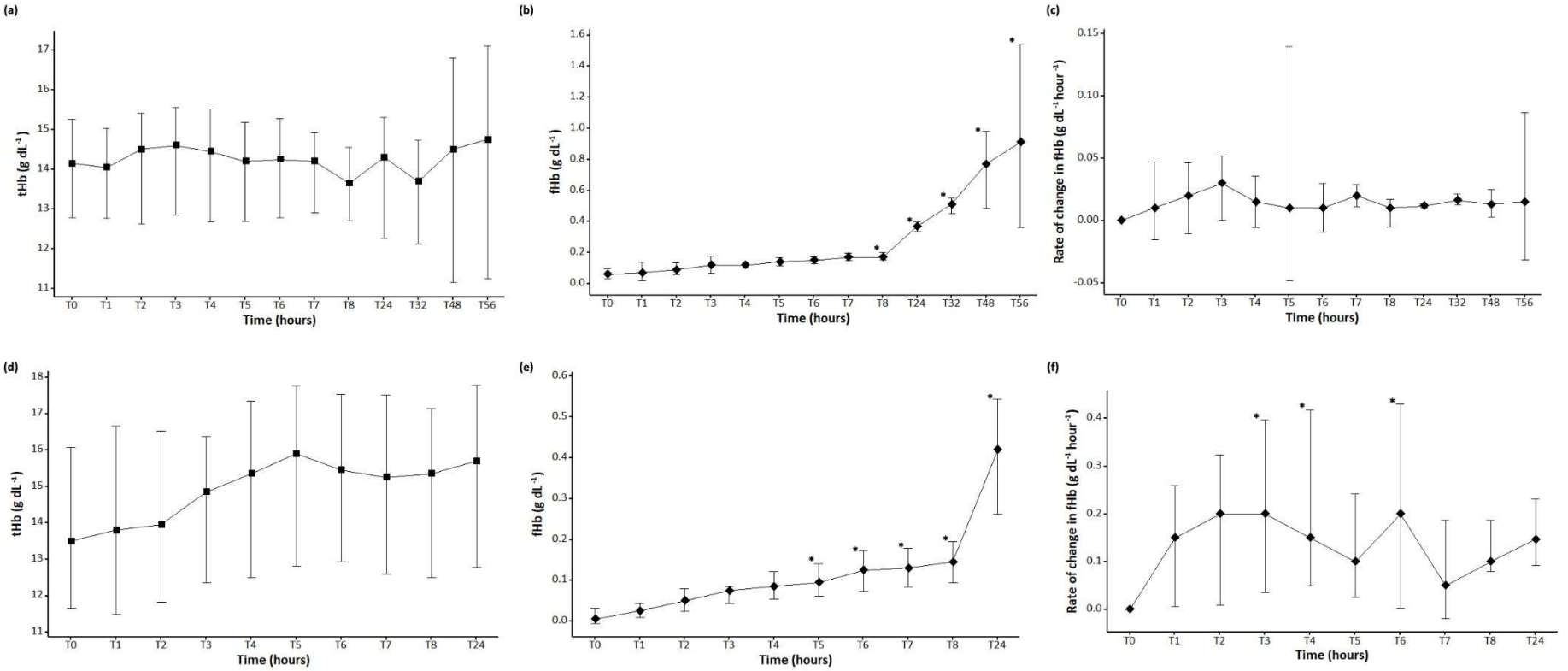

Figure 4 *Confidence interval plots (median ± 95% confidence interval) indicating the (a) total haemoglobin (tHb) (b) free plasma haemoglobin (fHb) concentration, (c) rate of change in free plasma haemoglobin, in the room air replicates; (d) total haemoglobin (e) free plasma haemoglobin, (f) rate of change in free plasma haemoglobin, in the oxygen replicates. For the room air replicates, 6 oxygenators were open to room air (fraction inspired oxygen 0.21) and given an ultra-low flow of stored canine whole blood at 0.02 L minute^{-1}. For the oxygen replicates, 6 extracorporeal oxygenators were given an ultra-low flow of oxygen (fraction inspired oxygen 1.0) at 0.03 L minute^{-1} and stored canine whole blood flowing at 0.02 L minute^{-1}. *Denotes statistical significance according to Dunnett's Test (p < 0,05).*

In the theoretical model describing the effects of an augmented DO_2 by oxygenators receiving ultra-low oxygen flow, contributing to the mixed venous oxygen content in dogs weighing 1 to 50 kg (**Figure 5a**), the highest DO_2 was effected in the low PaO_2 scenario, followed by the augmented DO_2. The unaugmented DO_2 virtually mirrored the critical DO_2 for every weight class. The lowest DO_2 was effected in the anaemic scenario where the DO_2 fell below the critical DO_2 in every weight class. However, the percentage change in DO_2 (**Figure 5b**), was the greatest in the anaemic scenario followed by the augmented DO_2 and lastly, the low PO_2 scenario. The percentage change in DO_2 in the anaemic scenario was the greatest between 1 – 20 kg, however, still appreciable up to 50 kg. The percentage change in DO_2 remained higher in the augmented DO_2 than the low PO_2 scenario up to approximately 10 kg, after which the values were similar to each other.

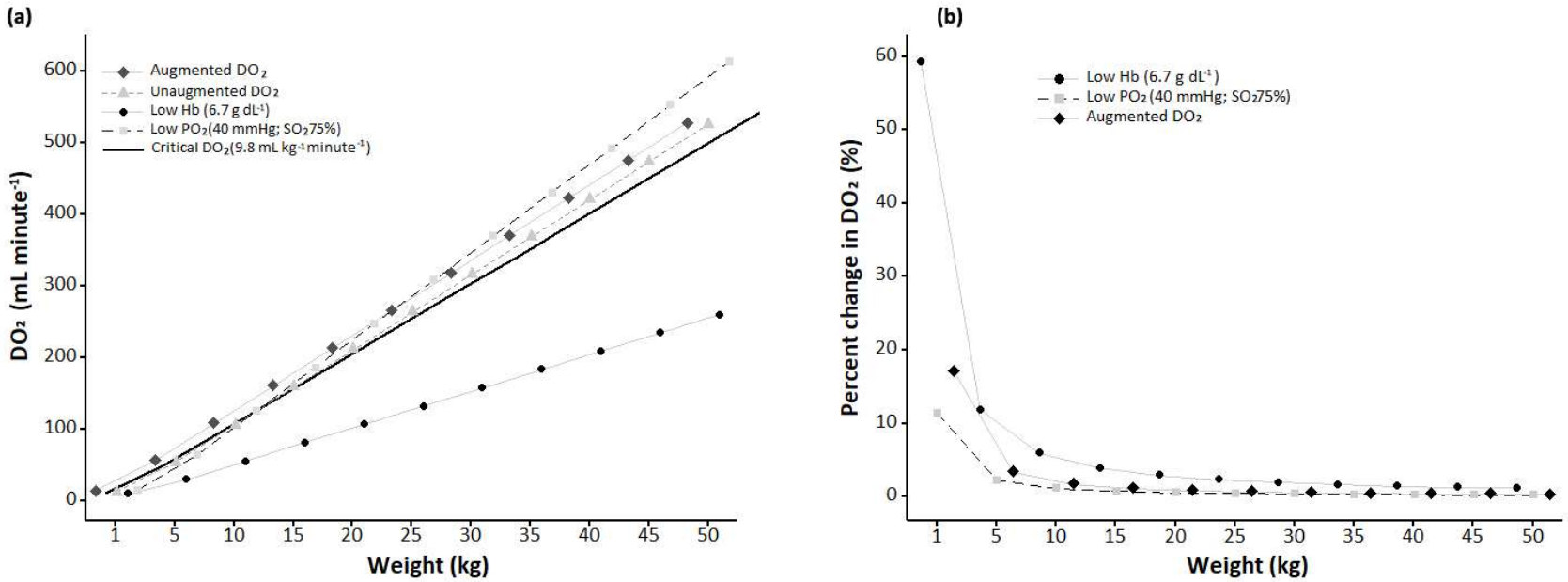

Figure 5 *(a) Theoretical model displaying the oxygen delivery (DO_2) during an unaugmented (no oxygen flow) and augmented (ultra-low flow of oxygen at 0.03 L minute^{-1}) state during an ultra-low flow of stored canine whole blood (0.02 L minute^{-1}) through an extracorporeal oxygenator. The augmented state was recalculated for two different scenarios of hypoxaemia, a low oxygen tension (Low PO_2) scenario (40 mmHg; oxygen haemoglobin saturation 75%) and an anaemic scenario (low Hb; haemoglobin concentration of 6.7 g dL^{-1}). The critical DO_2 for dogs was also plotted by weight for reference. (b) Percentage change in DO_2 of the augmented DO_2, the low PO_2 and the low Hb scenarios, from the unaugmented DO_2.*

Chapter four

4.1 Discussion

An *in vitro* ECMO circuit using ultra-low flow through the blood phase of the oxygenators with the gaseous phase either exposed to room air or an ultra-low oxygen flow, firstly, resulted in significant oxygenation of the blood, as reflected by the large increase in PaO_2, within an hour of commencing the blood and gas flow. Secondly, the blood carbon dioxide level, as reflected by the $PaCO_2$, consistently decreased over time in both the room air and oxygen groups. Thirdly, the degree of haemolysis within the room air and oxygen replicates was acceptable as the rate of change of fHb concentration never exceeded 0.05 g dL^{-1}. Lastly, an ultra-low flow ECMO model theoretically achieves a sufficient DO_2 under normal CaO_2 circumstances and in a hypoxic hypoxaemia scenario, but not in an anaemic hypoxaemia scenario. However, the ultra-low flow ECMO model makes the greatest impact on DO_2 in an anaemic hypoxaemia scenario. Therefore, the study objectives (apart from adequately demonstrating the longevity of the ultra-low flow ECMO configuration over 56 hours) were achieved, and the *null* hypothesis was rejected because there were significant differences in gas tensions and fHb concentration over time. Furthermore, the secondary objective of describing a theoretical model to predict the effects of DO_2 in dogs weighing from 1 kg to 50 kg was also met.

The oxygenation membrane within an oxygenator functions as a gaseous exchange interface between the blood and gaseous phases of the oxygenator (Allan et al. 2011). Gaseous exchange between the blood and gaseous phases is governed by Fick's law of diffusion and the oxygen carrying capacity of the blood (Allan 2011; Finney 2014). In terms of the oxygenation membrane, Fick's law of diffusion relates to the thickness of the oxygenation membrane, the thickness of the blood film passing through the blood phase of the oxygenator and the difference in gas tensions on either side of the membrane (Allan et al. 2011). The gas tension gradient generated across the membrane is related to the oxygen and carbon dioxide content of blood entering the oxygenator and the FiO_2 in the gas mixture supplied to the gaseous phase of the oxygenator (Finney 2014). Thus, constant blood flow through the

oxygenator is required to maintain the oxygen pressure gradient across the oxygenation membrane. Notwithstanding the PaO_2, it is the haemoglobin concentration of the blood flowing through the oxygenator is the major determinant of its oxygen carrying capability, as each 1 g dL^{-1} of haemoglobin contributes up to 1.34 mL of oxygen per dL of blood. The role of haemoglobin is juxtaposed against the oxygen tension within plasma, where every 1 mmHg contributes just 0.03 mL of oxygen per dL of blood (Dunn et al. 2016). Excessive blood volume and non-laminar flow within an oxygenator can impede gaseous exchange across a membrane by means of a ventilation-perfusion mismatch within the oxygenator similar to certain pulmonary disease processes such as ARDS, pulmonary contusions secondary to trauma, aspiration pneumonia, cardiac failure (Hopper & Powell 2003; Allen et al. 2011). Laminar flow in a tubular structure is characterised by movement fluid in a longitudinal, parabolic manner through the tube. Laminar flow is promoted by lower fluid densities, narrow tube diameters low fluid velocities and high fluid viscosity (Muir 2015). In the present study, it is speculated that the ultra-low blood flow, narrow intravenous tubing and viscosity of the blood due the physiological packed cell volume thereof promoted laminar flow.

Considering that the concentration of oxygen in room air is 21% (volume $volume^{-1}$) (Fuentes & Chowdhury 2021), therefore, at a barometric pressure of 651 mmHg the oxygen tension in room air is 137 mmHg. Considering Fick's law of diffusion, as applied to a membrane oxygenator, one would assume that an oxygenator exposed to room air would thus allow the blood flowing through to achieve a maximum PaO_2 of 137 mmHg. However, in the present study, the highest median PaO_2 achieved in the room air replicates was 218 mmHg, which should only be achievable with a barometric pressure of 1038 mmHg. The reason for this higher than expected PaO_2 is unknown, but a few speculations could be made. In the traditional ECMO circuit setup, the pump is positioned upstream of the oxygenator in the limb of the circuit draining the patient's blood, allowing it to drain the patient and simultaneously pump blood into the oxygenator (Ali & Vuylsteke 2019). In the present setup of the *in vitro* ECMO circuit, the peristaltic infusion pump was positioned downstream of the oxygenator. This setup was chosen as it was the most convenient method of interfacing pump compatible tubing with

oxygenator compatible tubing. It is theorised that this setup, by siphoning blood out of the oxygenator somehow created a greater than expected pressure gradient between the gas and blood phases of the oxygenator. This pressure gradient was possibly produced by reducing the pressure within the blood phase as a result of siphoning blood across the oxygenator. The Affinity Pixie oxygenators used in this study have an oxygenation membrane made from tightly packed propropylene in a small plastic housing (Medtronic Affinity Pixie Oxygenation System 2012). Furthermore, the oxygen inlet and outlets are 10 mm in diameter. The application of a pump across this small, mostly closed unit thus possibly created a microenvironment in which a higher than expected PaO_2 was achieved. A disadvantage of this setup is that it may cause cavitation in both the oxygenator and drainage limb of the circuit as compared to only the drainage limb in the traditional setup. Cavitation is a phenomenon where excessive negative pressure applied to blood may cause the dissolved gasses to be sucked out of solution, creating air emboli and shear strain on erythrocytes (Allen et al. 2011).

In the oxygen replicates, the highest expected PaO_2 with consideration of the barometric pressure was 651 mmHg. This median PaO_2 achieved in this group was more reflective of the barometric pressure, albeit still higher than expected (median PaO_2 of 694 mmHg). In both groups, the ultra-low blood flow through the oxygenator likely maximised contact time of the blood with the oxygenation membrane, thus creating optimum conditions for maximal oxygen transfer.

The effects of temperature on the solubility of gases within blood must also be considered. Although a water bath heated to 44°C was supplying warmed LRS to the heat exchangers within the oxygenators, the ambient temperature within the room was not measured but the central thermal control unit of the hospital was set to 22°C. It is possible that the temperature of the LRS decreased by the time it reached the oxygenator by transferring heat to the environment during the ultra-low flow rate. Therefore, it is likely that the blood within the oxygenator, which was refrigerated up to the point of use, did not reach a physiological temperature. Oxygen solubility in blood increases with a decrease in temperature , thus it is also possible that a large amount of oxygen was liberated when

the samples were analysed for blood gases at 37°C. This phenomenon may have given rise to the high PaO_2 values observed (Hedley-Whyte & Laver 1964). The effects of temperature are the most like explanation for the higher than expected PaO_2 values. When temperature correction formulae for PO_2 (Hansen et al. 1999; Merino 1999) were applied to the highest achieved PaO_2 value (218 mmHg at T8), the corrected value at 22°C (likely temperature of the blood due to set ambient temperature of the hospital and the assumption that the water phase was ineffective in heating the blood) was calculated to 135 mmHg. This value is extremely close to the predicted value of 137 mmHg, extrapolated from the effects of the ambient barometric pressure. Lastly, errors in blood gas analysis were also considered. However, the analyser regularly self-calibrated, was maintained according to manufacturer recommendations and the quality control was judiciously carried out. Furthermore, the analyser did not display any error messages during the course of the study. Moreover, the consistency in results reduced the suspicion of analyser malfunction.

Similar to oxygen exchange, the exchange of carbon dioxide across the oxygenation membrane is governed by Fick's law of diffusion and blood flow (Allan et al. 2011; Finney et al. 2014; Ficial et al. 2021). Carbon dioxide diffuses a lot more efficiently across the membrane than oxygen and thus builds up very quickly in the gaseous phase of the oxygenator where its removal becomes dependant on gas flow. Thus, a decrease in $PaCO_2$ is less dependent on blood flow as compared to oxygen exchange (Allan 2011 et al.; Finney 2014 et al.; Ficial et al. 2021). Therefore, it is not surprising that the rate of $PaCO_2$ decline was a lot quicker in the oxygen replicates as compared to the room air replicates. It is likely that a microenvironment of increased CO_2 tension built up within the oxygenator housing of the room air replicates, which slowly exited over time. Conversely, the oxygen flow within the oxygen replicates was sufficient to both create a gas tension gradient across the membrane for the exchange of CO_2 and to sweep it out of the oxygenator housing.

The most common cause of mechanical complication within an ECMO circuit is a thrombus formation within the circuit (Allen et al. 2011). The ECMO circuit exposes blood to an aberrant environment of

plastic tubing instead of the endothelium. Various blood proteins adhere to this foreign surface and act as an anchor for platelets which could cause their activation, initiating coagulation and thus the production of thrombi (Mulder et al 2017). These thrombi commonly occur in areas of turbulent flow within the circuit such as connection points, abrupt turns and changes in tubing diameter (Allen et al. 2011; Allan et al 2011). Microthrombi within the circuit are a relatively common occurrence and are usually of minor consequence (Allen et al. 2011). The consequences of thrombosis depend on the segment of the circuit affected and include cavitation, circuit rupture, and oxygenation membrane failure (Allen et al. 2011). Efficiency of gaseous exchange across the membrane function over time slowly declines due a process called ageing, which is the accumulation of microthrombi, proteins and cellular debris within the fibres of the membrane (Ficial et al. 2021). Oxygenator failure is the second commonest mechanical complication within an ECMO circuit, which occurs as a result of catastrophic thrombosis within the oxygenation membrane (Allen et al. 2011). In the present study, two cases of catastrophic circuit failure occurred as a result of thrombosis. However, this failure occurred unobserved overnight and thus, it is unknown whether it was caused by infusion pump suddenly stopping or high circuit pressures secondary to thrombosis stopping the pumps as a result of a decline in the activity of the UFH. In all replicates, the oxygenators were inspected at every sampling point and after decommissioning, for areas of thrombosis, none were ever detected, even in the 2 replicates that completely failed. Nonetheless, in the room air replicates, it is speculated that the oxygenation efficiency of the membranes declined after 8 hours as evidenced by the unchanged PaO_2 values at T24 onward, when compared to T0. However, the PaO_2 was maintained at clinically applicable levels for the 56 hours duration of the replicate. Conversely, the PaO_2 in the oxygen replicates remained significantly increased up to T24 hours when compared to T0. Thus, a cautious clinical recommendation of using the Affinity Pixie Oxygenator with ultra-low blood and oxygen flow, for use up to 24 hours, can be made.

Haemolysis is a relatively common complication of ECMO with a reported incidence of between 5% and 18% in human patients (Lehle et al. 2015; Omar et al. 2015; Appelt 2020). Haemolysis during

ECMO can be caused either due to patient related factors or more commonly, circuit related factors, also known as technical-induced haemolysis (Lehle et al. 2015; Appelt 2020). Patient related factors inducing haemolysis are due to metabolic abnormalities, which prematurely age erythrocytes (Appelt 2020). Technical induced haemolysis is related to the exposure of blood to the non-endothelial surface of the ECMO circuit and the shear stresses exerted on the erythrocytes. Therefore, circuits of excessive length and superfluous components expose blood to a larger than necessary non-endothelial surface area, increasing the likelihood of haemolysis (Williams et al. 2015). Shear stresses occur as a result of high pump flow rates, cavitation, high circuit pressures secondary to small paediatric components or mechanical shear from large thrombi within the circuit (Lehle et al 2015; Williams et al. 2015). In the traditional ECMO circuit, the pump is usually the greatest contributor to haemolysis. Roller pumps were originally used to drive the ECMO circuits, however this pump type has been associated with an increased incidence of haemolysis. Thus, centrifugal pumps, which have been shown to cause less haemolysis, have widely been adopted for use in ECMO circuits (Lequier et al. 2013). In the present study, linear peristaltic infusion pumps were used in a novel manner to drive the blood within the *in vitro* ECMO replicates. Thus, inferences can only be made from studies investigating the blood handling characteristics of these pumps during blood transfusions. Stiles & Raffe (1991) compared fHb concentration during canine whole blood transfusions of both fresh and 21-day old blood, using peristaltic infusion and rotary infusion pumps at different flow rates. It was found that the level of haemolysis significantly increased during transfusions of stored blood at higher rates. Moreover, similar to ECMO circuits, roller infusion pumps caused significant haemolysis during both fresh and stored blood transfusions. Similarly, Kusahara et al. (2015) found no significant haemolysis during transfusions of stored human whole blood at different rates using peristaltic infusion pumps. However, Stiles & Raffe (1991) used a maximum flow rate of 0.0033 L minute^{-1} and Kusahra et al. (2015) used a maximum flow rate of 0.005 L minute^{-1}. The quickest flow rate investigated was by Poder et al. (2017), where stored blood of varying ages was subjected to varying flow rates ranging from 0.0005 L minute to 0.0075 L minute^{-1}; it was concluded that haemolysis increases with blood age and

transfusion speed. However, the fHb concentration remained within clinically acceptable limits. Thus, the level of haemolysis in the present study was expected to be clinically significant due to the fact that stored blood was pumped at a significantly higher speed than the speeds investigated by Stiles & Raffe (1991), Kusahara et al. (2015) or Poder et al., (2017). In both the room air and oxygen replicates, the fHb concentration exceeded the recommended threshold of 0.05 g dL^{-1} (Lehle et al. 2015; Omar et al. 2015) for ECMO, by T3. However, these fHb concentration levels must be interpreted in light of the fact that healthy dogs catabolise fHb at a rate of 0.07 g kg^{-1} $hour^{-1}$ (Price et al. 1988). This catabolic rate in context of the infusion pump rate of 0.02 L $minute^{-1}$ equates to a maximum fHb concentration threshold of 0.006 g dL^{-1} kg^{-1} (0.02 L $minute^{-1}$ = 12 dL $hour^{-1}$; 0.07 g kg^{-1} $hour^{-1}$/12 dL $hour^{-1}$ = maximum fHb threshold). The fHb concentration in the room air and oxygen replicates exceeded this threshold by T3. However, the rate of change of fHb concentration never exceeded this threshold in the room air replicates. The fHb concentration and rate of change of fHb concentration were markedly greater in the oxygen replicates as compared to the room air replicates at similar timepoints. It is theorised that the supply of oxygen promoted the formation of reactive oxygen species which accelerated haemolysis in the oxygen replicates. However, in an *in vivo* research model investigating the additive effects of fHb on oxidative damage in a hyperoxic state found that fHb catabolism is accelerated in a hyperoxic environment (Jungner et al. 2017). Therefore, the results obtained may not be reflective of the progression of haemolysis and accumulation of fHb *in vivo*. Furthermore, the blood used in this study was stored and then exposed to room temperature for 56 hours in the room air replicates and 24 hours in the oxygen replicates. The cell membranes of erythrocytes within stored blood progressively undergo irreversible changes due to the ongoing depletion of energy substrates within the storage medium. Vesicles bud off the cell membranes resulting in the loss of cell membrane lipids, leading to a decreased deformability and increased propensity for lysis (Price et al. 1988). It is unknown exactly how the ambient temperature during this study influenced the development of haemolysis. Hughes et al. (2007) observed no significant haemolysis in human blood exposed to ambient temperatures for 72 hours, therefore the haemolysis caused by exposure to ambient

temperature may have been minimal. However, Hughes et al. (2007) made this observation with fresh blood as compared to the stored blood used in this study.

During pathological conditions characterised by a reduction in blood oxygen content (for example, hypoxic hypoxaemia or anaemic hypoxaemia) or hypermetabolic states characterised by an increase in VO_2 (for example, sepsis), cardiac output increases to meet tissue oxygen requirements. However, some degree of myocardial dysfunction may occur under these conditions (Adachi et al. 1976; Butler & Campbell 2010; Champion et al. 2011). Typically, intravenous fluids and catecholamine therapy are used to improve cardiac output and thus DO_2. However, the initiation of catecholamine therapy under these conditions may thus worsen cardiovascular performance due to increased myocardial oxygen requirements during catecholamine therapy (Butler & Campbell 2010; Peterson & Moses 2011). The theoretical model conceptualised, depicting DO_2 under various circumstances is seemingly sufficient to meet the minimum systemic metabolic oxygen requirements, except under anaemic conditions. Whilst this model depicts DO_2 under non-pathological circumstances, it does assume that the lungs are not functioning at all. Therefore, it is speculated that augmentation of mixed venous blood oxygen content under circumstances of cardiopulmonary dysfunction may maintain adequate myocardial oxygenation. Thus, an ultra-low ECMO configuration during hypoxic or anaemic hypoxaemia or hypermetabolic states, could improve outcomes compared to conventional therapy.

4.2 Limitations

This study is an *in vitro* study where stored blood, which is already in an aberrant physiological state, was subjected to a non-endothelial surface for a prolonged period of time. In an *in vivo* setting, even if stored blood were used to prime the circuit, it would only be at the initiation of the ECMO circuit, that stored blood would be subjected to circuit conditions.

In all replicates, blood was circulated in a single, closed circuit from a reservoir into and oxygenator and returned to the reservoir. In the absence of metabolically active tissues consuming oxygen and producing carbon dioxide, the PO_2 and PCO_2 values achieved in both the room air and oxygen

replicates are unlikely to be reflective of a metabolically active patient. Therefore, it is highly unlikely that oxygen supplied oxygenators in an ultra-low flow setting will achieve the PaO_2 and $PaCO_2$ values obtained in the oxygen replicates. Regardless, clinical benefit may be realised, especially in dogs weighing < 20 kg.

During the traditional application of ECMO, there are various sensors positioned throughout the circuit. These sensors measure pressures, flow, temperature, clotting parameters and oxygenation indices. These sensors function to inform care providers of efficient circuit function and alert them to impending malfunctions (Allen 2011). Most malfunctions occur as a result of clot formation (Allen et al. 2011). Therefore, UFH is used as the anti-coagulant of choice in ECMO patients, targeting an ACT of 160 – 220 seconds (Allen et al. 2011; McRae & de Perrot 2018). In the current study, UFH was added to the reservoir, however, its effect was not monitored. Therefore, it is difficult to determine the cause of failure of 2 of the room air replicates as well as the reduction in oxygenation efficiency at T24 onward in the room air replicates. Lastly, the lack of temperature monitoring within the circuit also makes it difficult to make inferences on the reasons for the higher than expected PO_2 levels in the room air replicates. Oxygen solubility in blood increases with a decrease in temperature, resulting in a lower PaO_2 if CaO_2 remains constant. Formulae can be applied to calculate this change in PaO_2 (Hansen et al. 1999; Merino 1999). Thus, had the temperature of the blood within the circuit been measured, the accuracy of the predicted achievable PaO_2 could be improved due to the consideration of factors other than solely the barometric pressure.

The monitoring of anticoagulation therapy is vitally important during ECMO to mitigate the risks of catastrophic haemorrhage and thromboembolic events (Allen et al. 2011; McRae & de Perrot 2018). During the present study, neither the baseline nor ongoing coagulation parameters were monitored. Therefore, it is difficult to draw conclusions as to the reasons for the observed thrombotic events in 2 of the room air replicates, especially considering the short duration (2 to 3 hours) of clinically observable anticoagulation in dogs after intravenously administered UFH (Green 1980). It is extremely

likely that effects of the UFH introduced into the system at the commencement of the replicate had likely waned by the time the thrombotic event occurred.

4.3 Conclusion and future research

The *in vitro* oxygen replicates carried out over 24 hours demonstrated the potential for clinical application of ultra-low flow ECMO in pathological states characterised by an oxygen debt. The results strongly indicate that an ultra-low flow ECMO system may improve DO_2 at least to the myocardium, by improving the oxygen content of blood returning to the heart. Whilst it is difficult to predict the degree of haemolysis to expect *in vivo* from the results obtained *in vitro*, the circumstances affecting haemolysis in the experimental replicates are likely to differ in the clinical setting. Furthermore, in a clinical setting, it will not be stored blood which is prone to haemolysis that will be circulating through the ECMO circuit; it will be a relatively small amount of the patient's blood from which most of the oxygen will already be consumed. Thus, the influence of stored blood in a hyperoxic environment is unlikely to translate into a clinical setting. Nevertheless, the experimental data obtained in this study indicates that an ultra-low flow ECMO system can be safely instituted clinically for at least 24 hours. However, an *in vitro* model cannot completely predict what will occur in a clinical setting. Therefore, future research is required to investigate the oxygenation efficacy and effects of ultra-low flow ECMO in healthy dogs. A pilot clinical study should focus on obtaining baseline data for augmentation of DO_2, haemolysis, coagulation and longevity of the oxygenation membrane.

Once a pilot study in healthy dogs has been completed, the ultra-low flow ECMO should be investigated in dogs where hypoxic hypoxaemia and anaemic hypoxaemia has been induced. These studies should simulate the interaction of the ultra-low flow ECMO circuit in the setting of a stressed cardiovascular, pulmonary and haemostatic system. Once these studies are complete, ultra-low flow ECMO could be applied in clinically applicable scenarios.

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Addendum

Animal Ethics Approval Certificate

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Veterinary Science
Animal Ethics Committee

11 May 2020

Approval Certificate with Conditions
New Application

AEC Reference No.:	REC200-19
Title:	An in-vitro Veno-Venous Extra Corporeal Membrane Oxygenation model using a volumetric infusion pump
Researcher:	Dr AR Kadwa
Student's Supervisor:	Dr R Buck

Dear Dr AR Kadwa,

The **New Application** as supported by documents received between 2019-11-03 and 2020-05-04 for your research, was approved by the Animal Ethics Committee on its quorate meeting of 2020-05-04.

Please note the following about your CONDITIONAL ETHICS APPROVAL: 1.5 % OF BODY WEIGHT OF BLOOD VOLUME TO BE COLLECTED PER ANIMAL ONLY.

1. The use of species is approved:

Species	Number
Dogs	12
Samples Blood	12 x (1.5% of bodyweight of blood volume per animal)

2. Ethics Approval is valid for 1 year and needs to be renewed annually by 2021-05-11.
3. Please remember to use your protocol number (REC200-19) 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

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Fakulteit Veeartsenykunde
Lefapha la Diseanse tša Bongakadiruiwa

Animal Ethics Approval Certificate Extension

Faculty of Veterinary Science
Animal Ethics Committee

14 April 2021

Approval Certificate with Conditions
Annual Renewal (EXT1)

AEC Reference No.: REC200-19
Title: An in-vitro Veno-Venous Extra Corporeal Membrane Oxygenation model using a volumetric infusion pump
Researcher: Dr AR Kadwa
Student's Supervisor: Dr R Buck

Dear Dr AR Kadwa,

The **Annual Renewal** as supported by documents received between 2021-03-11 and 2021-04-01 for your research, was approved by the Animal Ethics Committee on its quorate meeting of 2021-04-01.

Please note the following about your CONDITIONAL ETHICS APPROVAL: 1.5 % OF BODY WEIGHT OF BLOOD VOLUME TO BE COLLECTED PER ANIMAL ONLY.

1. The use of species is approved:

Species	**Number**
Dogs	12
Samples Blood	12 x (1.5% of bodyweight of blood volume per animal)

2. Ethics Approval is valid for 1 year and needs to be renewed annually by 2022-04-14.
3. Please remember to use your protocol number (REC200-19) 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.
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

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Section 20 Permit

agriculture,
forestry & fisheries

Department:
Agriculture, Forestry and Fisheries
REPUBLIC OF SOUTH AFRICA

Directorate Animal Health, Department of Agriculture, Forestry and Fisheries
Private Bag X138, Pretoria 0001

Enquiries: Mr Herry Gololo • Tel: +27 12 319 7532 • Fax: +27 12 319 7470 • E-mail: HerryG@daff.gov.za
Reference: 12/11/1/4/1

Abdur Rahmaan Kadwa
Department of Companion Animal Clinical Studies
University of Pretoria
Onderstepoort-0110
E-mail: abdur.kadwa@up.ac.za

Dear Abdur Rahmaan Kadwa,

RE: PERMISSION TO DO RESEARCH IN TERMS OF SECTION 20 OF THE ANIMAL DISEASES ACT, 1984 (ACT NO. 35 OF 1984)

Your application requesting permission under Section 20 of the Animal Disease Act, 1984 (Act No. 35 of 1984) to perform a research project or study, refers. I am pleased to inform you that permission is hereby granted to perform the following study, with the following conditions:

Conditions:

1. This permission does not relieve the researcher of any responsibility which may be placed on him/her by any other act of the Republic of South Africa;
2. Written permission from the Director: Animal Health must be obtained prior to any deviation from the conditions approved for this study under this Section 20 permit. Please apply in writing to HerryG@daff.gov.za;
3. The study must be conducted in compliance with the Veterinary and Para-Veterinary Professions Act 1982 (Act No. 19 of 82);
4. All potentially infectious materials utilised or generated during or by the study must be destroyed at completion of the study. A registered waste removal company must dispose the material generated from the study;

5. Ethical approval for the study must be obtained from the relevant authority before the study may start;
6. Any further use or distribution of the samples is subject to obtaining a separate Section 20 approval;
7. If required, an application for an extension must be made by the responsible researcher at least one month prior to the expiry of this Section 20 approval;

Title of research/study: An in-vitro Veno-Venous Extra Corporeal Membrane Oxygenation model using a volumetric infusion pump.

Researcher: Abdur Rahmaan Kadwa
Institution: University of Pretoria
Our ref Number: 12/11/1/1/8
Your ref: REC200-19
Expiry date: 31 December 2022

Kind regards,

DR. MPHO MAJA
DIRECTOR OF ANIMAL HEALTH
Date: 2020/06/18

SUBJECT: An in-vitro veno-venous Extra Corporeal Membrane Oxygenation model using a volumetric infusion pump

Data Collection Form

A.R.Kadwa – ECMO Research Data Collection Sheet

Date:		
Replicate type and number (1-6):	Experimental	Control

Blood Donor Details			
Name/ID:			
Breed:	Gender:	Age:	Weight:
Sedation used (drug(s) and dose(s)):			
Haematocrit prior to collection:	Blood smear comments/results:		
Volume of blood collected:	Donor reactions/complications:		

Equipment/Supplies Checklist				
Filled blood bag	Heparin bottle	23G Needle	1mL Syringe	Drip set x 2
Blood gas syringe x 16 (experiment)		Blood gas syringe x 8 (control)		
Eppendorf tube x 16 (experiment)		Eppendorf tube x 8 (control)		
fHb curvette x 16 (experiment)		fHb curvette x 8 (control)		
Pixie oxygenator		HemoCue Plasma Low/Hb		
HemoCue Plasma Low/Hb high control		HemoCue Plasma Low/Hb low control		
Reservoir		Water bath/incubator		
HemoCue Plasma Low/Hb, calibration performed				

Circuit Setup Details		
Oxygenator serial number/identifier:		
Prime composition and volume(s):		
Volume of blood added to reservoir:	Volume and type of heparin added:	Temperature of reservoir/water:
Initial pump flow rate:	Initial O_2 sweep rate:	Initial CO_2 flow/sweep rate:

Data collected									
Time point	Actual time	O_2 flow rate	CO_2 flow rate	Pump flow rate	Pre-oxygenator		Post-oxygenator		Collector initial
					Blood gas	fHb	Blood gas	fHb	
Baseline									
1 hour									
2 hours									
3 hours									
4 hours									
5 hours									
6 hours									
7 hours									
8 hours									
24 hours									
48 hours									
72 hours									

Publications

Title: Canine blood oxygenation using ultra-low blood flow in an *in vitro*, single-circuit, extracorporeal membrane oxygenator model

Authors: Abdur Rahmaan Kadwa, Roxanne Buck, Gareth Zeiler

Journal: in draft: American Journal of Respiratory and Critical Care Medicine

Presentations

Event: IVECCS 2022 - International Veterinary Emergency and Critical Care Symposium

Date: 07/09/2022 – 11/09/2021

Title: Canine blood oxygenation using ultra-low blood flow in an *in vitro*, single-circuit, extracorporeal membrane oxygenator model