Assessment of Microcirculatory Perfusion in Healthy Anesthetized Cats Undergoing
Ovariohysterectomy Using Sidestream Dark Field Microscopy

Thesis

Presented in Partial Fulfillment of the Requirements for the Degree Master of Science in
the Graduate School of The Ohio State University

By:

Michelle Goodnight B.S., D.V.M., M.A.

Major, United States Army Veterinary Corps

Graduate Program in Comparative and Veterinary Medicine

The Ohio State University

2010

Thesis Committee:

Edward S. Cooper, Advisor

Amy L. Butler

Richard Bednarski

Mary McLoughlin

Copyright by:

Michelle E. Goodnight

2011

Abstract

The microcirculation consists of vessels less than ten microns in diameter. Each microcirculatory unit has a feeder arteriole, capillaries and draining venules. Traditionally, when monitoring perfusion in patients, macrocirculatory parameters such as heart rate, blood pressure, cardiac output, lactate levels and central venous oxygen saturation have been utilized. Unfortunately, research in humans and dogs has demonstrated that these macrovascular parameters show poor correlation with changes at the microvascular level and thereby may poorly reflect tissue perfusion. Newer technologies, such as sidestream dark field microscopy (SDF) allow for direct imaging of the microcirculation and the ability to evaluate changes related to various stimuli, such as anesthesia, surgery, shock, and sepsis. This technology has been validated in humans, rodents and dogs, but has not yet been explored in cats. We sought to establish baseline clinical values for microcirculatory parameters in healthy, anesthetized cats using SDF and to determine if surgical manipulation alters these values during ovariohysterectomy.

Eighteen healthy cats presenting for elective ovariohysterectomy were anesthetized using a standardized protocol. Three 20-second microcirculatory videos were obtained from the sublingual mucosa at three intervention points: after induction but

before placement of the first towel clamp, after ligation of the first ovarian pedicle and after placement of the final skin suture and towel clamp removal. At each time point, macrovascular parameters (heart rate, respiratory rate, blood pressure), pulse oximetry, end tidal carbon dioxide, and end tidal inhalant anesthetic concentrations were recorded. In addition, SDF videos of the microcirculation were obtained from the sublingual mucosa using previously established consensus criteria.

Videos were assessed for quality; only those deemed acceptable were included. Qualifying videos were analyzed by a single observer blinded to intervention point. Total vessel density (TVD), proportion of perfused vessels (PPV), perfused vessel density (PVD) and microvascular flow index (MFI) were determined using vascular analysis software. Microvascular parameters were analyzed for significant changes between intervention points and correlation with macrovascular parameters.

Twelve cats were included in final video analysis; six were removed for poor video quality. There was no significant variation in TVD, PPV and PVD with surgical manipulation, however MFI showed a significant increase from baseline. The MFI had independent positive correlation with mean arterial pressure, heart rate, respiratory rate and systolic blood pressure and had negative correlation with temperature [$p < 0.05$]. However, when multivariate analysis was conducted, these correlations failed to retain

significance. Pearson correlation showed no relationship between length of anesthesia and changes in microcirculatory parameters.

These results show that surgical tissue manipulation did not significantly alter microvascular vessel density or the proportion of perfused vessels, although tissue manipulation did increase MFI. The length of time under anesthesia did not affect the microcirculatory parameters in the study cats. As documented in other species, changes in macrovascular parameters do not correlate well to changes in microcirculatory perfusion parameters.

This study demonstrated that SDF can be successfully utilized in cats, allowing real-time imaging of the sublingual microvasculature. This technology has potential as a tool in experimental and clinical monitoring of microcirculatory changes. However, there are several potential limitations to its use, such as the difficulty in obtaining high quality images, the need for general anesthesia and the time consuming nature of the offline video analysis. Future experimental and clinical investigation is warranted, including assessment of microvasculature changes in disease states like shock, sepsis, congestive heart failure and diabetes mellitus.

Dedication

Thank you to Dr. Amy Butler, my program mentor and inspiration for this project. Without her efforts on my behalf, this paper would not have been possible. Also thank you to Dr. Ed Cooper for his continued support for this project and assistance with manuscript preparation, and Drs. Richard Bednarski and Mary McLoughlin, who assisted in obtaining cases and served on my thesis committee. A special thank you to Dr. Julien Guillaumin for providing a different perspective on critical care and presenting concepts in a way that made sense to my brain. Finally, thank you to Dr. Ann Peruski for all her support, encouragement and commiseration during our residency.

I am proud to currently serve in the United States Army Veterinary Corps. The U.S. Army provided funding for my Residency and Masters programs, an investment which I hope they will find advantageous.

I am forever indebted to my wonderful husband Ryan, fabulous children Nathaniel and Isabelle, and my extended family and friends for their love, support, and encouragement during the past three years. I could not have done it without them.

Acknowledgements

Special thanks to the Anesthesia and Surgery Departments and the Small Animal Operative Practice Laboratory personnel at the Ohio State University Veterinary Medical Center for all their patience and assistance during data collection for this project.

VITA

1997.....B.S. Chemistry and Biological Science, Marshall University
1998.....M.A. Biological Science, Marshall University
2002.....Doctor of Veterinary Medicine, Virginia-Maryland
 Regional College of Veterinary Medicine
2002.....Commissioned Officer, United States Army Veterinary Corps
2011.....Residency in Small Animal Emergency and Critical Care
 Medicine, The Ohio State University

FIELD OF STUDY

Major Field: Comparative and Veterinary Medicine

Table of Contents

	Page
Abstract	ii
Dedication	v
Acknowledgements	vi
Vita	vii
List of Figures	x
List of Tables	xi

Chapter 1: Literature Review

1.1 The microcirculation	1
1.2 Imaging the microcirculation	10
1.3 The effect of anesthesia on microcirculation	17
1.4 Changes in microcirculation induced by surgical tissue manipulation	24

Chapter 2: The Experiment

2.1 Introduction.....	32
2.2 Materials and methods	35
2.3 Results.....	40
2.4 Discussion and conclusion.....	42
Bibliography	56
Appendix A: List of proprietary materials.....	63
Appendix B: Glossary of microvascular terms.....	64

List of Figures

Figure	Page
1.1 A microcirculatory unit.....	27
1.2 A microcirculatory unit.....	27
1.3 The cyclooxygenase pathway.....	28
1.4 The renin-angiotensin-aldosterone system.....	28
1.5 The orthogonal polarization spectral imaging microscope.....	29
1.6 The sidestream dark field microscope.....	29
1.7 Calculating total vascular density.....	30
1.8 Calculating microcirculatory flow index.....	30
2.1 Microcirculatory flow index at each intervention point.....	52

List of Tables

Table	Page
1.1 Mediators of vasoregulation.....	31
2.1 Monitored variables.....	53
2.2 Microvascular parameters.....	53
2.3 Univariate analysis of microcirculatory flow index and intervention point.....	54
2.4 Univariate analysis of microvascular and monitored variables.....	54
2.5 Comparative microcirculatory parameters.....	55

CHAPTER 1

LITERATURE REVIEW

1.1 The microcirculation

The microcirculatory unit is comprised of arterioles feeding into a capillary bed that is drained by venules. The feeder arterioles are highly muscular throughout their length, while the terminal metarterioles have intermittent bands of smooth muscle. True capillaries have walls that lack musculature and are one endothelial cell thick attached to a basement membrane. A precapillary sphincter is located between the arteriole and the capillary bed (Figures 1.1 & 1.2). Arterioles and venules in the microcirculation have a diameter less than 100 microns, while the capillaries are less than 10 microns in diameter. Altogether, the microcirculation represents the largest vascular surface area in the body, and is vital for the effective delivery of nutrients to the cells and removal of waste products from the tissue beds.^[1, 2]

The basal requirements of tissue beds vary based on their metabolic rate, nutrient availability and accumulation of waste products. Given that each capillary bed has unique requirements that may change independent of nearby capillary beds or systemic tissue needs, there are many local regulators of microcirculatory flow. Typically, these changes in perfusion occur at the precapillary sphincter, the lowest level necessary to achieve required flow through a capillary bed.^[2, 3] Independent regulation of flow based on local tissue needs results in selective capillary perfusion and the potential for microcirculatory

shunting. These local blood flow regulators are often able to overcome systemic vasoconstriction to maintain perfusion in individual capillary beds, a process referred to as autoregulation.^[2] Given the expansive nature of the capillary circulation, cardiac output would be insufficient to maintain forward flow if every capillary bed were to open simultaneously. Thus the ability to adjust capillary perfusion through local and systemic changes is essential for modulating cardiac workload.^[1, 2] This regulation of individual capillary bed blood flow occurs both rapidly and in a more chronic fashion.^[2]

Rapid control of microcirculatory flow is mediated at a local level through autoregulation.^[2] There are many environmental conditions and substances with active vasomotor effects at the local level (Table 1.1).^[1-3] Vasoactive substances can be divided into local and systemic control, each level encompassing conditions and substances promoting vasoconstriction or vasodilation. Local environmental conditions that promote vasoconstriction include tissue alkalosis, hypothermia, hyperoxia and elevated calcium levels.^[2, 3] Important locally acting vasoconstrictors include thromboxane A₂, endothelium-derived constricting factors and the endothelins. Thromboxane A₂ has an extremely short half-life (roughly 30 seconds).^[4] It is produced by many tissues and activated platelets, often in response to endothelial injury, via the cyclooxygenase pathway (Figure 1.3) and stimulates smooth muscle contraction.^[4, 5] Similarly, the endothelium-derived constricting factors and endothelins are produced by endothelial cells in response to alterations in the local cellular environment, such as endothelial injury, and promote vascular smooth muscle constriction.^[2, 3]

Conversely, there are many conditions and substances that promote vascular smooth muscle relaxation leading to vasodilation. Environmental conditions that promote vasodilation include tissue acidosis, elevated temperature, hypoxia and elevated potassium or magnesium levels.^[2, 3, 6] One important local vasodilator is nitric oxide (NO). The endogenous form of nitric oxide synthetase (eNOS) is responsible for maintaining a basal level of NO produced and vascular tone. The inducible form of nitric oxide synthetase (iNOS) is produced by the endothelial cells under many circumstances. In patients suffering from anemia, decreased red blood cell mass and oxygen carrying capacity results in tissue hypoxia and increased NO production. An increase in blood viscosity, which occurs in diseases such as polycythemia vera, results in elevated shear stress on the vascular endothelium, increasing iNOS activity and levels of NO. Another important activator of iNOS is the inflammatory cascade, including mediators such as interferon gamma and cytokines, as well as the lipopolysaccharide (LPS) released by gram negative bacteria. This marked upregulation of iNOS and resultant vasodilation plays a major role in the development of vasodilatory shock associated with gram negative sepsis. In situations where both interferon gamma and lipopolysaccharide (LPS) are present, the iNOS pathway can be excessively upregulated, resulting in cytotoxic levels of NO.^[7-9] Another potent vasodilator, prostacyclin, is produced by endothelial cells via the cyclooxygenase pathway (Figure 1.3). Induction of the cyclooxygenase pathway and increased prostaglandin synthesis occurs secondary to increased shear stress on the endothelium, inflammation and hypoxia.^[10, 11] Many other vasodilatory mediators are released during hypoxia. Decreased amounts of adenosine triphosphate and increased

amounts of adenosine diphosphate, adenosine and lactate develop in tissues that are unable to maintain aerobic metabolism.^[2, 3] Carbon monoxide, a byproduct of hemoglobin breakdown, and carbon dioxide, a byproduct of cellular respiration, also act as a local vasodilators.^[1-3]

Separate from local vasomotor regulation, vascular tone can be influenced by systemically released substances. Catecholamines are important systemic vasoconstrictor agents released at increased rates when the sympathetic nervous system is activated. This occurs via central nervous system control of the systemic vasculature in response to baroreceptors and chemoreceptors in the aortic arch and carotid sinuses.^[3, 12] The baroreceptors respond to changes in the distension of the vessels. When the baroreceptors are triggered by increased stretch of the vessel, they inhibit vasoconstriction through downregulation of the sympathetic nervous system and stimulation of the vagal system, resulting primarily in decreased heart rate and, to a limited extent, vasodilation.^[3]

Clusters of chemoreceptors in the aorta and carotid sinuses, organized into carotid bodies and aortic bodies, play a role in vasomotor tone through chemosensitive cells that respond to hypoxia, hypercapnia or acidemia. Stimulation of chemoreceptors causes excitation of the vasomotor center in the brain, resulting in release of catecholamines and systemic vasoconstriction. These effects are most important once the systemic arterial pressure drops below 80 mmHg, and allow individual capillary beds to maintain perfusion despite decreased systemic blood pressure.^[12]

Maintenance of vascular tone is not the only factor influencing arterial blood pressure and tissue perfusion.^[13] The fluid volume within the vascular space also

contributes to maintenance of appropriate blood pressure and perfusion. In normal animals, the renin-angiotensin-aldosterone system (Figure 1.4) is integral in maintaining appropriate vascular volume, as well as contributing to systemic vascular tone.^[13] When blood flow through the kidney decreases, the macula densa detects a decrease in ultrafiltrate flow in the renal tubules. The macula densa signals the release of renin from the juxtaglomerular apparatus. Renin then converts angiotensinogen into angiotensin I. As it moves through the lungs, angiotensin I is converted into angiotensin II. Angiotensin II acts on vasculature to cause vasoconstriction and on the proximal tubules to cause sodium and water retention, leading to increased blood volume and arterial pressure.^[13]

Angiotensin also causes the release of aldosterone from the adrenal glands, which acts on the distal tubule to increase sodium retention, and release of antidiuretic hormone (also called arginine vasopressin) from the posterior pituitary, which causes vasoconstriction and acts on the cortical and medullary collecting ducts to increase water retention.^[13] Other stimuli for antidiuretic hormone release include decreased stretch of the aortic and carotid receptors, increased serum osmolality detected by the osmoreceptors in the hypothalamus and in response to increased cholecystokinin levels.^[13, 14]

Systemically acting vasodilators include the kinins, adrenomedullin and atrial natriuretic peptide (ANP). The kinins include bradykinin and lysylbradykinin, and are activated by the kallikreins. They typically regulate blood flow at the tissue level, but can also be found circulating in the bloodstream.^[3] In addition to causing arteriolar dilation, bradykinin increases capillary permeability, thereby increasing the delivery of nutrients

to the tissue bed. Histamine, which is released when tissues are damaged or in allergic reactions, also acts as a vasodilator and increases capillary permeability.^[2] Adrenomedullin increases the production of NO while ANP acts to antagonize numerous vasoconstrictor agents, both exerting their influence on the vascular tone indirectly.^[3]

In essence, there are many factors affecting microcirculatory and systemic vascular tone. The systemic regulators, such as catecholamines, input from baroreceptors and chemoreceptors, and the renin-angiotensin-aldosterone system, are responsible for controlling delivery of blood to the precapillary sphincter across different tissue beds. Once blood arrives at the capillary bed, local substances such as endothelin and nitric oxide act to maintain flow through the capillary bed independent of systemic changes to perfusion.^[1-3, 6-14]

It is important to note sympathetic innervation exists for the arterial tree and much of the venous system, but is not present at the capillary or small venule level.^[3, 12] The absence of innervations and musculature in the true capillaries means flow through each capillary bed is regulated by the hemodynamic pressures generated via the precapillary sphincter and the post capillary venules. A single capillary bed can be fed by multiple arterioles, a situation that allows flow through the capillary bed to increase in the range of 200-500% without any significant change in the overall arteriolar pressure.^[15] This can result in preservation of microcirculatory flow to specific tissue beds during periods of transient hypotension, which may occur during shock or anesthesia. For example, it has been demonstrated that sublingual microcirculatory flow is maintained during positive

pressure ventilation^[16] and cerebral microcirculatory flow is maintained during hemorrhagic shock.^[17]

The interplay of the local and systemic mediators of vascular tone ideally serve to maintain appropriate blood flow to the tissues. In many tissues, such as the brain and kidneys, the local regulatory systems take precedence over changes in systemic hemodynamics.^[1-3, 6, 12] However, when tissue hypoxia is prolonged, systemic blood pressure is not restored quickly enough, or systemic derangements such as sepsis develop, there is reduction in functional capillary density and heterogeneity of microvascular flow.^[6] This process is related to a decreased responsiveness to vasoactive agents, termed endothelial stunning, and is one reason macrovascular parameters do not correlate well to microvascular perfusion.^[6, 18]

Monitoring heart rate, mean arterial blood pressure, and, if possible, cardiac output, can provide a representation of changes occurring at the macrovascular level. However, the differences in innervation, musculature, and local environment mean that microcirculatory flow is only loosely correlated to macrovascular hemodynamics.^[6, 17-19] Mean arterial pressure is determined by cardiac output and systemic vascular resistance. Cardiac output is determined by heart rate and stroke volume (the amount of blood ejected from the heart with each contraction).^[6] The systemic factors described previously maintain the mean arterial pressure during normal circumstances and provide blood flow to the feeder arterioles.^[1-3, 6, 12] The local regulatory factors described previously regulate the amount of blood traveling through the feeder arterioles and work to sustain appropriate microvascular perfusion during compensated shock and transient

hypotensive episodes, such as those occurring during anesthesia.^[17, 20-22] In other circumstances, such as sepsis or heart failure, local control is unable to maintain normal microvascular perfusion.^[23-26]

Numerous studies have demonstrated the lack of correlation between macrovascular and microvascular parameters.^[17, 18, 21, 23, 27, 28] In one example, Dubin et al demonstrated that microvascular parameters worsened under the influence of norepinephrine when the microcirculation was initially normal. However, when microcirculatory flow is significantly altered due to decreased systemic blood pressure, norepinephrine improved microcirculatory perfusion.^[29] This inability to predict microvascular perfusion based upon macrovascular parameters results in a need to use specialized techniques to directly assess and monitor microcirculatory flow in our patients. Studies in dogs have established normal values for microcirculatory perfusion parameters^[20] and demonstrated that they do not correlate well with macrovascular hemodynamic parameters.^[21] Developing a technique to evaluate the microvasculature in cats is important because macrovascular changes during shock and sepsis are often very different than those seen in dogs. For instance, cats often develop bradycardia during shock states, rather than exhibiting a tachycardic response as is seen in dogs.^[30]

When evaluating literature related to microvascular perfusion in cats, all available studies are based on research rather than clinical populations. One study showed that in cats cerebral blood flow can be increased secondary to a transient vasodilation of the parynchamal microvessels in response to adrenomedullin.^[19] Another study found that inducing ischemia in the feline cerebral parynchema via microembolism resulted in

arteriolar vasodilation that was not regulated by nitric oxide production.^[31] Both these studies involved surgically exposing the brain for imaging of the microcirculation using intravital microscopy techniques.^[19, 31] Other studies of feline microcirculation focus on skeletal muscle.^[32, 33] Rather than imaging the microcirculatory flow, these papers focused on changes in permeability related to endothelin-1^[32] or nitric oxide.^[33] Finally, experiments using cat lungs have demonstrated changes in blood pressure of vessels between 30 and 50 micrometers related to hyperoxia^[34] and hypoxia.^[35] To date, there are no published studies concerning non-invasive microcirculatory imaging of feline microcirculation in clinical patients.

1.2 Imaging the microcirculation

Traditionally, monitoring of a patient's hemodynamic state has been conducted at the macrovascular level by monitoring heart rate, respiratory rate, blood pressure, oxygen content and lactate, among other parameters. While these parameters may reflect systemic cardiovascular regulation, they do not necessarily reflect what is occurring on the microcirculatory level.^[27] The upstream parameters, namely heart rate, respiratory rate, cardiac output and blood pressure, provide us information about delivery of blood to the precapillary sphincter, but may not correlate to the amount of flow reaching the capillary beds.^[17, 18, 27] Downstream parameters, such as lactate, central venous oxygen saturation (S_vO_2), base excess and pH, have also been investigated as potential markers for microcirculatory perfusion,^[36, 37] However, numerous studies show that changes in downstream parameters are not predictive of microcirculatory perfusion, particularly in septic patients.^[38-42] Fortunately, the development of newer technologies has allowed the in vitro imaging and monitoring of the microcirculation.

Initially, imaging of the microcirculation was conducted by surgically implanting a “window” at a given location, such as the dorsal skinfold tissue window chamber in rodents.^[43] This window is then used for advanced imaging of the blood vessels underlying the window. Such windows can be used to study angiogenesis in implanted tumors, in different levels of oxygen and under differing exposures to vasoactive substances and growth factors.^[43] Multiple intravital microscopy methods to image the blood vessels have been described.^[44-46] Conventional microscopes and inverted microscopes are used to obtain an image. The light source can be conventional

transillumination, which highlights the red blood cells well when used with a green filter, via refractive contrast through the tissues using oblique transillumination, or via fluorescence contrast administered to label the plasma.^[44] These techniques can be modified using different cameras and computer processing equipment.^[43, 44]

The images obtained using intravital microscopy are analyzed at a later time. All vessels with red blood cell movement are identified. Then vessels through which red blood cells are moving at least once every thirty seconds are identified. The functional capillary density is the percentage of vessels that have red blood cell passage at least every thirty seconds.^[47] Kerger and colleagues demonstrated that the functional capillary density (FCD) is the only parameter, whether macrocirculatory or microcirculatory, significantly associated with survival in hamsters undergoing hemorrhagic shock, and a >50% decrease in FCD was predictive of decreased tissue oxygen content and a metabolic state less compatible with survival.^[47] The difficulty with using conventional intravital videomicroscopy in clinical patients is that it requires surgical exposure of the tissue of interest.

In 1999, Warren Groner and his colleagues described a new technique for imaging the microcirculation, orthogonal polarization spectral imaging (OPS).^[48] With this technology, light of a specified wavelength is polarized to one plane and used to illuminate the target (Figure 1.5). When the light hits its target, it is either absorbed or reflected. The reflected, depolarized light is detected by a charge-coupled device (CCD) videocamera. This information is then translated by a computer into an image for display. Using this technique, a wavelength of 530 nm is equally reflected by oxy- and

deoxyhemoglobin, and one pixel is equivalent to one micrometer.^[48] Groner and his colleagues showed that OPS imaging provides information comparable to intravital microscopy.^[48] In addition, this technique can be performed using a solid organ or non-keratinized surface to provide microcirculatory information about sites not accessible to intravital microscopy.^[48] Since its introduction, OPS has been utilized to determine microvascular flow in numerous situations, such as mesenteric hypoxia, cutaneous burns, sepsis, congestive heart failure and hypothermic resuscitation.^[23-26, 49-52] Despite its widespread use to investigate microcirculatory flow in multiple situations,^[23-26, 28, 49-52] OPS was still being validated as a semi-quantitative method for assessing the microcirculation as recently as 2005.^[53]

Part of the ongoing challenge in directly imaging the microcirculation is the ability to consistently obtain a clear image. OPS made an enormous contribution to microcirculatory analysis, but obtaining quality videos can be difficult, as any motion of the probe or target causes blurring of the image.^[52, 54, 55] Recently, a related technique was developed using polarized light and indirect illumination of the target tissue.^[55, 56] Termed sidestream dark field imaging (SDF), this new technique provides illumination of the target tissue through a ring of light emitting diodes (LEDs) surrounding a light guide (Figure 1.6). The LEDs provide 530 nm wavelength light that penetrates the tissue and scatters. Light that scatters into the light guide is focused via lenses onto a CCD chip. This information is then translated into a video image for recording and later analysis. Because the light guide is optically isolated from the LEDs and the light source enters the tissue from the sides, the effect of surface reflection is negated, providing a clearer image

than obtained using OPS imaging techniques.^[55] Additionally, the microvascular perfusion parameters obtained using SDF are comparable to those obtained using OPS.^[55, 57] Microvascular perfusion parameters are obtained using the same technique for video analysis of images from either OPS or SDF systems.^[58]

In 2005, eight experts in the study of microvascular hemodynamics created consensus criteria for the acquisition and analysis of microcirculatory images using OPS or SDF.^[58] It was agreed that “scoring of the microcirculation should include an index of vascular density, assessment of capillary perfusion and a heterogeneity index.”^[58] The panel provided five key points for optimal image acquisition: image five sites per organ, avoid pressure artifact, eliminate secretions from the imaged surface, adjust focus and contrast appropriately and obtain high quality recordings.^[58]

Once recorded, the videos are analyzed offline for multiple variables. Total vessel density (TVD) is derived by superimposing a grid of three equidistant horizontal and three vertical lines over the image (Figure 1.7). TVD is determined based upon the number of vessels crossing these lines divided by the length of those lines. This value is comparable in either OPS or SDF.^[58] The total vessel density provides an indication of the number of capillaries available for perfusion in the imaged capillary bed. It is important to note that because both OPS and SDF technologies require the presence of hemoglobin for detection, only vessels that contain red blood cells at some point in the video have the potential to be detected.^[58]

When analyzing OPS images, the number of blood vessels that have detectable red blood cell transit at least once every thirty seconds is determined. The functional

capillary density (FCD) is then calculated as the number of capillaries with red blood cell transit at least once every thirty seconds divided by the total vessel density then multiplied by one hundred.^[47] This value is comparable to the perfused vessel density obtained when analyzing SDF videos.^[58]

When analyzing SDF videos, flow categories are assigned to each vessel (absent (0), intermittent (1), sluggish (2), normal (3) or hyperdynamic (4) flow). The proportion of perfused vessels (PPV) is calculated as $[100 \times (\text{total number of vessels} - [\text{number of vessels with no flow} + \text{number of vessels with intermittent flow}]) / \text{total number of vessels}]$. The perfused vessel density (PVD) is calculated by multiplying TVD by PPV.^[58]

In both imaging techniques, the microcirculatory flow index (MFI) is obtained by dividing the visual field into quadrants (Figure 1.8). Each quadrant is then assigned a flow index value based on the average vessel flow in that section. The value from each quadrant is then averaged to determine the MFI for the video. The MFI provides an assessment of the heterogeneity of flow in the imaged capillary bed, which is a reflection variability of blood flow through visible capillaries in adjacent tissues.^[59]

Although these systems have been well utilized in human and research settings, scientific reports detailing utilization in dogs are limited^[20, 21] and there are none relating to cats. Silverstein and colleagues used SDF to establish normal microvascular assessment parameters in healthy, anesthetized dogs. In their study, the microcirculation was readily imaged. They determined TVD, PPV, PVD and MFI for healthy anesthetized dogs.^[20] Another study, by Peruski and colleagues, sought to assess the microcirculation

of dogs during hemorrhagic shock. This study detected a significant decrease in microvascular variables following hemorrhage. With the ability to detect significant changes in the microcirculation following massive, acute blood loss, SDF was shown to be a modality that may have clinical relevance in veterinary patients.^[21] In consideration of feline patients, published studies relating to non-invasive direct or indirect imaging of the microcirculation are sparse. In one study, Leinonen and colleagues used contrast-enhanced ultrasound to analyze perfusion in the abdomen of ten healthy, anesthetized cats.^[60] Two other papers describe the use of specialized magnetic resonance imaging to assess cortical neurovasculature in anesthetized cats.^[61, 62] Although these techniques allow for assessment of perfusion patterns and thus indirect evaluation of microcirculation, they are unable to directly image and assess individual capillary beds.

Studies designed to assess specific vascular beds have been conducted in the cat.^[63-67] Two studies evaluated choroidal microcirculation in Abyssinian cats, one using radiolabeled microspheres and the other using histopathologic analysis of enucleated eyes from the same cats.^[63, 64] Additional studies approach assessment of microvasculature in the central nervous system using invasive surgical techniques.^[65-67] In one of these studies, a craniectomy was performed and a laser-Doppler flowmeter used to evaluate changes in cerebral microcirculatory flow in response to electro-acupuncture. This method provides a quantitative assessment of microvascular flow based on perfusion units, but does not allow imaging of individual vessels.^[65] Fluorescence videomicroscopy was used to evaluate cerebral microcirculatory alterations after air-embolism in anesthetized cats in another study. This required a craniotomy and specially labeled red

blood cells for analysis. This method does allow identification of individual cells and vessels in the microcirculation.^[66] Yet another study involved surgical implantation of a specially constructed spinal window to allow direct videomicroscopy of the pial vessels. This method allowed identification of arterioles down to 11 μm in diameter.^[67]

Unfortunately, all these studies entailed surgical implantation of imaging windows or cannot provide information concerning individual capillary beds. To allow assessment of individual capillary beds in clinical patients, other techniques are required. The newer microvascular imaging techniques, such as OPS and SDF, allow imaging of the microcirculation with resolution which allows for identification of individual red blood cells.^[55] These types of imaging allow monitoring of the microcirculation in diverse situations,^[23-26, 28, 48-52] and are validated for use in dogs.^[20] To date, no studies have been published evaluating the use of either OPS or SDF imaging techniques to image the microcirculation in cats.

1.3 The effect of anesthesia on microcirculation

It is well known that anesthetic drugs can alter vascular tone and change macrocirculatory parameters such as heart rate and blood pressure.^[68, 69] The drugs typically used in general anesthesia can be categorized as preanesthetic, induction, maintenance and local anesthetic agents. Common preanesthetic agents include anticholinergics, phenothiazines, benzodiazepines, α -2 agonists and opioids. Induction medications may be administered via injection (propofol, ketamine/valium, barbiturates, etomidate, etc.), oral (ketamine) or inhalant (isoflurane, sevoflurane, halothane, desflurane, etc.). Maintenance anesthetics are routinely inhalant, but may also be injectable.^[68, 69] Local anesthetics, such as lidocaine, ropivacaine and bupivacaine, are salts of weak bases that act to stabilize neuronal membranes and reduce signal transmission by blocking sodium channels and thus perception of painful stimuli.^[68, 70]

Preanesthetic agents may be administered to minimize patient stress and anxiety, as well as to minimize the cardiopulmonary effects and reduce induction and maintenance anesthetic requirements. Phenothiazines such as acepromazine and chlorpromazine provide sedation and suppress the sympathetic nervous system by blocking dopamine receptors in the basal root ganglia and limbic system. They produce a dose-dependent α_1 adrenergic blockade, causing a peripheral vasodilation and reflex increase in heart rate. Bradycardia can develop as the patient becomes more relaxed. The phenothiazines provide some muscle relaxation, antiemesis, antiarrhythmic effect, have antihistaminic effect and potentiate the effects of many analgesics. Administration of

phenothiazines can amplify the cardiopulmonary depressant effects of other medications.^[68, 69, 71]

As preanesthetics, opioids provide analgesia plus or minus sedation and can decrease the dose of induction agent required to obtain general anesthesia. Numerous opioid receptors have been identified, including μ , κ , σ , δ and ϵ . Commonly used opioid medications can be classified as full μ -agonists, partial μ -agonists or κ -agonist/ μ -antagonists.^[71] The μ receptors are primarily found in the central nervous system (CNS), and are responsible for modulating the perception of pain. κ receptors are located deep in the cerebral cortex and spinal cord and provide analgesia when stimulated. σ receptors can cause dysphoria when activated. δ and ϵ receptors have been identified but their actions are not fully understood at this time.^[71] Effects of opioids include sedation, euphoria, dysphoria, nausea, vomiting and constipation. Activation of the μ receptors can increase vagal tone, leading to bradycardia. Response to a adrenergic stimulation may be blunted, leading to peripheral vasodilation and baroreceptor inhibition.^[71] Administration of some opioids, such as morphine or meperidine, causes histamine release leading to vasodilation, nausea and vomiting.^[71] Opioid medications are also known to cause hyperthermia in some species, including cats.^[71] At published doses opioids can also exert a respiratory depressant effect. Pure and partial μ -agonists can be reversed to varying degrees using medications such as naloxone.^[68, 69, 71]

Induction agents are used to render an animal unconscious so that distressing or painful procedures can be performed. Propofol is a rapidly acting anesthetic medication with rapid clearance that causes CNS depression via potentiation of GABA_A receptor

activity, and possibly through actions on the endocannabinoid system.^[72, 73] It provides smooth induction but can significantly suppress ventilation and may cause prolonged apnea. Alterations in heart rate are minimal but propofol is a potent vasodilator and can cause a transient drop in blood pressure. Propofol has a dose-dependent hemodynamic depressive effect, likely due to its action as a sodium channel blocker.^[69, 71, 74] Propofol decreases myocardial contractility by reducing calcium availability in the myocardium, and has a direct effect on vascular tone through NO release and decreases baroreceptor sensitivity.^[75] At anesthetic doses, propofol decreases cerebral blood flow and reduces cerebral oxygen demand. Propofol also possesses anti-seizure activity comparable to barbiturates.^[69, 71, 74]

Ketamine is an NMDA-receptor antagonist often used in combination with a benzodiazepine, such as diazepam, to induce anesthesia. Ketamine is a dissociative anesthetic causing profound amnesia and analgesia. With large doses, hallucinations, agitation and seizures occur. Anesthetic doses of ketamine increase cerebral blood flow and may increase cerebral oxygen demand by increasing the metabolic rate. It is believed the cardiovascular effects of ketamine, namely increased heart rate and blood pressure, occur due to centrally located adrenergic receptor stimulation secondary to the release of epinephrine and norepinephrine.^[76] Ketamine has a negative inotropic effect if there is downregulation of the sympathetic response and may be arrhythmogenic in some patients.^[77] Ketamine may also produce an apneustic respiratory pattern, increases heart rate and blood pressure, thus increasing myocardial workload and oxygen demand.^{[69, 71,}

^{74]} These effects are likely related to the catecholamine release and the related increase in sympathetic outflow.^[76]

Inhalant anesthetic agents may be used for induction and maintenance of general anesthesia. These volatile gases include the chlorofluorocarbons, halogenated ethers such as isoflurane and sevoflurane, and the halogenated hydrocarbons such as halothane. They depress the central nervous system and decrease sympathetic tone. Halothane depresses the body-temperature regulating centers, often causing hypothermia. All chlorofluorocarbons cause dose dependent respiratory and cardiovascular depression. In the heart, they interfere with calcium movement across the sarcoplasmic reticulum membrane, decreasing release of calcium. They also decrease the sensitivity of the contractile mechanisms to what calcium is available. These actions lead to direct myocardial depression.^[78] This propensity to decrease calcium availability also affects the vascular tree, leading to vasodilation. Progressive vasodilation is documented with isoflurane anesthesia, and often causes hypotension. Although cardiac output is typically maintained, it may decrease in a dose-dependent fashion.^[69, 71, 79]

Macrovascular parameters are traditionally used to assess cardiovascular status during general anesthesia,^[68, 69] yet these variables do not necessarily correlate to changes in microcirculatory flow.^[18] Numerous studies have evaluated the effect of anesthetic agents on the microcirculation.^[22, 80-85] Turek and colleagues present a concise overview of recent studies relating to anesthesia and the microcirculation. The effect of anesthesia on the microcirculation varies depending upon the species, the anesthetic, the fluid status of the patient and the vascular bed being imaged.^[22]

On a macrovascular level, ketamine leads to vasoconstriction, increasing systemic vascular resistance.^[68, 69] On a microvascular level, the effect of ketamine on the microcirculation has been assessed in a rat model of endotoxemia.^[80] In this study, rats were anesthetized using pentobarbital anesthesia and a laparotomy performed. Intravital microscopy was used to image the intestinal microcirculation. Intravital microscopy was performed and functional capillary density was obtained. This study found no statistically significant alterations in the intestinal microcirculation associated with the administration of ketamine in pentobarbital anesthetized rats.^[80]

Rat skeletal microcirculation and its response to varying doses of fentanyl was evaluated by Brookes, et al.^[81] In this study, a dorsal skinfold microcirculatory window was implanted for imaging of the microcirculation. Each animal was exposed to saline, low-dose and high-dose fentanyl at separate anesthetic events. Although the mean arterial pressure decreased, intravenous fentanyl resulted in significant arteriolar constriction of the imaged microvascular beds at both low and high doses.^[81] It is proposed fentanyl may act on different μ receptor subtypes in the imaged vascular beds to cause vasoconstriction. The investigators note that fentanyl causes vasoconstriction in some microvascular beds, such as the renal and mesenteric vasculature, while producing vasodilation in the hindquarters.^[81] With such a different response in varied capillary beds, it is possible the vasoconstriction and vasodilation occurs in such a manner that SVR remains unchanged. In addition, it was also demonstrated that morphine acts on peripheral μ opioid receptors in the endothelium to produce vasodilation.^[81]

The effect of general anesthesia on human skin microcirculation has been investigated using laser Doppler flowmetry.^[82] This study found that general anesthesia with propofol, fentanyl and midazolam produced a reduction in systolic, diastolic and mean blood pressure and an increase in basal skin perfusion. Heart rate and skin temperature were unchanged by the anesthetic protocol.^[82] Although it can provide an assessment of the microcirculatory flow in tissues, laser Doppler flowmetry does not allow assessment of individual vessels and vessel density in the target tissue.^[86]

Propofol's effect on microcirculation has been investigated in both human skin^[83] and rabbit pial microvessels.^[84] In humans, OPS imaging of the sublingual microcirculation was used to assess changes related to intravenous administration of propofol. This study found a significant decrease in the total vessel density, as well as a decrease in the proportion of perfused small vessels.^[83] A slightly different effect was noted when propofol was topically applied to the pial vasculature in healthy rabbits using a closed cranial window technique. This study showed no change in pial arterioles at topical propofol concentrations of 10^{-8} to 10^{-5} . However, at propofol application of 10^{-4} , a slight dilation was detected in large and small arterioles and small and large venules.^[84] Application of the carrier solution without propofol did not have any effect on the microvasculature.^[84] Thus it appears that although propofol is a potent systemic vasodilator, it has minimal vasodilatory effects on the pial microvasculature.^[84]

Long-term inhalant anesthesia with isoflurane was shown to have minimal cardiovascular effects in mice, with macrovascular variables such as heart rate and mean arterial pressure remaining stable over prolonged periods. Despite published evidence

revealing a lack of correlation between macrovascular and microvascular parameters,^[6, 18, 21, 29, 36-39] this anesthetic is put forward as an acceptable general anesthetic for intravital microscopy in mice.^[85]

1.4 Changes in microcirculation induced by surgical tissue manipulation

Initially, when tissues are injured, either through trauma or surgical interventions, there is a short period of hemorrhage followed very quickly by local vasoconstriction that lasts five to ten minutes. After the initial vasoconstriction and initiation of coagulation, moderate vasodilation and increased vessel permeability may develop. This local vasoconstriction and subsequent vasodilation is mediated by the release of catecholamines, histamine, serotonin, and bradykinin from the damaged tissue, platelets and activation of the sympathetic nervous system.^[87]

Although it is possible to monitor and regulate macrovascular parameters in anesthetized patients undergoing surgical procedures, these parameters do not necessarily correlate with microvascular perfusion.^[18] Recently, studies focusing on the microcirculation in surgical patients have been performed. In one study, Jhanji and colleagues demonstrated alterations (decreased PPV and MFI) in sublingual microcirculatory flow of patients undergoing major abdominal surgery were associated with postoperative complications, regardless of the macrovascular stability of the patient.^[88] The advent of OPS and SDF technologies provide us with the ability to assess sublingual microcirculation in surgical patients. As knowledge in this field expands, it has been discovered there can be significant derangement in microcirculatory function in surgical patients.^[89] For instance, patients undergoing cardiopulmonary bypass often experience decreased sublingual MFI while undergoing bypass surgery. Once returned to the intensive care unit, patient's sublingual MFI returned to presurgical levels.^[89] Non-

cardiac surgeries, such as those involving increased intraabdominal pressure, aortic clamping or vasoactive medications negatively impact microvascular perfusion.^[90]

In a recent study, laser-Doppler flowmetry and photospectrometry were used to assess changes in microcirculatory flow related to surgical repair of intracranial aneurysms. A significant change in microcirculatory flow was detected in 33 – 46 % of patients undergoing this procedure. A severe decrease (>50% change from baseline) in all microvascular parameters studied, or a solitary change in capillary venous blood flow, correlated with reduced cerebral perfusion and worse post-operative neurological deficits.^[91]

Laser Doppler has also been used to assess changes in digital microcirculation after foot and ankle surgery.^[92] Microvascular flow was measured in the first digit of the foot with the toe at the level of the heart and then in a dependent position before and after the surgical procedure. There was a significant decrease in the foot's ability to tolerate postural changes in capillary pressure and blood flow in the 96 hours after surgery, indicating the foot's normal vasoconstrictive response to increased capillary pressure is impaired following surgery.^[92]

Perhaps the most extensively studied surgery relating to altered hemodynamics is the cardiac bypass procedure. A recent study used angiography and cardiac magnetic resonance to attempt and determine the extent of damaged cardiac tissue compared to myocardial tissue that may be salvaged. This imaging technique allowed for better identification of compromised regions and improved outcomes.^[93] Other studies approach the microcirculation using SDF technology. One study analyzed pulsatile versus non-

pulsatile perfusion during the cardiac bypass procedure. Using SDF, the investigators determined there was no difference in microvascular parameters between the two groups.^[94] Yuruk and colleagues demonstrated that perioperative red blood cell transfusion increases microcirculatory parameters in the sublingual capillary beds of humans undergoing cardiac bypass. Concurrent spectrophotometry of the same tissues showed an increase in microcirculatory hemoglobin and oxygen saturation.^[95] Finally, Boerma et al used SDF imaging to evaluate rectal microcirculatory alterations in patients recovering from cardiopulmonary bypass procedures. The microcirculatory flow index was comparable to previous studies, but the proportion of perfused vessels was decreased compared to previously reported normal values. The absence of a significant arterial to rectal CO₂ gradient led to the conclusion that the alterations in perfusion did not lead to rectal mucosal hypoxia in these patients.^[96]

Many surgical situations, particularly cardiac bypass, aortic clamping and those increasing abdominal pressure, decrease microvascular perfusion parameters.^[88-97] These studies seem to show that the microcirculation is altered even when macrovascular parameters are normal^[88] or the patient is awake.^[92] Given this evidence in human medicine, it is our hypothesis that surgical tissue manipulation, such as that occurring during routine ovariectomy, will result in a decrease in microvascular perfusion parameters in cats.

Figure 1.1: A microcirculatory unit

Figure 1.2: A microcirculatory unit

Figure 1.3: The cyclooxygenase pathway

Figure 1.4: The renin-angiotensin-aldosterone system

Critical Care

Figure 1.5: The orthogonal polarization spectral imaging microscope

Figure 1.6: The sidestream dark field microscope

Figure 1.7: Calculating total vascular density (TVD) for a sidestream dark field microscopy (SDF) image

Figure 1.8: Calculating microcirculatory flow index (MFI) for a sidestream dark field microscopy (SDF) image

Vasoconstriction	Vasodilation
Thromboxane A ₂	Prostacyclin
Endothelins	Endothelium-derived hyperpolarizing factor
Endothelium-derived constricting factor 1	Nitric oxide
Endothelium-derived constricting factor 2	Histamine
Hypothermia	Carbon dioxide
Vasopressin	Tissue hypoxia
Angiotensin II	Acidosis
Epinephrine/Norepinephrine	Elevated tissue K ⁺ , lactate, adenosine diphosphate (ADP), adenosine
Hyperoxia	Decreased tissue adenosine triphosphate (ATP)
Alkalosis	Elevated temperature
	Kinins

Table 1.1: Mediators of vasoregulation

CHAPTER 2

THE EXPERIMENT

2.1 Introduction

The microcirculation, encompassing vessels less than 100 microns, comprises the largest surface area of the vasculature and is instrumental in providing adequate nutrients and oxygen to the tissues.^[1-3] It has been demonstrated that maintenance of functional capillary density (FCD), a reflection of microcirculatory flow and tissue perfusion, is important for tissue survival.^[47] While there is some measure of systemic regulation of capillary flow, much of microcirculation is controlled locally through production of substances such as nitric oxide, bradykinin, histamine, carbon dioxide, endothelin, and thromboxane A₂.^[1, 2] Given the significant impact of local regulation, upstream macrovascular parameters such as heart rate (HR), mean arterial blood pressure (MAP) and cardiac output (CO) and downstream macrovascular parameters, such as lactate, central venous oxygen saturation (S_vO_2), base excess and pH may not accurately reflect microvascular perfusion.^[18, 29, 36-39]

Specialized microvascular imaging techniques, including sidestream dark field microscopy (SDF), have been used in rodents, humans and dogs to establish normal microperfusion parameters.^[20, 26, 83] The same techniques have been used

to investigate changes induced by many clinical situations, such as shock, sepsis and tumor angiogenesis.^[24-28, 43, 44, 88, 89] The SDF microscope uses LED lights around a light guide. The LEDs provide 530 nm wavelength light that penetrates the tissue and scatters. Light that scatters into the light guide is focused via lenses onto a CCD chip. This information is then translated into a video image for recording and later analysis. Consensus criteria have been established for imaging and quantifying the microcirculation using this technology in human patients,^[58] including determination of total vessel density (TVD), proportion of perfused vessels (PPV), perfused vessel density (PVD) and microvascular flow index (MFI). The TVD reflects the potential vasculature available for perfusion. The PPV reflects the percentage of visible capillaries that have continuous flow. The PVD reflects the density of vessels actually receiving continuous blood flow and is equivalent to the functional capillary density (FCD) reported in studies using orthogonal polarization spectral imaging. The MFI is a reflection of the heterogeneity of blood flow in the imaged capillary bed.^[47, 58]

Direct imaging of the microcirculation with SDF has not previously been explored in cats. We sought to determine an acceptable technique and establish baseline clinical values for microcirculatory parameters in healthy, anesthetized cats using this technology, determine whether surgical manipulation alters these values during ovariectomy and determine whether changes in microcirculatory values correlate to changes in macrocirculatory parameters.

Our hypotheses are that surgical manipulation will result in significant reduction in microvascular parameters and the measured microvascular parameters will not correlate with changes in macrovascular parameters, such as arterial blood pressure and heart rate.

2.2 Materials and Methods

All procedures were performed with approval from the Ohio State University Hospital Executive Committee. Eighteen healthy cats presenting for elective ovariohysterectomy were enrolled in the study. The patients presented to the Veterinary Medical Center one day prior to surgery and were examined for clinical evidence of disease. Their weight, packed cell volume and total plasma protein were determined. All cats were fasted overnight and allowed free access to water.

Anesthesia and Instrumentation:

Each cat was given acepromazine^A (0.05 mg/kg), ketamine^B (5 mg/kg) and hydromorphone^C (0.1 mg/kg) intramuscularly for premedication. The cats were instrumented with an intravenous catheter (20 to 24 gauge) in a cephalic vein, general anesthesia was induced with propofol^D to effect (avg: 4.9 mg/kg; range: 2.5 to 8.2 mg/kg). The cats were orotracheally intubated and maintained on isoflurane gas^E administered in 100% oxygen via non-rebreathing system in fifteen cats and circle rebreathing system in three cats. Lactated Ringer's solution^F (LRS) was administered IV to all cats through the duration of the procedure with a calculated rate of 5 mL/kg/hr. Continuous monitoring of heart rate, end-tidal carbon dioxide concentration (EtCO₂), end-tidal inhalant anesthetic concentration, pulse oximetry (SpO₂), and electrocardiography was performed using either the LifeWindow^G and PetMap^H monitors (14 cats) or the Cardell^I and Poet^J monitors (4 cats).

Experimental design:

All cats were positioned in dorsal recumbency in the anesthesia preparation room. Their ventral abdomens were clipped and aseptically prepared for elective ovariohysterectomy. The cats were then transported to the operating room, placed in dorsal recumbency and the cats were instrumented. A routine ovariohysterectomy was performed by third or fourth year veterinary students under direct supervision of the surgical faculty and residents using approved surgical protocol.

Data was collected at three designated time points during the procedure. Preoperative data was obtained after instrumentation and prior to application of the first towel clamp. Intraoperative data was collected after the first ovarian pedicle was exposed and clamped. Postoperative data was collected after the last skin suture was placed and all towel clamps had been removed.

Measurement of monitored variables

Macrovascular variables measured included heart rate, systolic (SAP), diastolic (DAP), and mean arterial pressure (MAP). Additionally, respiratory rate (RR), end-tidal carbon dioxide concentration ($E_t\text{CO}_2$), end-tidal anesthetic concentration ($E_t\text{Anes}$), temperature and pulse oximetry values were recorded. Any fluids or additional medications administered were documented. Prior to anesthesia, packed cell volume (PCV) and total protein (TP) were obtained for each cat as presence of PCV/TP outside normal reference ranges would serve as criteria for exclusion from the study.

Measurement of microvascular parameters

At each time period, videos of the microcirculation were recorded using the videomicroscope.^K The established technique in dogs involves application of the probe to the mucogingival junction above a canine tooth.^[20] However, pilot studies revealed that the size of the videomicroscope and conformation of the feline mouth preclude this approach. Instead, application to the sublingual mucosa was utilized. Based on previously established consensus criteria, three videos of 20 second duration were obtained from adjacent areas of the sublingual mucosa at each time point. If there was concern for overall video quality at the time of acquisition, additional videos were obtained. The risk of vessel compression was minimized by gentle application of the videomicroscope and subjective assessment of the larger vessels to ensure flow was not being compromised as has been previously recommended.^[58]

Videos were assessed for image resolution, amount of motion and presence of pressure artifact by one study investigator (ESC). Any videos assessed as poor quality based on those parameters were excluded from the final analysis. Acceptable videos were those videos with minimal to no drift, good resolution, appropriate contrast and absence of pressure artifact. Marginal videos were those that had moderate amounts of drift, were slightly out of focus or had poor contrast. Unacceptable videos had a large amount of drift, were completely unfocused or had crush or contact artifacts. Any subjects which did not have at least two videos of acceptable quality at any of the time points were removed

from analysis. In cats with only one marginal quality video at one time point, all three videos for each intervention point were still included for analysis.

A separate investigator (MEG) analyzed all videos using specialized vascular analysis software^L and was blinded as to the study time point at which the videos were obtained. Microcirculatory parameters were established using consensus criteria as previously described.^[58] The TVD was derived by superimposing a grid of three equidistant horizontal and three vertical lines over the image and then dividing the number of vessels crossing these lines by the length of those lines.^[58] Flow categories were assigned to each vessel (absent (0), intermittent (1), sluggish (2), normal (3) or hyperdynamic (4) flow). The PPV was calculated as $[100 \times (\text{total number of vessels} - [\text{number of vessels with no flow} + \text{number of vessels with intermittent flow}]) / \text{total number of vessels}]$. The PVD was calculated by multiplying TVD by PPV.

The MFI was obtained by dividing the visual field into quadrants. Each quadrant was then assigned a flow index value based on the average vessel flow in that section. The value from each quadrant was then averaged to determine the MFI.^[36] All videos of acceptable quality were included in the analysis. An average value for TVD, PPV, PVD and MFI was determined at each time point using accepted videos.

Statistical analysis

The data for each variable at each time point were analyzed for normality using the Kolmogorov-Smirnov test. Although data from three of the 39 variable

and time point combinations were not distributed normally, we reported all data as mean $\pm$ standard deviation for consistency. The non-normally distributed data occurred for TVD at induction, PPV at induction, and MFI at pedicle ligation.

A linear mixed effect model was used to perform univariate analysis of association between the intervention point and microvascular parameters (TVD, PPV, PVD and MFI). Univariate analysis of the association between microvascular parameters and other measured variables was also performed. Finally, a covariate analysis of microvascular parameters that had reached significance was performed between that variable, the intervention point and the other measured variable to control for the surgical event. For all tests, $p < 0.05$ was considered statistically significant.

2.3: Results

Eighteen cats were initially enrolled in the study. The median age was 0.5 years [2.5 months – 2.5 years] with a median weight of 2.62 kilograms [1.2 – 4.0]. Videos from all eighteen cats initially enrolled in the study were assessed for quality and stability of the image. Twelve cats were included in the final video analysis; six had to be excluded owing to video quality.

All values for the monitored variables (Table 2.1) and microvascular parameters (Table 2.2) are presented. Over time, the end-tidal carbon dioxide concentration, MAP, SAP, DAP, HR and RR increased. The end-tidal anesthetic concentration decreased and the temperature and pulse oximetry did not vary. These changes were not independently evaluated for statistical significance.

Univariate linear regression was performed to identify significant changes in microvascular parameters relative to surgical intervention points. There was not significant variation in TVD, PPV or PVD. There was a significant relationship between MFI and surgical intervention point (Table 2.3, Figure 2.1).

Univariate analysis of microvascular variables in relation to the monitored variables was performed. TVD, PPV and PVD did not vary with surgical manipulation or changes in the monitored variables. The MFI had an independent positive correlation with MAP, heart rate, respiratory rate and SAP. There was an independent negative correlation between MFI and temperature (Table 2.4).

However, when controlled for surgical event in multivariate analysis, these values fail to reach significance.

The relationship between TVD, PPV, PVD, MFI and duration of anesthesia was analyzed using Pearson correlation. None of the parameters evaluated varied significantly in relation to time of anesthesia.

2.4: Discussion

Sidestream dark field microscopy was used to successfully assess the microcirculation in healthy, anesthetized cats allowing for establishment of the technique and determination of baseline clinical values for microvascular perfusion parameters. In our population of cats, the TVD and PVD were higher and the PPV and MFI were lower than values reported in dogs or humans (Table 2.5).^[20, 26, 83]

Based upon these values, it appears cats have higher vessel densities but a lower proportion of perfused vessels and more heterogenous flow than either dogs or humans. Previous studies in cats using intravital microscopy do not indicate any differences in their microcirculation compared to humans or dogs.^[60-67] The lower MFI in cats indicates more heterogeneity of flow compared to that normally seen in dogs and humans. This could be due to either a true difference in microcirculatory regulation between the species, or it could be related to anesthetic protocols, investigator differences related to imaging techniques or video analysis.

Based on previously published human data showing that microvascular parameters decreased in patients undergoing abdominal, foot and cardiac bypass procedures,^[88-96] we expected surgical tissue manipulation would lead to a decrease in microvascular perfusion parameters. However, we did not find significant changes in TVD, PPV or PVD and actually found an increase in MFI, thereby failing to prove our hypothesis. In an effort to explain this finding, it is

possible the vasodilatory effects of general anesthesia, or blunting of the sympathetic response counteracted any potential systemic vasoconstrictive effect of surgical tissue manipulation. Another possibility is that systemic stimuli from the surgery, such as increased sympathetic tone, was unable to overcome local autoregulation of tissue blood flow.^[1-3, 12] Or, the type surgery performed (routine ovariectomy) did not trigger enough of a systemic response to affect the microcirculation. Regardless of the cause, these findings suggest that SDF could potentially be used to detect microcirculatory changes associated with various interventions (e.g. fluid resuscitation) in surgical patients with minimal impact from the surgical intervention itself.

The only significant change in microcirculatory parameters in relation to intervention time point was an increase in the MFI between the induction time point and surgical manipulation as well as an increase in MFI between the induction time point and the end of surgical intervention. This indicates an increase in the homogeneity of flow through the microvasculature. An increase in MFI between the induction time point and subsequent time points indicates an increase in capillaries that are experiencing continuous blood flow. This may indicate more vasodilation in the capillary bed leading to increased homogeneity of flow. Possible reasons for this could be vasodilation related to the inhalant anesthetic agent, alterations in sympathetic tone, or systemic release of vasodilators such as histamine, bradykinin and nitric oxide from the surgically manipulated tissue. Increasing vasodilation would lead to more consistent flow

through individual capillaries, though if this were the case one might expect to see an increase in the other microvascular parameters.

Systemically, changes in temperature, pH, cardiac output and blood pressure can lead to activation of the sympathetic nervous system, the renin-angiotensin-aldosterone system and release of anti-diuretic hormone as mechanisms to maintain perfusion on the macrocirculatory level.^[1-3, 6, 12-14] Based on this information, and previously reported human and canine information,^[18, 21, 29, 36-39] we hypothesized there would not be a correlation between macrovascular and microvascular parameters. To assess this, selected macrocirculatory parameters were monitored to determine if there was a correlation between changes in microcirculation and any changes on the macrocirculatory level.

Although there was a significant correlation between MFI and temperature, heart rate, respiratory rate and systolic blood pressure individually, once controlled for the intervention point, none of these correlations reached significance. This indicates that although the monitored variables changed alongside the microvascular parameters, the only statistically significant influence was that of surgical intervention, not the relationship between the macrovascular and microvascular parameters. This failure to reach significance substantiates findings in other species that macrovascular parameters cannot be used to predict changes in microcirculatory flow.

These findings support the notion that macrovascular parameters provide an indication of systemic cardiovascular regulation but they do not necessarily

reflect what is occurring on the microcirculatory level.^[27] The upstream parameters, namely heart rate, respiratory rate, cardiac output and blood pressure, provide us information about delivery of blood to the precapillary sphincter, but may not correlate to the amount of flow reaching the capillary beds.^[17, 18, 27] Downstream parameters, such as lactate, central venous oxygen saturation (S_vO_2), base excess and pH, are not predictive of microcirculatory perfusion, particularly in septic patients, as evidenced by a number of previously published studies.^[36-42]

Autoregulation of microvascular perfusion provides a great deal of autonomy to individual capillary beds.^[1-3, 12] Specific capillary beds, such as those in the brain and kidneys, have additional regulatory mechanisms, such as the myogenic reflex and tubuloglomerular feedback.^[1-3, 12] These mechanisms are likely responsible for the lack of correlation between macrovascular and microvascular parameters, and may explain why we did not find a correlation in our patients. Additionally, due to these autoregulatory mechanisms, different capillary beds may have markedly different blood flow in the same macrovascular environment. Despite this potential variation, studies in humans demonstrate statistically significant correlation between microcirculatory parameters in the sublingual capillary bed and perfusion in the gastric mucosa.^[40, 41]

The cats in our study were under general anesthesia, which is known to cause numerous changes in perfusion.^[22, 68-71, 74, 79-85] The cats were premedicated using hydromorphone, acepromazine and ketamine. Hydromorphone is a pure μ agonist opioid medication. Activation of the μ receptors can increase vagal tone,

leading to bradycardia. Response to an adrenergic stimulation may be blunted, leading to peripheral vasodilation and baroreceptor inhibition.^[68, 69, 71] Acepromazine is a phenothiazine sedative, and may cause a dose-dependent α_1 adrenergic blockade, causing a peripheral vasodilation and reflex tachycardia.^[68, 69, 71] Ketamine is an NMDA-receptor antagonist that may increase cerebral blood flow and oxygen demand by increasing the metabolic rate. These cardiovascular effects occur due to centrally located adrenergic receptor stimulation secondary to the release of epinephrine and norepinephrine.^[76] Ketamine has a negative inotropic effect in patients with decreased sympathetic tone and may be arrhythmogenic in some patients,^[77] and may also produce an apneustic respiratory pattern, increased heart rate and blood pressure, and increased myocardial workload and oxygen demand.^[69, 71, 74] These effects are likely related to the catecholamine release and the related increase in sympathetic outflow.^[76]

General anesthesia was induced using propofol and maintained with isoflurane. Propofol has a dose-dependent hemodynamic depressive effect. It decreases myocardial contractility by reducing calcium availability in the myocardium. Propofol also has a direct effect on vascular tone through NO release and decreases baroreceptor sensitivity.^[68, 69, 71, 75] Although propofol causes a profound vasodilation, it is rapidly metabolized by the liver and cleared. As such, it would not have been present at the time of video acquisition. Although the direct effects may not have continued through video acquisition, blunting of the sympathetic response by other medications, such as the hydromorphone, may

have negatively impacted the cats ability to recover from the vasodilation associated with propofol administration.

Finally, isoflurane causes dose dependent respiratory and cardiovascular depression. Progressive vasodilation can lead to hypotension, but cardiac output is typically maintained with isoflurane anesthesia, although CO may decrease in a dose-dependent fashion.^[69, 71, 79] Hodgson and colleagues found that in cats anesthetized with 2.0 times minimum alveolar concentration (MAC), arterial blood pressure and total peripheral resistance were reduced although cardiac index was maintained.^[90] The MAC at which autonomic response is blocked by inhalant anesthetics (MAC-BAR) is lowered by the administration of opioid medications. In cats anesthetized using isoflurane, 0.5 times MAC caused significant depression of the baroreceptor reflex.^[98]

It is possible that depression of the autonomic response by the isoflurane anesthesia counteracted any potential vasoconstriction related to surgical stimulation, leading to the increased in MFI seen in the study cats. The cats were maintained in the hospital overnight prior to the surgery. It is also possible the cats were subclinically dehydrated prior to anesthesia and rehydration lead to the increase in homogeneity of microvascular flow between the induction and pedicle ligation time points. Another possibility is that the increase in MFI at the surgical intervention time points reflects a species variation in response to surgical tissue manipulation between cats and humans.

There are some limitations to this study. For a number of reasons it is not practical or possible to utilize the SDF in non-anesthetized cats. Based on the size of the probe compared to the size of a typical cat mouth and the impact of motion artifact on video quality, the ability to obtain quality videos is a challenge, even in dogs,^[21, 30] and is operator dependent. Any type of pigmentation, too much or little saliva or the presence of hemorrhage or foreign material, such as vomitus, in the mouth is detrimental to video quality. The SDF probe is too large to place on the gingival mucosa over the canine tooth, as reported in dogs.^[20] It is more feasible to place the SDF probe on the sublingual mucosa to obtain an image without significant artifact, a technique previously described in humans.^[23-26, 28, 56, 57]

Studies using sublingual SDF imaging of the microcirculation in surgical patients have shown correlation between the microcirculatory parameters and outcome. Jhanji and colleagues used sublingual SDF to show that decreased PPV and MFI in patients undergoing major abdominal surgery were associated with postoperative complications, regardless of the apparent macrovascular stability of the patient.^[88] It has also been demonstrated that non-cardiac surgeries, such as those involving increased intraabdominal pressure, aortic clamping or vasoactive medications, negatively impact sublingual microvascular perfusion.^[90] These studies seem to show that the microcirculation is altered even when macrovascular parameters are normal^[88] or the patient is awake.^[92] The results of

these studies in humans indicates sublingual SDF imaging could be used as a marker for systemic changes in our patients.

In order to obtain videos acceptable for analysis, the probe must be in constant steady contact with the tissue, as even slight movement could result in significant motion artifact. Application of too much pressure can collapse capillaries, causing underestimation of microvascular parameters. There is effort to control for this by only obtaining video when there was adequate flow in large venules (as these would likely be the first to collapse with excessive pressure).^[58] If this is noted, the pressure applied to the tissue should be reduced to provide a more accurate image of the microvasculature.^[58] Despite careful video acquisition, six of the eighteen cats were removed from the study due to poor video quality. Some cats were very difficult to image and multiple attempts to obtain high quality images failed to procure acceptable videos. Other cats appeared to have adequate videos at the time of capture yet on later analysis enough videos were deemed unacceptable to require removal from the study population.

Another limitation of this study is the need to store the videos for offline analysis at a later time. This analysis is time-consuming, taking an average of one to two hours per video for this study, and includes some degree of subjectivity on the part of the observer. This subjectivity is controlled for by blinding the observer as to study time and/or group of the analyzed videos until all analysis is complete, and by performing intraobserver variability analysis.^[58] Despite this

limitation, there is the possibility operators will become adept at bedside subjective interpretation of real-time videos. Should this level of skill and comfort develop, treatment decisions could be made to improve the microcirculation prior to completion of video analysis.

This study is important because it demonstrates that SDF can be successfully utilized to obtain microcirculatory images and analysis in healthy, anesthetized cats. The length of general anesthesia did not significantly affect microcirculatory parameters, meaning future work can be conducted in anesthetized cats with minimal concern about the length of the procedure. There are many potential research applications for this technique in the future. Cats display different macrovascular responses to shock than other species, often presenting with bradycardia. This technology could be used to investigate if microcirculatory differences in shock occur in cats, as has been demonstrated in dogs.^[21] SDF imaging can be used to investigate microcirculatory changes in sepsis to determine if cats respond to therapy similarly to humans. It can also be used to evaluate microcirculatory changes associated with congestive heart failure and diabetes mellitus to determine if there are any similarities to those documented human patients.^[25, 26, 28, 93-96] The information obtained could be used to allow for development of optimal treatment for cats with these disease, as well as serve as model for investigation in human medicine.

Conclusion:

Surgical tissue manipulation did not significantly alter TVD, PPV or PVD. However, MFI significantly increased from induction compared to pedicle ligation and skin closure. Changes in macrovascular parameters did not correlate well to changes in TVD, PPV or PVD, but a univariate association was found with MFI. Once controlled for intervention point, none of the macrovascular parameters maintained significance. Cats appear to have greater microvascular density but more heterogeneous flow than reported in previously published studies in humans and dogs. SDF allowed for real-time imaging of the sublingual microvasculature in cats. This technology has potential as a tool in experimental and clinical monitoring of microcirculatory changes. However, anesthesia is required for microcirculatory imaging in cats and obtaining images with acceptable quality can be challenging.

Figure 2.1: Microcirculatory flow index at each intervention point. The red line represents the mean values.

	Induction	Pedicle ligation	Skin closure
EtCO ₂ mmHg	46 ± 10	43 ± 9	40 ± 11
EtAnes mmHg	1.5 ± 0.5	1.3 ± 0.4	1.4 ± 0.4
MAP mmHg	55 ± 17	83 ± 31	87 ± 17
Temperature °F	95.6 ± 2.6	94.4 ± 2.2	95.3 ± 2.2
SpO ₂ %	98 ± 2	98 ± 2	98 ± 2
HR	112 ± 18	138 ± 28	158 ± 29
RR	10 ± 3	11 ± 7	20 ± 10
SAP mmHg	80 ± 16	110 ± 25	114 ± 20
DAP mmHg	39 ± 19	63 ± 32	64 ± 22

Table 2.1: Monitored variables.

EtCO₂: End-tidal carbon dioxide; **EtAnes:** End-tidal anesthetic concentration; **MAP:** Mean arterial pressure; **SpO₂:** Pulse oximetry; **HR:** Heart rate; **RR:** Respiratory rate; **SAP:** Systolic arterial pressure; **DAP:** Diastolic arterial pressure.

	Induction	Pedicle ligation	Skin closure
TVD mm/mm ²	47.73 ± 8.39	47.95 ± 7.64	47.98 ± 5.61
PPV %	88 ± 6	92 ± 7	92 ± 3
PVD mm/mm ²	43.01 ± 9.00	44.85 ± 7.11	44.50 ± 5.18
MFI	2.33 ± 0.33	2.72 ± 0.27*	2.64 ± 0.27*

Table 2.2: Microvascular parameters.

TVD: Total vessel density; **PPV:** Proportion of perfused vessels; **PVD:** Perfused vessel density; **MFI:** Microcirculatory flow index.

*= Significant difference ($p < 0.05$) from baseline value

	Surgical event	Value	95% CI	p-value
MFI	Induction	2.33	2.14 – 2.53	0.0025*
	Pedicle ligation	2.72	2.56 – 2.87	
	Skin closure	2.64	2.48 – 2.80	
	Induction vs. pedicle ligation	-0.38	-0.59 – -0.17	0.0010*
	Induction vs. skin closure	-0.31	-0.52 – -0.087	0.0083*
	Pedicle ligation vs. skin closure	0.08	-0.14 – 0.29	0.47

Table 2.3: Univariate analysis of MFI v. intervention point.

MFI: Microcirculatory flow index. *= $p < 0.05$

	TVD	PPV	PVD	MFI
EtCO₂	0.24	0.89	0.21	0.38
EtAnes	0.30	0.25	0.78	0.39
MAP	0.46	0.17	0.83	0.014*
Temperature	0.66	0.089	0.42	0.044*
SpO₂	0.45	0.51	0.78	0.52
HR	0.42	0.057	0.23	0.0078*
RR	0.93	0.38	0.86	0.0053*
SAP	0.64	0.16	0.77	0.0083*
DAP	0.24	0.32	0.65	0.054

Table 2.4: Calculated p-values for univariate analysis of microcirculatory parameters versus monitored variables.

TVD: Total vessel density; PPV: Proportion of perfused vessels; PVD: Perfused vessel density; MFI: Microcirculatory flow index; EtCO₂: End-tidal carbon dioxide; EtAnes: End-tidal anesthetic concentration; MAP: Mean arterial pressure; SpO₂: Pulse oximetry; HR: Heart rate; RR: Respiratory rate; SAP: Systolic arterial pressure; DAP: Diastolic arterial pressure. *= $p < 0.05$

Parameter	Humans	Dogs	Cats
TVD (mm/mm ²)	7.4 (0.2)	24 [17-30]	47.73 (8.39)
PPV %	92.7	100 [94-100]	88.19 (5.95)
PVD (mm/mm ²)	6.9 (0.8)	24 [17-30]	43.01 (9.00)
MFI	3.0	3 [2-3]	2.33 (0.33)

Table 2.5: Comparative microvascular parameters. Human and cat values

reported as mean (SD). Dog values reported as median [range]. ^[28,37,57]

TVD: Total vessel density; PPV: Proportion of perfused vessels; PVD: Perfused vessel density; MFI: Microcirculatory flow index.

Bibliography

1. Ganong, W.F., *Dynamics of Blood & Lymph Flow*, in *Review of Medical Physiology*, W.F. Ganong, Editor 2005, Lange-McGraw-Hill: New York. p. 577-596.
2. Guyton, A.C., *Local and Humoral Control of Blood Flow by the Tissues*, in *Textbook of Medical Physiology*, A.C. Guyton, Editor 2011, Elsevier, Inc.: Philadelphia. p. 191-200.
3. Ganong, W.F., *Cardiovascular Regulatory Mechanisms*, in *Review of Medical Physiology*, W.F. Ganong, Editor 2005, Lange-McGraw-Hill: New York. p. 597-610.
4. Nakahata, N., *Thromboxane A₂: physiology/pathophysiology, cellular signal transduction and pharmacology*. *Pharmacology & therapeutics*, 2008. **118**(1): p. 18-35.
5. Brass, L., *Understanding and Evaluating Platelet Function*. *Hematology*, 2010(Am Soc Hematol Educ Program): p. 387-396.
6. Muir, W.W., III, *Trauma: physiology, pathophysiology, and clinical implications*. *Journal of veterinary emergency and critical care*, 2006. **16**(4): p. 253-263.
7. Bryan, N.S., K. Bian, and F. Murad, *Discovery of the nitric oxide signaling pathway and targets for drug development*. *Front Biosci*, 2009. **14**: p. 1-18.
8. Xiao, Z., Z. Zhang, and S.L. Diamond, *Shear stress induction of the endothelial nitric oxide synthase gene is calcium dependent but not calcium activated*. *J Cell Phys*, 1997. **171**: p. 205-211.
9. Choi, J.W., et al., *Iron deficiency anemia increases nitric oxide production in healthy adolescents*. *Annals of hematology*, 2002. **81**(1): p. 1-6.
10. Caughey, G.E., et al., *Roles of Cyclooxygenase (COX)-1 and COX-2 in Prostanoid Production by Human Endothelial Cells: Selective Up-Regulation of Prostacyclin Synthesis by COX-2*. *J Immunol*, 2001. **167**: p. 2831-2838.
11. Camacho, M., et al., *Hypoxia upregulates PGI-synthase and increases PGI release in human vascular cells exposed to inflammatory stimuli*. *Journal of lipid research*, 2011. **52**(4): p. 720-31.
12. Guyton, A.C., *Nervous Regulation of the Circulation and Rapid Control of Arterial Pressure*, in *Textbook of Medical Physiology*, A.C. Guyton, Editor 2011, Elsevier, Inc: Philadelphia. p. 201-211.
13. Guyton, A.C., *Dominant Role of the Kidney in Long-Term Regulation of Arterial Pressure and in Hypertension: The Integrated System for*

- Pressure Control*, in *Textbook of Medical Physiology*, A.C. Guyton, Editor 2011, Elsevier, Inc: Philadelphia. p. 213-228.
14. Guyton, A.C., *Regulation of Extracellular Fluid Osmolarity and Sodium Concentration*, in *Textbook of Medical Physiology*, A.C. Guyton, Editor 2011, Elsevier, Inc: Philadelphia. p. 345-360.
 15. Dinnar, U., *Metabolic and Mechanical Control of the Microcirculation*, in *Interactive Phenomena in the Cardiac System* 1993, Plenum Press: New York. p. 243-254.
 16. Lauten, A., et al., *Effect of mechanical ventilation on microvascular perfusion in critical care patients*. Clin Hemorheol Microcirc, 2010. **45**: p. 1-7.
 17. Wan, Z., et al., *The cerebral microcirculation is protected during experimental hemorrhagic shock*. Critical care medicine, 2010. **38**(3): p. 928-32.
 18. De Backer, D., J.A. Ortiz, and D. Salgado, *Coupling microcirculation to systemic hemodynamics*. Curr Opin Crit Care, 2010. **16**: p. 250-254.
 19. Takao, M., et al., *Transient vasodilatory effects of adrenomedullin on cerebral parynchomal microvessels in cats*. Neurosci Lett, 1999. **268**: p. 147-150.
 20. Silverstein, D.C., A. Pruett-Saratan, 2nd, and K.J. Drobatz, *Measurements of microvascular perfusion in healthy anesthetized dogs using orthogonal polarization spectral imaging*. Journal of veterinary emergency and critical care, 2009. **19**(6): p. 579-87.
 21. Peruski, A.M. and E.S. Cooper, *Assessment of microcirculatory changes by use of sidestream dark field microscopy during hemorrhagic shock in dogs*. Am J Vet Res, 2011. **72**(4): p. 438-445.
 22. Turek, Z., et al., *Anesthesia and the microcirculation*. Seminars in cardiothoracic and vascular anesthesia, 2009. **13**(4): p. 249-58.
 23. Koh, I.H., et al., *Microcirculatory evaluation in sepsis: a difficult task*. Shock, 2010. **34 Suppl 1**: p. 27-33.
 24. De Backer, D., *Microvascular Blood Flow Is Altered in Patients with Sepsis*. Am J Resp Crit Care Med, 2002. **166**(1): p. 98-104.
 25. De Backer, D., et al., *Microvascular alterations in patients with acute severe heart failure and cardiogenic shock*. Am Heart J, 2004. **147**(1): p. 91-99.
 26. Elbers, P.W., et al., *Microvascular hemodynamics in human hypothermic circulatory arrest and selective antegrade cerebral perfusion*. Critical care medicine, 2010. **38**(7): p. 1548-53.
 27. Casserly, B., R. Read, and M.M. Levy, *Hemodynamic monitoring in sepsis*. Critical Care Clinics, 2009. **25**(4): p. 803-823.
 28. De Backer, D. and M.-J. Dubois, *Assessment of the microcirculatory flow in patients in the intensive care unit*. Curr Opin Crit Care, 2001. **7**: p. 200-203.

29. Dubin, A., et al., *Increasing arterial blood pressure with norepinephrine does not improve microcirculatory blood flow: a prospective study*. Critical care, 2009. **13**(3): p. R92.
30. de Laforcade, A.M. and D.C. Silverstein, *Shock*, in *Small Animal Critical Care Medicine*, D.C. Silverstein and K. Hopper, Editors. 2009, Saunders Elsevier: St. Louis. p. 41-45.
31. Yamaguchi, S., T. Yamakawa, and H. Niimi, *Microcirculatory responses to repeated embolism reperfusion in cerebral microvessels of cat a fluorescence videomicroscope study*. Clin Hemorheol Microcirc, 2000. **23**: p. 313-319.
32. Bentzer, P., S. Holbeck, and P.O. Grande, *Endothelin-1 reduces microvascular fluid permeability through secondary release of prostacyclin in cat Skeletal muscle*. Microvascular research, 2002. **63**(1): p. 50-60.
33. Persson, J., U. Ekelund, and P.O. Grande, *Endogenous nitric oxide reduces microvascular permeability and tissue oedema during exercise in cat skeletal muscle*. Journal of vascular research, 2003. **40**(6): p. 538-46.
34. Nagasaka, Y., et al., *Effect of pulmonary blood flow on microvascular pressure profile determined by micropuncture in perfused cat lungs*. J Appl Physiol, 1984. **77**: p. 1834-1839.
35. Toga, H., et al., *Effect of hypoxia on pulmonary blood flow segmental vascular resistance relationship in perfused cat lungs*. J Appl Physiol, 1998. **84**: p. 1003-1010.
36. Hadian, M. and M.R. Pinsky, *Evidence-based review of the use of the pulmonary artery catheter: Impact data and complications*. Critical care, 2006. **10** (Suppl 3): p. S8.
37. Rivers, E., et al., *Early goal-directed therapy in the treatment of severe sepsis and septic shock*. N Eng J Med, 2001. **345**(19): p. 1368-1377.
38. Chawla, L.S., et al., *Lack of equivalence between central and mixed venous oxygen saturation*. Chest, 2004. **126**(6): p. 1891-1896.
39. Varpula, M., et al., *Mixed venous oxygen saturation cannot be predicted by central venous oxygen saturation in septic shock*. Intensive care medicine, 2006. **32**(9): p. 1336-1343.
40. Creteur, J., *Gastric and sublingual capnometry*. Curr Opin Crit Care, 2006. **12**(3): p. 272-277.
41. Creteur, J., et al., *Sublingual capnometry tracks microcirculatory changes in septic patients*. Intensive Care Medicine, 2006. **32**(4): p. 516-523.
42. Kerger, H., et al., *Systemic and subcutaneous microvascular PO2 dissociation during 4h hemorrhagic shock in conscious hamsters*. Am J Physiol Heart Circ Physiol, 1996. **66**(3): p. H827-H836.
43. Makale, M., *Noninvasive Imaging of Blood Vessels*. Methods in Enzymology, 2008. **444**: p. 175-199.
44. MacDonald, I.C. and A.F. Chambers, *Intravital Videomicroscopy in Angiogenesis Research*. Methods in Enzymology, 2008. **444**: p. 201-230.

45. Svensjo, E., *The hamster cheek pouch as a model in microcirculation research*. Eur Resp J Suppl, 1990. **12**: p. 595-600.
46. Lunt, S.J., et al., *Application of intravital microscopy in studies of tumor microcirculation*. J Biomed Opt, 2010. **15**(1:011113): p. 1-14.
47. Kerger, H., et al., *Systemic and microcirculatory effects of autologous whole blood resuscitation in severe hemorrhagic shock*. Am J Physiol Heart Circ Physiol, 1999. **276**(45): p. H2035-H2043.
48. Groner, W., et al., *Orthogonal polarization spectral imaging a new method for study of the microcirculation*. Nat Med, 1999. **5**: p. 1209-1212.
49. Lupi, O., et al., *Orthogonal polarization technique in the assessment of human skin microcirculation*. Int J Dermatol, 2008. **47**(5): p. 425-431.
50. Bracht, H., et al., *Orthogonal polarization spectroscopy to detect mesenteric hypoperfusion*. Intensive Care Med, 2008. **2008**(34): p. 10.
51. Goertz, O., et al., *Orthogonal polarization spectral imaging: a tool for assessing burn depths?* Annals of plastic surgery, 2010. **64**(2): p. 217-21.
52. Lindert, J. and e. al., *OPS imaging of the human microcirculation: a short technical report*. J Vasc Res, 2002. **39**(4): p. 368-372.
53. Boerma, E.C., et al., *Quantifying bedside-derived imaging of microcirculatory abnormalities in septic patients: a prospective validation study*. Critical care, 2005. **9**(6): p. R601-6.
54. Cerny, Z., Z. Turek, and R. Parizkova, *Orthogonal Polarization Spectral Imaging: A Review*. Physiol Res, 2007. **56**: p. 141-147.
55. Goedhart, P., et al., *Sidestream dark field imaging a novel stroboscopic LED ring based imaging modality for clinical assessment of the microcirculation*. Opt Express, 2007. **15**(23): p. 15101-15114.
56. Ince, C., *Sidestream dark field imaging: An improved technique to observe sublingual microcirculation*. Crit Care, 2005. **2005**(Suppl 1): p. 72.
57. De Backer, D., et al., *Monitoring the microcirculation in the critically ill patient: current methods and future approaches*. Intensive care medicine, 2010. **36**(11): p. 1813-25.
58. De Backer, D., et al., *How to evaluate the microcirculation: report of a round table conference*. Critical care, 2007. **11**(5): p. R101.
59. DeBacker D, et al. *How to evaluate the microcirculation: report of a round table conference*. Critical Care, 2007. **11**, R101 DOI: 10.1186/cc6118.
60. Leinonen, M.R., et al., *Quantitative contrast enhanced ultrasonographic analysis of perfusion in the kidneys, liver, pancreas, small intestine, and mesenteric lymph nodes in healthy cats*. Am J Vet Res, 2010. **71**(11): p. 1305-1311.
61. Zhao, F., et al., *Cortical layer-dependent BOLD and CBV responses measured by spin-echo and gradient-echo fMRI: insights into hemodynamic regulation*. NeuroImage, 2006. **30**(4): p. 1149-60.
62. Bolan, P.J., et al., *In vivo micro-MRI of intracortical neurovasculature*. NeuroImage, 2006. **32**(1): p. 62-9.

63. Nilsson, S.F.E., et al., *Ocular Blood Flow and Retinal Metabolism in Abyssinian Cats with Hereditary Retinal Degeneration*. Invest Ophthalmol Vis Sci, 2001. **42**(5): p. 1038-1044.
64. May, C.A. and K. Narfstrom, *Choroidal microcirculation in Abyssinian cats with hereditary rod-cone degeneration*. Experimental eye research, 2008. **86**(3): p. 537-40.
65. Zhang, D., et al., *Correlation of the Cerebral Microvascular Blood Flow with Brain Temperature and Electro-Acupuncture Stimulation*. J Tradit Chin Med, 2010. **30**(4): p. 243-248.
66. Yamaguchi, S., T. Yamakawa, and H. Niimi, *Cerebral microvascular responses to air embolism-reperfusion in the cat using fluorescence videomicroscopy*. Clin Hemorheol, 2003. **28**: p. 59-70.
67. Bertalanffy, H., et al., *A new model for in vivo observation of the feline spinal microcirculation: The closed spinal window*. Neurosurg, 1994. **34**(2): p. 316-322.
68. Muir, W.W., III, *Drugs Used for Preanesthetic Medication*, in *Handbook of Veterinary Anesthesia*, W.W. Muir, III, Editor 2007, Mosby, Inc.: St. Louis. p. 23-50.
69. McKelvey, D., *Small Animal Anesthesia & Analgesia*. 2 ed2000, St. Louis: Mosby.
70. Muir, W.W., III, *Local Anesthetic Drugs and Techniques*, in *Handbook of Veterinary Anesthesia*, W.W. Muir, III, Editor 2007, Mosby: St. Louis. p. 51-71.
71. Plumb, D.C., *Veterinary Drug Handbook*. 3 ed1999, Ames: Iowa State University Press.
72. Trapani, G., et al., *Propofol in anesthesia. Mechanism of action, structure-activity relationships and drug delivery*. Curr Med Chem, 2000. **7**(2): p. 249-271.
73. Fowler, C.J., *Possible involvement of the endocannabinoid system in the actions of three clinically used drugs*. Trends Pharmacol Sci, 2004. **25**(2): p. 59-61.
74. Muir, W.W., III, *Specific Intravenous Anesthetic Drugs*, in *Handbook of Veterinary Anesthesia*, W.W. Muir, III, Editor 2007, Mosby: St. Louis. p. 140-163.
75. Hutchens, M.P., S. Memtsoudis, and N. Sadovnikoff, *Propofol for sedation in neuro-intensive care*. Neurocrit Care, 2006. **4**(1): p. 54-62.
76. Craven, R., *Ketamine*. Anesthesia, 2007. **62**(Suppl 1): p. 48-53.
77. Bovill, J.G., *Intravenous anesthesia for the patient with left ventricular dysfunction*. Seminars in cardiothoracic and vascular anesthesia, 2006. **10**(1): p. 43-48.
78. Steffey, E.P., *Inhalation anesthetics*, in *Veterinary Pharmacology and Therapeutics*, H.R. Adams, Editor 2001, Blackwell Publishing: Ames. p. 184-212.

79. Muir, W.W., III, *Pharmacology of Inhalation Anesthetic Drugs*, in *Handbook of Veterinary Anesthesia*, W.W. Muir, III, Editor 2007, Mosby: St. Louis. p. 177-194.
80. Lehmann, C.H., et al., *Ketamine does not affect intestinal microcirculation in pentobarbital anesthetized rats during experimental endotoxemia*. *Lab Anim*, 2006. **41**: p. 55-62.
81. Brookes, Z.L.S., N.J. Brown, and C.S. Reilly, *The Dose-Dependent Effects of Fentanyl on Rat Skeletal Muscle Microcirculation In Vivo*. *Anesthesia and analgesia*, 2003. **96**: p. 456-462.
82. Landsverk, S.A., et al., *The effects of general anesthesia on human skin microcirculation evaluated by wavelet transform*. *Anesthesia and analgesia*, 2007. **105**(4): p. 1012-9, table of contents.
83. Koch, M., et al., *Effects of propofol on human microcirculation*. *British journal of anaesthesia*, 2008. **101**(4): p. 473-8.
84. Shibuya, K., et al., *The direct effects of propofol on pial microvessels in rabbits*. *J Neurosurg Anesthesiol*, 2009. **21**(1): p. 40-46.
85. Szczesny, G., et al., *Long-term anaesthesia using inhalatory isoflurane in different strains of mice—the haemodynamic effects*. *Lab Anim*, 2003. **38**: p. 64-69.
86. Stefanovska, A., M. Bracic, and H.D. Kvernmo, *Wavelet analysis of oscillations in the peripheral blood circulation measured by laser Doppler technique*. *IEEE Trans Biomed Eng*, 1999. **46**(10): p. 1230-1239.
87. Fossum, T.W., *Surgery of the Integumentary System*, in *Small Animal Surgery*, T.W. Fossum, Editor 2007, Mosby Elsevier: St. Louis. p. 159-160.
88. Jhanji, S., et al., *Microvascular flow and tissue oxygenation after major abdominal surgery: association with post-operative complications*. *Intensive care medicine*, 2009. **35**(4): p. 671-7.
89. Vellinga, N.A.R., C. Ince, and C. Boerma, *Microvascular dysfunction in the surgical patient*. *Curr Opin Crit Care*, 2010. **16**: p. 377-383.
90. Hodgson, D.S., et al., *Cardiopulmonary effects of anesthesia induced and maintained with isoflurane in cats*. *Am J Vet Res*, 1998. **59**(2): p. 182-185.
91. Klein, K.U., et al., *Measurement of Cortical Microcirculation during Intracranial Aneurysm Surgery by Combined Laser-Doppler Flowmetry and Photo-Spectrometry*. *Neurosurg*, 2011.
92. Attar, F., et al., *Perioperative changes in the microcirculation in feet after foot and ankle surgery*. *The Journal of foot and ankle surgery : official publication of the American College of Foot and Ankle Surgeons*, 2007. **46**(4): p. 238-41.
93. Ortiz-Perez, J.T., et al., *Determinants of myocardial salvage during acute myocardial infarction: evaluation with a combined angiographic and CMR myocardial salvage index*. *JACC. Cardiovascular imaging*, 2010. **3**(5): p. 491-500.

94. Elbers, P.W., et al., *Direct observation of the human microcirculation during cardiopulmonary bypass: effects of pulsatile perfusion*. Journal of cardiothoracic and vascular anesthesia, 2011. **25**(2): p. 250-5.
95. Yuruk, K., et al., *Blood transfusions recruit the microcirculation during cardiac surgery*. Transfusion, 2011. **51**(5): p. 961-7.
96. Boerma, C., et al., *Rectal microcirculatory alterations after elective on-pump cardiac surgery*. Minerva Anest, 2011. **77**(0): p. 1-6.
97. den Uil, C.A., et al., *Impaired sublingual microvascular perfusion during surgery with cardiopulmonary bypass: a pilot study*. The Journal of thoracic and cardiovascular surgery, 2008. **136**(1): p. 129-34.
98. Sellgren, J., et al., *The effects of propofol, methohexitone and isoflurane on the baroreceptor reflex in the cat*. Acta Anaesthesiol Scand, 1992. **36**(8): p. 784-790.

Appendix A: List of proprietary materials

^AAcepromazine – Butler Schein Animal Health, Dublin, OH

^BKetamine (Ketaset®) – Fort Dodge Animal Health, Fort Dodge, IA

^CHydromorphone - Baxter Healthcare, Deerfield, IL

^DPropofol – APP Pharmaceuticals, LLC, Schaumburg, IL

^EIsoflurane – VetOne, Meridian, ID

^FLactated Ringer’s Solution – Baxter Healthcare, Deerfield, IL

^GLifewindow 6000 – Life Medical Equipment, Miami, FL

^HpetMap – Ramsey Medical Inc., Tampa, FL

^ICardell Veterinary Monitor 9405 – Midmark Corp., Versailles, OH

^JPoet IQ II Anesthetic Gas Monitor – Criticare Systems Inc., Waukesha, WI

^KMicroscan – MicroVision Medical, Amsterdam, The Netherlands

^LAVA 3.0 MicroScan Analysis Software, MicroVision Medical, Amsterdam, The Netherlands

Appendix B: Glossary of Microvascular Terms

Functional Capillary Density (FCD): A measure of the capillary density in a microvascular image obtained using intravital microscopy or orthogonal polarized spectral imaging. The number of blood vessels that have detectable red blood cell transit at least once every thirty seconds is determined. FCD is then calculated as the number of capillaries with red blood cell transit at least once every thirty seconds divided by the total vessel density then multiplied by one hundred. This value reflects the number of vessels being perfused in the imaged capillary bed.

Microcirculatory flow index (MFI): MFI is obtained by dividing the visual field into quadrants. Each quadrant is then assigned a flow index value (absent (0), intermittent (1), sluggish (2), normal (3) or hyperdynamic (4) flow) based on the average vessel flow in that section. The value from each quadrant is then averaged to determine the MFI for the video. The MFI provides an assessment of the heterogeneity of flow in the imaged capillary bed.

Perfused vessel density (PVD): PVD is calculated by multiplying TVD by PPV. This value reflects the number of vessels being perfused in the imaged capillary bed and is comparable to FCD.

Proportion of perfused vessels (PPV): Flow categories are assigned to each vessel (absent (0), intermittent (1), sluggish (2), normal (3) or hyperdynamic (4) flow). PPV is calculated as $[100 \times (\text{total number of vessels} - [\text{number of vessels with no flow} + \text{number of vessels with intermittent flow}]) / \text{total number of vessels}]$. This reflects the percentage of detected vessels that experience consistent red blood cell flow during the imaging period.

Total vessel density (TVD): TVD is derived by dividing the video into quadrants and is determined based upon the number of vessels crossing these lines divided by the length of those lines. This value is comparable in either orthoganol polarized spectral imaging (OPS) or sidestream dark field microscopy (SDF). The total vessel density provides an indication of the number of capillaries available for perfusion in the imaged capillary bed. It is important to note that because both OPS and SDF technologies require the presence of hemoglobin for detection, only vessels that contain red blood cells at some point in the video have the potential to be detected.