Carbon Dioxide Pneumoperitoneum and Laparoscopic Ovariectomy in the Domestic Rabbit (Oryctolagus cuniculus)

by

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Abstract

Carbon Dioxide Pneumoperitoneum and Laparoscopic Ovariectomy in the Domestic Rabbit (*Oryctolagus cuniculus*)

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Laparoscopic surgeries are increasingly popular in exotic companion mammals, but information about their benefits or disadvantages is limited. The aims of this thesis are: 1) to assess the working space achieved with carbon dioxide pneumoperitoneum in rabbits; 2) to evaluate the cardiorespiratory effects of pneumoperitoneum; and 3) to compare intra-operative and post-operative outcomes between laparoscopic and open ovariectomy in rabbits.

For the first two cross-over studies, six female New Zealand White rabbits were randomly assigned to sequences of intra-abdominal pressures (IAP). Computed tomography (CT) was used to measure working space after insufflation to 4, 8, and 12 mm Hg. From 4 to 8 mmHg, there was a 19% increase in working space, whereas from 8 to 12 mm Hg the increase was only 6.9%. The order of IAPs affected working space.

To assess the effect of pneumoperitoneum on cardiorespiratory parameters, the following outcomes were measured at 0, 4, and 8 mm Hg: blood pressures (ABP); cardiac output, cardiac and stroke volume indices (CI, SVI); heart rate; end-tidal CO₂ (ETCO₂); blood gases (PaCO₂, PaO₂); peak inspiratory pressure (PIP); and peripheral oxygen saturation (SpO₂). Heart rate, SpO₂, and ABP were unaffected by IAP. For PaO₂, an interaction effect was seen between IAP
and pressure sequence. PaCO\textsubscript{2} increased at 8 mm Hg, and ETCO\textsubscript{2} and PIP were greater with each IAP. IAP decreased cardiac output and CI.

Intra-operative and post-operative outcomes between laparoscopic (LapOVE) and open ovariectomy (OVE) were compared in female New Zealand White rabbits randomly allocated to surgical treatments (n=6 per group). Surgical and anaesthetic time were longer and incision length was shorter for LapOVE versus OVE. There were no significant differences in post-operative outcomes between the treatments, including ethograms evaluated by a blinded observer. Surgical complications associated with LapOVE included intestinal perforation, subcutaneous emphysema, and seroma formation. Incisional dehiscence occurred in both groups.

In summary, pneumoperitoneal pressures should be limited to 8 mm Hg in rabbits. When performing ovariectomy in rabbits, laparoscopy may not provide as marked of an advantage over open approaches as in other mammals. However, further evaluation of laparoscopic techniques is warranted for this species.
Dedication

I would like to dedicate this thesis to the numerous clinicians and veterinary technicians that strive daily to provide the best level of veterinary care for exotic companion mammals. We are colleagues working together to expand our knowledge about exotic animal medicine and surgery, and I am proud to be included in this wonderful community of veterinarians. Let us continue aiming for the best techniques to improve the lives of our patients and their dedicated owners every day!
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Firstly, I would like to thank the Ontario Veterinary College (OVC) Pet Trust for their financial support with the research comprising this thesis. Additional financial support was provided by a University of Guelph Undergraduate Work-Study program and the Tasha scholarship which provided funding for this graduate program of study.

I would also like to acknowledge the assistance and expertise provided by John Phillips in using the Materialise software to obtain pneumoperitoneal volumes for Chapter 3, and would like to acknowledge Medtronic Canada for lending the Ligasure™ generator used for laparoscopic surgeries in Chapter 5.

I am equally grateful to Dr. Matthew Leach for his time in discussing behavioral expression in rabbits.

There are many individuals that have contributed over the years to the development of this thesis and providing support for the work that was undertaken. A huge gratitude is owed to Samantha Dobson and Megan Freedman for their assistance with assessing post-operative parameters in rabbits after ovariectomy. Many hours were put into measuring fecal production, food consumption, performing physical exams, and evaluating video footage! Their assistance was paramount in making this study successful.

The staff of the University of Guelph Central Animal Facility was also wonderful in providing animal care and assistance with anesthetic procedures, and I am grateful for their time and attention to detail.

I would like to acknowledge the many interns, residents, and veterinary technicians with whom I have had the pleasure of working over the years of this program. They have been
inspirational and have assisted me while I needed time to delve into research that pulled me away from clinical duties.

My deepest gratitude also goes to my supervisors and graduate committee members who were an integral part of making this thesis possible, from its conception to its completion. Thank you for the advice, words of wisdom, and continued inspiration. It has been a pleasure to learn from you and I hope that I will continue to have the opportunity to work alongside you.

Finally, I would like to express my sincerest love and appreciation for the support provided by my accomplished and dear husband, Dr. Boyko Kabakchiev, my parents, Ursula and Bruno Ursprung, and the rest of my close family. I love you and I could not have finished this without your daily support and words of encouragement!

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<tr>
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<th>Description</th>
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<tbody>
<tr>
<td>AIC</td>
<td>Akaike information criterion</td>
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<tr>
<td>ACZM</td>
<td>American College of Zoological Medicine</td>
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<tr>
<td>ACVS</td>
<td>American College of Veterinary Surgeons</td>
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<tr>
<td>ABP</td>
<td>Arterial blood pressure</td>
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<tr>
<td>BP</td>
<td>Blood pressure</td>
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<tr>
<td>CO₂</td>
<td>Carbon dioxide</td>
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<tr>
<td>CI</td>
<td>Cardiac index</td>
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<tr>
<td>CO</td>
<td>Cardiac output</td>
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<tr>
<td>CT</td>
<td>Computed tomography</td>
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<tr>
<td>CIₙt</td>
<td>Confidence interval</td>
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<tr>
<td>DBP</td>
<td>Diastolic blood pressure</td>
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<td>ETCO₂</td>
<td>End-tidal carbon dioxide</td>
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<td>FGS</td>
<td>Facial grimace scale</td>
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<tr>
<td>HR</td>
<td>Heart rate</td>
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<tr>
<td>IAP</td>
<td>Intra-abdominal pressure</td>
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<tr>
<td>IM</td>
<td>Intramuscular</td>
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<tr>
<td>IV</td>
<td>Intravenous</td>
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<tr>
<td>LapOVE</td>
<td>Laparoscopic ovariectomy</td>
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<tr>
<td>LiDCO</td>
<td>Lithium dilution cardiac output system</td>
</tr>
<tr>
<td>MBP</td>
<td>Mean blood pressure</td>
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<tr>
<td>MIS</td>
<td>Minimally invasive surgery</td>
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<tr>
<td>OR</td>
<td>Odds ratio</td>
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<tr>
<td>OVE</td>
<td>Ovariectomy</td>
</tr>
<tr>
<td>OHE</td>
<td>Ovariohysterectomy</td>
</tr>
<tr>
<td>PaCO₂</td>
<td>Partial pressure of arterial carbon dioxide</td>
</tr>
<tr>
<td>PaO₂</td>
<td>Partial pressure of arterial oxygen</td>
</tr>
<tr>
<td>pCO₂</td>
<td>Partial pressure of carbon dioxide</td>
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<tr>
<td>PIP</td>
<td>Peak inspiratory pressure</td>
</tr>
<tr>
<td>SpO₂</td>
<td>Peripheral oxygen saturation</td>
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<tr>
<td>SD</td>
<td>Standard deviation</td>
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<tr>
<td>SVI</td>
<td>Stroke volume index</td>
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<tr>
<td>SC</td>
<td>Subcutaneous</td>
</tr>
<tr>
<td>SVR</td>
<td>Systemic vascular resistance</td>
</tr>
<tr>
<td>SBP</td>
<td>Systolic blood pressure</td>
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Chapter 1: Introduction

1.1 Laparoscopic Surgery

Minimally-invasive surgical (MIS) techniques involving endoscopic visualization have been rapidly developing since their initial use in human medicine about 30 years ago. MIS is now becoming popular in veterinary medicine as clientele and clinicians seek less invasive approaches to common surgical procedures. The purpose of this thesis is to objectively assess the use of laparoscopic surgery, using laparoscopic ovariectomy as a model, in the domestic rabbit (Oryctolagus cuniculus).

Laparoscopic approaches have been compared to conventional open surgical approaches in humans and companion mammals including canines and felines. The potential benefits include decreased post-operative morbidity and faster recovery. Exotic small mammals, especially rabbits, represent a growing population of companion animals for which owners request an increasing level of veterinary care. MIS is a field that requires further investigation in pet rabbits. Rabbits are known to be extremely sensitive to pain and stress, which may promote potentially life-threatening complications in this species, such as gastrointestinal ileus and anorexia. If it is possible to decrease the post-operative healing time, morbidity, and pain, it will significantly benefit pet rabbits and their owners. However, there are few MIS studies performed in pet rabbits, and none that compare laparoscopic surgery to conventional open surgery in detail.
1.2 Abdominal Insufflation

With minimally-invasive laparoscopic procedures it is important to consider the effects of insufflating the abdomen to cause pneumoperitoneum. Pneumoperitoneum is required with laparoscopy to provide the necessary working space to both visualize internal structures and accommodate endoscopic instruments. However, insufflation increases the intra-abdominal pressure and affects ventilation and cardiovascular parameters in the patient. It is necessary to optimize the intra-abdominal pressure in order to provide good visualization and instrumentation, while minimizing adverse effects. This is dependent on the size of the animal, but also on the pressure of insufflated gas. Artificial pneumoperitoneum alters physiological parameters under anaesthesia and leads to corresponding changes in anaesthetic protocols. Also, the anticipated working space generated at different pressures is important to know as it directly affects the ability to triangulate endoscopic instruments and perform a variety of minimally-invasive procedures. Despite being important considerations for laparoscopic procedures, these have not been formally assessed in rabbits beyond the application for human pediatric surgery. The first part of this investigation into the benefits and drawbacks of laparoscopic surgery in rabbits will aim to look at the features of carbon dioxide (CO₂) pneumoperitoneum in this species (see Chapters 3 and 4).

1.3 Comparison of Ovariectomy Techniques

In this thesis, ovariectomy is performed in rabbits as a model for other abdominal surgeries. Ovariectomy involves the removal of ovaries bilaterally by sealing and transecting the
ovarian pedicle and separating the ovaries from the uterine horns. It is performed for surgical castration in domestic animals including pet rabbits. Since this procedure is common, uncomplicated, and has been described in rabbits previously it was selected as the best surgical procedure for developing and perfecting laparoscopy in a novel species. Ovariectomy is highly recommended by most veterinarians for companion animal species in order to prevent problems associated with hormonally-driven diseases, behavioural problems, and overpopulation. Specifically in rabbits, it is used as a preventative measure for uterine adenocarcinoma and to limit behavioural problems. Therefore, it is a procedure that is frequently performed by clinicians and is relevant to the larger veterinary community. Further details about laparoscopic ovariectomy will be described in Chapter 5.
Chapter 2: Review of Literature

2.1 Pneumoperitoneum

2.1.1 Abdominal Insufflation

Laparoscopic procedures in both veterinary and human medicine are performed after insufflating the abdomen to expand the peritoneal cavity and provide space around abdominal organs. After being anaesthetized, small surgical skin incisions are made to allow for placement of the laparoscopic ports and the abdomen is expanded using carbon dioxide gas.\(^1\) The volume of space provided by this insufflation is called the “working space”.\(^2\) If this space is too large, the associated internal pressure may adversely affect cardiovascular and respiratory function as well as local tissue perfusion; if the working space is too small, it will not allow sufficient visualization and instrument triangulation for laparoscopic surgery. There is therefore a trade-off between the working space and adverse effects of pressure. It is recommended to minimize the intra-abdominal pressure (IAP) required to provide adequate working space.\(^3\) The ideal intra-abdominal pressure to provide sufficient working space has been investigated in feline and porcine models,\(^4,5\) but is currently unknown for rabbits.

The most common gas employed for pneumoperitoneum today is CO\(_2\). Carbon dioxide insufflation can lead to diffusion of CO\(_2\) into the bloodstream, resulting in hypercapnia and acidosis.\(^2,6\) Other gases (ex. helium, argon, air, nitrogen, and nitrous oxide) have been investigated in the past, but overall have been found to be inferior to CO\(_2\). Helium and argon gases are less soluble than CO\(_2\) and risk formation of gas emboli, and nitrous oxide can be flammable when using electrocautery.
Newer techniques, such as abdominal wall lifting, are still under investigation in the veterinary literature as an alternative to abdominal insufflation. In these cases, the abdominal wall is raised and pneumoperitoneum occurs when air enters the abdomen via the laparoscopic ports. The abdominal working space becomes a triangular tent shape rather than a rounded dome; the working space formed by lift laparoscopy has previously been reported as smaller than with traditional insufflation when using an IAP of 12 mm Hg. In contrast, a recent study on rabbit cadavers comparing abdominal wall lifting to CO\textsubscript{2} insufflation at 10 mm Hg found that there was no significant difference in the abdominal heights between the groups, and there was adequate working space to perform laparoscopic-assisted ovariohysterectomy with both techniques. This technique needs to be examined in greater detail for its application in pet animals. Lift laparoscopy may be preferred in patients with cardiovascular or respiratory compromise and conditions that can be exacerbated by hypercapnia, changes in vascular perfusion, and thoracic compression that occur during abdominal insufflation.

### 2.1.2 Intra-abdominal Pressure

The current recommendation for intra-abdominal pressure to be applied during laparoscopic procedures in companion animal practice is 6-10 mm Hg. Pressures of up to 12 mm Hg have been considered safe in dogs, but beyond that several important concerns arise. One concern is that this increased pressure will affect abdominal organ perfusion, venous return and preload, and increase thoracic compression. A systematic review found that pneumoperitoneum causes decreased splanchnic perfusion as IAP increases, and subsequently results in tissue ischemia and oxidative stress with reperfusion. All abdominal organs have the
potential to be affected by ischemia as a result of pneumoperitoneum; for example, CO₂ insufflation to an IAP of 12 mm Hg in rabbits has been shown to cause oxidative stress to ovarian tissue.¹⁴ The use of lower pneumoperitoneal pressures or ischemic pre-conditioning (i.e. transiently elevated IAP between periods of lower IAP) have been advocated to minimize this effect.¹⁵,¹⁶ The effects of pneumoperitoneal pressure on cardiac output, blood pressure, and ventilation are described in greater detail below (Section 2.1.3 and 2.1.4). Decreased thoracic compliance with higher IAP causes a decrease in permissible tidal volume or increase in peak inspiratory pressure. These ventilatory effects are exacerbated by using CO₂ insufflation, since the carbon dioxide diffuses into the bloodstream causing hypercapnia. Increased ventilatory rates are required to limit the acidosis caused by CO₂ and its effect on metabolic processes.

Despite the possible negative consequences associated with elevated intra-abdominal pressures during laparoscopy, pneumoperitoneum is important to allow visualization and surgical manipulation of abdominal structures. If the IAP is too low, it may not be possible to efficiently access the surgical site, triangulate the endoscopic instruments, and avoid iatrogenic trauma to abdominal organs. Furthermore, the abdominal wall tension decreases as IAP decreases; there may be more abdominal compression or shifts in working space during laparoscopic manipulation which can interfere with the procedure being performed.¹⁷ Feline laparoscopic procedures have been performed at IAPs as low as 4-6 mm Hg, with good surgical visualization and tissue handling.¹⁸,¹⁹ These pressures need to be assessed in other companion animal species, including rabbits, since body size, abdominal organ fill, and abdominal wall distensibility will vary.
Ultimately, the intra-abdominal pressure to be used in any individual case depends on several factors. The surgical procedure will determine how much space is needed to visualize and access the organ of interest. The surgeon’s skill level may allow for lower pressures or only short periods of higher IAP. The instrumentation available for laparoscopy will also determine how much pneumoperitoneal space or working space is needed for the procedure. Finally, the patient’s health status may determine whether higher IAPs are contraindicated. If greater working space is required for the surgery, but higher pneumoperitoneal pressures are deleterious for the patient, pre-stretching has been shown to improve working space in pigs. Pre-stretching involves the application of high insufflation pressures initially, followed by lower IAPs for the remainder of the procedure. This will concurrently provide ischemic pre-conditioning and decrease the risk of oxidative stress that occurs with reduced perfusion to abdominal organs.

2.1.3 Effect of Pneumoperitoneum on Cardiovascular Parameters

Due to the growing use of laparoscopy in humans and veterinary patients, much research has been focused on the anaesthetic management of these patients. The induction of pneumoperitoneum can influence hemodynamic parameters in these patients; in particular, it decreases cardiac output and increases systemic vascular resistance (SVR). The decrease in cardiac output occurs as a result of decreased preload and stroke volume with increasing IAP. Even though CO₂ insufflation and resultant hypercapnia may increase heart rate, the decrease in stroke volume is sufficient to affect the cardiac output accordingly.

A change in blood pressure (BP) can also be seen, although the trends vary between studies. In some cases, BP increases with IAP as a result of SVR; in other cases, BP decreases
due to declines in cardiac output.\textsuperscript{6} The hemodynamic effects of IAP will be discussed in more detail in Chapter 4 of this thesis.

In general, at low to moderate IAPs of less than 12 mm Hg in humans and canines and less than 8 mm Hg in felines, the hemodynamic effects are minimal.\textsuperscript{4,12,23} However, prolonged IAP at 8 mm Hg in rabbits has been shown to significantly decrease cardiac index (i.e. the cardiac output per kilogram of body weight).\textsuperscript{22} Therefore, the duration of a procedure needs to be taken into account when determining the cardiovascular consequences of pneumoperitoneum. Similarly, patient positioning (Trendelenburg or reverse Trendelenburg) can affect perfusion and venous return to the heart. The patient’s health status will also determine whether pneumoperitoneum is contraindicated or which intra-abdominal pressures are appropriate to use. For example, patients with anemia, hypovolemia, or under cardiovascular disease will need to be monitored more closely for any adverse effects.

\section*{2.1.3.1 Cardiac Output Measurement in Domestic Animals}

Cardiac output (CO) can be estimated using several different techniques.\textsuperscript{24} The Fick or modified Fick technique provides an estimation of oxygen consumption by tissues as a measure of CO. It can be challenging to obtain these measurements and is, therefore, not commonly used.

The pulmonary artery catheter thermodilution technique assesses a change in blood temperature from the right atrium to the pulmonary artery, in order to assess blood flow through the right ventricle. This technique is very effective, but more invasive than other
methodologies since it requires placement of a pulmonary artery catheter. Transpulmonary thermodilution avoids this concern by using a peripheral arterial catheter instead.

In veterinary patients, lithium dilution (using the LiDCO™ system) is used with peripheral vessels as a less-invasive alternative to pulmonary artery catheter thermodilution. Lithium dilution has been validated in dogs, cats, and horses; however, its use in rabbits has not been compared to other techniques.\textsuperscript{25-30} Lithium chloride is administered via an intravenous catheter. A sensor placed by a peripheral arterial catheter is used to measure lithium concentrations as blood is withdrawn from the catheter. These concentrations measured over time are used to calculate CO. For most techniques, a 20-25\% change in cardiac output is generally considered clinically significant.\textsuperscript{24}

Cardiac output estimates can also be obtained continuously using arterial pressure waveform analyses; however, this technique requires calibration with transpulmonary thermodilution or lithium dilution measurements. It assumes that the waveforms produced by changes in pulse pressure will reflect CO. Echocardiography and echo-Doppler have also been used to measure ventricular blood volumes or flow velocities in order to estimate stroke volume and CO. Finally, recent studies into bioimpedance have assessed the use of this technique for stroke volume estimation in humans. Electrical currents are passed across the thorax, and changes in stroke volume and blood flow result in predictable changes in electrical conductivity. Despite these various additional techniques for cardiac output assessment, the most commonly used in veterinary medicine are still transpulmonary thermodilution and lithium dilution. Lithium dilution will be discussed further in this thesis for the estimation of cardiac output in rabbits at varying intra-abdominal pressures.
2.1.4 Effect of Pneumoperitoneum on Respiratory Parameters

In addition to changes in cardiovascular parameters, the effect of pneumoperitoneum on ventilation should be considered. As previously mentioned, insufflation of the abdomen results in compression of the thoracic volume and subsequent decreases in pulmonary compliance. As lung volume decreases, the peak inspiratory pressure will increase unless tidal volume is lowered to compensate for this change. Laparoscopic surgeries are usually performed in patients that can be intubated and mechanically ventilated; as such, the tidal volume and ventilatory pressure can be controlled by the anaesthetist. Changes in ventilation patterns with thoracic compression can cause ventilation-perfusion mismatch. In severe cases, patients with pulmonary disease may be at risk of pneumothorax if alveolar pressure becomes too high. In these cases, the duration and pressure of pneumoperitoneum should be minimized, and monitoring of blood gas parameters may be necessary.

In addition to the compressive effects on the thorax, CO₂ insufflation also causes increased partial pressure of carbon dioxide (pCO₂) in the blood. Consequently, there is a decrease in blood pH and an increase in end-tidal carbon dioxide (ETCO₂). Increased ventilation (tidal volume and rate) is required to help manage this diffusion of CO₂ into the blood and prevent the development of hypoxemia that occurs with reduced blood pH (Bohr effect). Factors such as patient positioning, duration of pneumoperitoneum, and the use of CO₂ as opposed to other gases for insufflation will influence the ventilatory effects that are observed with pneumoperitoneum. Chapter 4 of this thesis will investigate these effects in more detail for rabbits.
2.1.5 Additional Adverse Effects of Pneumoperitoneum

Additional concerns that occur as a result of pneumoperitoneum include: elevations in intracranial pressure, which may be deleterious in patients with brain lesions or head trauma; increased potential for bacterial translocation from intra-abdominal infections or the gastrointestinal tract; and increased risk of metastases from neoplastic disease.\textsuperscript{21,32-35} However, other studies have shown that bacterial translocation and bacteremia can occur during laparotomy as well, and may be a consequence of surgical manipulation rather than abdominal insufflation.\textsuperscript{36,37} Similarly, metastases after laparotomy may occur with a similar incidence of those seen after pneumoperitoneum and laparoscopy, and incision site metastases are reported in both surgical approaches.\textsuperscript{38-40} It is also interesting to note that humidified and heated CO\textsubscript{2} insufflation can be used to mitigate the spread and survival of human colorectal cancer and gastric cancer cells in the abdomen, which would suggest protective effects in metastasis or carcinomatosis of certain neoplastic diseases.\textsuperscript{41,42} An in vitro study on canine transitional cell carcinoma cell lines found that CO\textsubscript{2} pressures of 10 mm Hg significantly decreased pH and cell viability, although this effect did not persist for more than a couple of days.\textsuperscript{43}

Another important complication of pneumoperitoneum is gas embolism. High incidences of subclinical emboli have been reported in the literature.\textsuperscript{44,45} Fortunately, concerns are less commonly seen with the current practice of using soluble CO\textsubscript{2} for insufflation, as opposed to using helium or nitrous oxide.\textsuperscript{6} Nevertheless, spontaneous fatal CO\textsubscript{2} gas embolism has been reported.\textsuperscript{46-48}
2.2 Laparoscopy in Companion Animals

2.2.1 Laparoscopic Surgeries

Laparoscopic techniques are well-developed in canine and feline patients for both routine procedures, such as organ biopsies\(^4\), ovariectomy/ovariohysterectomy\(^19,50-54\), cryptorchidectomy\(^55\), and cystotomy\(^56\), and more complex surgeries, such as adrenalectomy\(^57,58\) or cholecystectomy\(^59,60\). In 2010, a survey of members of the American College of Veterinary Surgeons found that 86% of small animal surgeons, 99% of large animal surgeons, and 98% of residents had performed minimally-invasive surgeries.\(^61\) Specific surgical techniques have been described in the literature, but the benefits of MIS over traditional open surgical approaches are still not well-characterized in veterinary patients.

Laparoscopic surgery requires specialized equipment and abdominal insufflation.\(^1\) As opposed to open surgical approaches, body wall incisions are small and internal structures are visualized with the aid of an endoscope. Endoscopes exist in various diameters and lengths, depending on the required use and the preference of the surgeon.\(^62\) They can be rigid or flexible, and can have an angled (30°) or a flat (0°) tip to allow for different fields of view and flexibility when looking around the abdomen. Endoscopes are connected to light cables and endoscopic videocameras, and can be linked to a monitor which the surgeon uses to visualize the surgical field (Figure 1). Endoscopes are usually contained within examination or operating sheaths, the latter allowing for adjacent placement of instruments such as forceps, needles, or scissors.

Laparoscopic surgeries are approached by making incisions to allow for placement of cannulae. These cannulae will be the ports through which the endoscope and other required instruments enter the abdomen. Different techniques for cannulation have been described;
Figure 1: Equipment used for a rabbit undergoing laparoscopic surgery. The endoscope, held by the surgeon at the back of this image, is connected to a light cable and video monitor to allow the surgeons to visualize the internal abdominal contents. Between the video monitor and the anaesthetic monitor, a mechanical insufflator delivers CO₂ via insufflation tubing connected to the cannula.
however, the most common approach is the open Hasson or modified Hasson technique. In this approach, the fascia over the linea alba is elevated away from internal organs and a scalpel is used to partially puncture through the fascia and peritoneum. A trocarized cannula is threaded into position, and the trocar is then removed from within the cannula. Carbon dioxide insufflation can be provided via the cannulae, with an insufflation line attached to a luer-lock adapter valve. Additional cannulae are placed with trocars after the abdomen is visualized with the endoscope to ensure that no organs are inadvertently located below entrance points.

Laparoscopic surgery requires specialized training since dexterity and fine motor coordination differs from techniques used in traditional open surgeries. However, studies show that medical and veterinary students and practitioners can become proficient at these techniques with appropriate instruction. Moreover, rabbits are commonly used as training models for human pediatric laparoscopic surgeries.

### 2.2.2 Potential Advantages of Laparoscopic Surgeries

In humans, MIS is associated with decreased post-operative morbidity, decreased incidence of infection and adhesions, and shortened hospitalization stays. A specific challenge seen in veterinary studies is a lack of well-defined post-operative parameters to examine. Commonly used outcome measures include: surgical time; post-operative rates of infection and adhesion; visual analogue pain scales; differences in serum cortisol, glucose, and C-reactive protein levels; and post-operative activity as compared to traditional open approaches. A retrospective assessment of dogs that were ovariectomized found lower wound healing complications with laparoscopy. A separate prospective case series in dogs and cats identified a
lower post-operative infection rate with MIS as compared to open surgeries; however, confounding factors such as patient preparation and length of the procedure may have contributed to this difference, thus a clear benefit could not be confirmed.\textsuperscript{76} Despite earlier findings that adhesion formation in rabbits with laparoscopy versus laparotomy was similar,\textsuperscript{77,78} a more recent surgical model of uterine horn resection confirmed lower adhesion rates with laparoscopy based on clinical scores and histologic findings.\textsuperscript{79} Furthermore, surgical adhesions in rabbits treated with laser adhesiolysis had a better response when performed laparoscopically as compared to those performed with a laparotomy approach.\textsuperscript{80} It has been shown that increased CO\textsubscript{2} flow rates and higher IAP during pneumoperitoneum for laparoscopy contribute to the formation of adhesions; therefore, insufflation needs to be maintained at lower flow rates and pressures whenever possible.\textsuperscript{81}

Pain in animals is generally assessed by examining food and water intake, activity level, physiologic responses, subjective pain scales, ethograms and facial grimace scales (FGS), and analgesiometry to assess response to painful stimuli.\textsuperscript{82,83} Each parameter of pain expression has its advantages and disadvantages. Some assessments, such as pain scales and behaviour ethograms, are subjective and qualitative, and as a result, may be biased in part by the observer and environmental conditions.\textsuperscript{84} Blinding, control of conditions, and validation studies are very important with these assessments; however, in many cases well-formulated validation studies are lacking in the veterinary literature.\textsuperscript{82} Additionally, some drugs that may be used for analgesia can cause sedation, thereby influencing an animal’s behaviour. Consumption, activity levels, and physiologic responses, although quantifiable, may not be the most consistent reflection of pain in animals.\textsuperscript{85} These parameters are frequently influenced by other factors, including stress.
associated with restraint and use of anaesthetic or other drugs; therefore, they are commonly applied in combination with pain scales or behavioural changes. Analgesiometry is also used in the literature to assess responses to painful stimuli; however, it is more a reflection of nociception than perceived pain. As such, it is not usually used in isolation for pain assessment of animals.

In dogs and cats with laparoscopic procedures, several studies have demonstrated decreased post-operative pain scores and physiologic inflammatory and stress markers (i.e. C-reactive protein, glucose, and cortisol). Similarly, decreased surgical stress markers (i.e. cortisol, epinephrine, and TNF-α) were found in rabbits up to 24 hours after laparoscopic ovariohysterectomy as opposed to open surgery. Other studies in dogs have also demonstrated decreased post-operative adhesion formation and increased post-operative activity, likely due to reduced pain, in ovariectomized dogs after laparoscopic surgery as compared to open surgery.

Despite the possible benefits of laparoscopic surgeries shown by some research in dogs and cats, comparisons of surgical time still vary across studies with many noting that laparoscopic procedures may be longer than traditional open procedures. This information is important if increased surgical and anaesthetic time results in increased risk to the patient. Nevertheless, this must be interpreted cautiously because it is possible that the surgeons involved in the study were more experienced with the open technique, and with sufficient practice these surgical times may become more equivalent.
2.2.3 Potential Complications of Laparoscopic Surgeries

In addition to the risks previously mentioned in association with abdominal insufflation, laparoscopic surgeries carry potential for surgical complications that are not seen with laparotomy. Subcutaneous emphysema occurs with a relatively high incidence. In humans undergoing laparoscopic surgery, reported rates of emphysema are approximately 0.43-2.3%, with greater odds in patients with improper cannula placement, higher end-tidal CO₂ levels, and longer surgical times. In another study, 8 out of 100 and 19 out of 100 patients developed subcutaneous emphysema as a result of insufflation to pressures of 10 mm Hg and 12 mm Hg, respectively. Therefore, higher insufflation pressures may contribute to emphysema formation. Subcutaneous emphysema is also commonly reported in veterinary species after a variety of laparoscopic surgical techniques, with reported prevalence of approximately 6-12%. When using CO₂ insufflation, emphysema will resolve spontaneously; however, additional analgesia may be warranted for a patient that develops emphysema intra- or post-operatively.

Trauma and hemorrhage of abdominal organs is reported in companion animals as well as humans. The most common traumatic injury in dogs and cats is splenic laceration occurring during abdominal access. The incidence of splenic laceration ranges between 7-18% in various studies. In some cases, conversion to an open approach may be necessary to manage the bleeding.

2.2.4 Laparoscopy in Rabbits

Laparoscopy has been described in rabbits, although the published reports are limited to a cases descriptions and review articles. For pet rabbits, laparoscopic biopsies, cryptorchid
castration, laparoscopic ovariectomy, and laparoscopic-assisted ovariohysterectomy are described in the literature.

Rabbit anatomy, physiology, and behaviour differ from that of dogs and cats. In general, rabbits are smaller in size, but possess a larger gastrointestinal system and a smaller thoracic volume as previously discussed.\textsuperscript{110} This makes laparoscopic procedures more challenging. There tends to be less available working space in the abdomen, making visualization of structures and the ability to manipulate instruments more difficult. Insufflation of the abdomen with carbon dioxide may also have more adverse effects on respiratory and cardiovascular parameters, including visceral perfusion.

Another important difference between rabbits and other companion animals is that rabbits display pain in a more subtle manner. The expression of pain, discomfort, or stress results in decreased activity, visual pain cues (ex. the facial grimace scale)\textsuperscript{113,114}, depressed appetite, and changes in gastrointestinal motility.\textsuperscript{84} Functional ileus in rabbits, also called gastrointestinal stasis in the literature, can result in severe sequelae if left untreated, including shock or death.\textsuperscript{115} Rabbits will benefit from laparoscopic approaches if they are confirmed to decrease the post-operative healing time, morbidity, and pain as compared to traditional open approaches. Some authors report subjective advantages in rabbit recovery post-operatively, but this has not be formally examined.\textsuperscript{112}

\subsection*{2.2.5 Laparoscopic Ovariectomy}

Laparoscopic ovariectomy procedures are frequently performed in veterinary medicine, because these procedures are highly recommended but elective; therefore, they are usually
performed in otherwise healthy patients. Ovariectomy prior to sexual maturity is an important component of preventative medicine in female rabbits since there is a high prevalence of uterine adenocarcinoma if they are left intact (as high as 80%). Development of uterine adenocarcinoma is promoted by the presence of sex hormones in intact females. If uterine pathology is present at the time of ovariectomy, the disease can continue to develop. Although neoplasia prevalence increases with age, with the majority of cases being found in females over 3 years of age, there has been a report of uterine adenocarcinoma in one 5-month-old rabbit. A large retrospective study on necropsy and biopsy findings identified endometritis and endometrial hyperplasia as early as 6 months of age. Therefore, ovariectomy without hysterectomy is not recommended for rabbits older than 6 months.

Laparoscopic ovariectomy techniques described in rabbits include 2- and 3-port approaches, and a SILS port (for single incision laparoscopic surgery) has been described in performing laparoscopic-assisted ovariohysterectomy. For the 2-portal approach, a subumbilical cannula is placed along midline for the endoscope and a cranial port is placed for grasping forceps. After grasping the ovarian tissue, a transabdominal suspension suture is used to raise the ovary against the body wall. A vessel sealing device is then used to seal and transect the ovarian pedicle. The 3-portal approach is described in more detail in Chapter 5 of this thesis. Previously reported advantages of the 3-portal approach include improved visualization of ovaries under the rabbit intestinal tract, better tissue manipulation with additional instrumentation, and broader application to other surgical procedures in this species.
2.3 Post-operative Evaluation of Animals

As previously mentioned, laparoscopy may benefit rabbits if it can be shown to decrease post-operative pain and morbidity when compared to laparotomy. However, this assessment may be challenging in a species that does not overtly show signs of stress or pain. Prey species commonly express a “conservation-withdrawal” reflex resulting in immobility and a crouched posture when stressed during handling; this can cause difficulty in evaluating pain. In order to evaluate pain in rabbits, several post-operative parameters need to be assessed simultaneously including: food consumption, fecal production, changes in vital parameters, blood glucose and cortisol measurements, facial grimace scale scores, and behavioural ethograms. While some of these parameters are quantitative (ex. food consumption, fecal production, vital parameters, glucose, and cortisol) and allow for objective assessment of an animal, they can be strongly influenced by restraint techniques as well as anaesthetic or analgesic drug use. Therefore, these parameters are frequently combined with FGS and behavioural scores to assess the expression of pain in rabbits.

FGS scores have been validated in rabbits for assessment of pain expression. Several behavioural ethograms have also been suggested and demonstrated to reflect post-operative responses in rabbits. Ethograms assess changes in activity level, posture and locomotion, response to palpation, grooming, and behaviours specifically associated with pain expression (ex. abdominal pressing, flinching, or wincing). FGS scores and ethograms may be affected by observer bias and require blinding techniques. They can also be sensitive to changes in environmental conditions and sedative or analgesic drug use. Careful consideration of study design to prevent confounding effects is important when assessing behavioural responses in
animals. Post-operative evaluation of rabbits, including behavioural ethograms, is described in more detail in Chapter 5.

2.4 Conclusion

The advantages and disadvantages of pneumoperitoneum need to be evaluated for each individual case when electing to pursue MIS. Potential concerns with pneumoperitoneum include depression of visceral perfusion, changes to infection and metastatic rates, the occurrence of gas emboli, and the effects on hemodynamic and ventilation parameters. Nevertheless, it is still the most commonly employed technique to allow for organ visualization and surgical manipulation in the abdomen. It is important to be able to optimize working space while minimizing the deleterious cardiovascular and respiratory effects of high IAP. One of the goals of this thesis is to determine recommendations for clinical use of pneumoperitoneum in rabbits undergoing laparoscopic procedures.

Further research is also needed to examine the value of laparoscopic surgery over traditional open approaches in rabbits, including any differences in post-operative pain and complications. In dogs, cats, and humans, laparoscopic approaches have been associated with decreased pain scores, increased post-operative activity, shortened hospitalization stays, and lower incidences of infection or adhesions. Using ovariectomy as a surgical model, this thesis aims to provide further insight and objectively determine the value of laparoscopy as a surgical technique for pet rabbits in veterinary practice.
Chapter 3: Effects of Intra-Abdominal Pressure on Laparoscopic Working Space in Domestic Rabbits (*Oryctolagus cuniculus*)

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3.1 Introduction

Minimally invasive surgery (MIS) with endoscopic techniques has been gaining popularity in companion animal medicine.\(^{60,61,110,111}\) Laparoscopic approaches for canine and feline patients for routine and complex surgical procedures are well-described.\(^{19,54,55,59,60,125}\) MIS procedures modified for use in some exotic small mammals, particularly domestic rabbits, have also been described.\(^{106,108-110}\) Laboratory rabbits have also been used in research for pediatric laparoscopic surgical techniques medicine.\(^{64,65}\) Despite the widespread use of laparoscopy in rabbits, the human and veterinary medical literature is lacking a detailed assessment of optimal intra-abdominal pressures for use in this species.

When creating a pneumoperitoneum for laparoscopic surgery, it is critical to understand which intra-abdominal pressure (IAP) will provide the greatest increase in working space while
minimizing adverse effects on cardiorespiratory function and local tissue perfusion.\textsuperscript{21.126-128} Working space refers to the volume of abdominal space created by insufflation to allow for visual access and instrument handling during laparoscopic procedures. It is dependent on fixed factors (e.g. patient size, gastrointestinal contents, and presence of organomegaly), as well as factors that can be adjusted (e.g. IAP, patient ventilation, and muscle tone effects of the anesthetic protocol).\textsuperscript{5} These factors need to be taken into account when evaluating the effect of a variable such as IAP on the working space.

Working space has been investigated in pigs and cats\textsuperscript{4,5}, but to the authors’ knowledge, there are currently no recommendations for optimal working space in rabbits. In one study,\textsuperscript{4} working space in cats was assessed by changes in abdominal width, height, and circumference at various IAPs. Significant increases in abdominal circumference were identified with IAP increases from 4 to 8 and from 8 to 15 mm Hg; nevertheless, the authors concluded that an IAP of 15 mm Hg, compared with 8 mm Hg, did not provide a clinically important difference in working space to justify its use, and found that the higher pressure was associated with increased mean arterial blood pressure and partial pressure of arterial CO\textsubscript{2} (PaCO\textsubscript{2}). A study\textsuperscript{5} in pigs used computed tomography (CT) to measure the intra-abdominal volume at pressures of 0, 5, 10, and 15 mm Hg. The CT-measured volume increased by approximately 93\% between pressures of 5 and 10 mm Hg and by approximately 19\% between pressures of 10 and 15 mm Hg; therefore, the increase in working space in pigs was deemed beneficial with an increase in IAP up to, but not beyond, 10 mm Hg.

The objective of this study was to evaluate the effect of changes in IAP (from 4 to 12 mm Hg) created with CO\textsubscript{2} insufflation on the working space in rabbits as assessed by measurement
with abdominal CT. These pressures were selected based on the recommendations for other companion animal species, as well as previous reports about laparoscopic procedures applied in rabbits. On the basis of the previously described studies in cats and pigs, we hypothesized that the working space for laparoscopic procedures would increase with increases in IAP, although not necessarily in a linear manner.

3.2 Materials and Methods

**Animals** – On the basis of sample size calculation ($\alpha = 0.05; \beta = 0.2; \text{Cohen } d = 1.4$), 6 specific-pathogen free female New Zealand White rabbits (*Oryctolagus cuniculus*) obtained from a commercial source were used in the study. The median body weight was 3.39 kg (range, 3.19 to 3.6 kg) and all rabbits were 4 to 5 months of age. The rabbits underwent physical examination prior to general anesthesia and were deemed healthy, with the exception of one rabbit. This rabbit was found to have a heart murmur, which was identified on echocardiographic examination as being caused by tricuspid dysplasia. This was not considered likely to affect abdominal volume with insufflation, and the rabbit was not excluded from the study. The study protocol was reviewed and approved by the Animal Care Committee of the University of Guelph in accordance with guidelines set by the Canadian Council on Animal Care.

**Anesthesia** – Food was withheld from the rabbits for 1.5 to 2.5 hours prior to premedication. Midazolam ($1 \text{ mg/kg}$) and buprenorphine hydrochloride ($0.05 \text{ mg/kg}$) were administered intramuscularly. After 15 minutes, a 22-gauge intravenous catheter was placed in a lateral
auricular vein and crystalloid fluids were administered IV during the anesthetic procedure at 5 mL/kg/h. Propofol (8 to 11 mg/kg) was administered IV to induce anesthesia. The rabbits were intubated with 4-mm uncuffed endotracheal tubes by use of an over-the-endoscope technique and were connected to non-rebreathing Bain circuits delivering isoflurane at concentrations of 2.5% to 3.5% in oxygen to maintain anesthesia. Manual intermittent positive-pressure ventilation was used to keep ETCO₂ concentration < 50 mm Hg, as measured by a microstream capnograph, when the rabbits were not breathing spontaneously. The ETCO₂, oxygen saturation as measured by pulse oximetry, heart rate as measured by an ultrasonic doppler probe, respiratory rate, and rectal temperature were monitored during the anesthesia. Temperature was intermittently measured with a digital thermometer, and warming was provided as needed using a forced-air system.

**Experimental procedure** – A balanced cross-over design was used for the following IAPs: 4, 8, and 12 mm Hg. The pressures were ordered into 6 distinct random sequences with two 3-by-3 Latin squares generated by use of statistical software. The 6 rabbits were randomly assigned to an IAP sequence with the same software.

Each rabbit was placed in dorsal recumbency on the CT table, and a 16-slice CT scan of the abdomen and thorax (slice thickness, 0.625 mm; 120 kVp; 140 mA) was performed to obtain baseline data prior to cannula placement. The pitch was 0.938:1 and rotation time was 1 second. After cannula placement as described in subsequent text, IAPs were created with a mechanical CO₂ insufflator according to the sequence assigned to each rabbit. The CT scans were performed after the assigned pressure was maintained for ≥ 15 minutes. Propofol (1.5 to 3
mg/kg) was administered as needed to slow respiratory rates or induce apnea for the scans. Scans were performed with each rabbit in dorsal, left lateral oblique, and right lateral oblique recumbency with wedges placed to achieve an approximate 45° angle of the ventral midline to the table surface. Cranially and caudally oriented endoscopic intra-abdominal images were recorded at each pressure using a 2.7-mm sheathed rigid endoscope⁹ for subjective visual assessment of laparoscopic working space. After CT scans were performed, the abdomen was purged of CO₂ for 5 minutes prior to insufflating to the next IAP in the assigned sequence. After all scans, the abdomen was purged of CO₂, the cannula was removed, and the linea alba and skin were apposed with 4-0 polydioxanone suture⁹ in simple interrupted and continuous intradermal patterns, respectively.

**Cannula placement** – The ventral portion of the abdomen was routinely clipped of fur and aseptically prepared. Abdominal access was gained with a modified Hasson technique. Briefly, a skin incision was made immediately caudal to the umbilicus with a No. 15 scalpel blade. Subcutaneous fat was bluntly dissected, and stay sutures of 4-0 polydioxanone⁹ were placed in the body wall on either side of the linea alba. The stay sutures were used to lift the body wall, and a small incision was made with a No. 15 scalpel blade prior to inserting a 5-mm plastic cannula with a blunted trocar⁹ into the abdomen. Plastic cannulas were used to minimize artifacts during CT scanning. A 2.7-mm sheathed rigid endoscope⁹ was briefly passed through the cannula to ensure appropriate placement into the abdomen.
Post-anesthetic management – Meloxicam\(^c\) (1 mg/kg subcutaneously, SC) was administered to all rabbits prior to recovery from anesthesia. Flumazenil\(^b\) (0.025 mg/kg; half of the volume IV and half SC) was administered to partially reverse the sedative effects of midazolam given in the premedication to speed up recovery so that rabbits could be returned to the animal facility with decreased supervision. Buprenorphine (0.05 mg/kg SC) was administered 8 to 9 hours after the initial premedication dose. Intravenous catheters were removed prior to returning the rabbits to their housing facility. Vital signs were assessed twice daily for 3 days after anesthesia to ensure appropriate recovery. Meloxicam\(^c\) (1 mg/kg orally every 24 hours) and buprenorphine (0.05 mg/kg SC) were provided for analgesia on an individual basis as deemed necessary.

Working Space Calculation – A 3-D medical segmentation and imaging software program\(^{t,u}\) was used to convert DICOM-formatted CT images into 3-D models with the pneumoperitoneal gas isolated from abdominal viscera and gastrointestinal gas (Figure 2). The pneumoperitoneal gas was segmented with settings from a low of –1024 to a high of –910 Hounsfield units. Laparoscopic working space volumes were then calculated in cubic millimeters, which were converted to liters. These measurements were determined by an individual trained in use of the software and blinded to the IAPs used for each image captured.

Statistical Analysis – Data were analyzed with statistical software.\(^1\) A mixed linear regression model was created with working space volume as the outcome measure; rabbit identification number was a random effect in the cross-over design study, and pressure order, position (dorsal vs right or left lateral oblique recumbency), and IAP were fixed effects. Residual plots were used
to assess linearity, homoscedasticity, and normality of residuals and to visually examine the data for outliers. Quantile plots of the residuals were also used to assess normality. All assumptions of linear mixed models were verified for the fitted model. The fixed effect variable of rabbit position did not improve the fit of the model on the basis of Akaike Information Criterion and was therefore removed from the final model. A type III analysis of variance was performed on the fixed effects and post-hoc comparisons were performed with a Tukey adjustment. Values of $P < 0.05$ were considered significant. Figures were created with a data visualization package of the statistical software.\textsuperscript{129}

### 3.3 Results

Positioning of rabbits (dorsal vs left or right lateral oblique recumbency) did not significantly ($P = 0.357$) affect the laparoscopic working space volume (Figure 3). A significant ($P < 0.001$) interaction effect was detected between treatment order (i.e., the order of the 3 different IAPs used in the model) and IAP (Figure 4). This effect could not be interpreted in any meaningful manner when comparing individual means; however, the following results were reported from the model including the interaction term. There was a significant ($P < 0.001$) effect of IAP on working space volume. The working space volumes achieved at each IAP are reported (Table 1); mean working space volume was 19% greater with an IAP of 8 mm Hg, compared with 4 mm Hg ($P < 0.001$) and was 6.9% greater with an IAP of 12 mm Hg, compared with 8 mm Hg ($P < 0.001$). The order of treatment also had a significant ($P < 0.001$) effect on working space volume. For an IAP of 4 mm Hg, working space volume was lower when applied
first in the order rather than when applied second or third (Figure 4). For an IAP of 8 mm Hg, a
difference in volume was seen when applied second as compared to third in the sequence;
however, the volume did not sequentially increase with increasing order. Representative CT
(Figure 2) and endoscopic images (Figure 5) were used to depict working space available with
the 3 experimental IAPs.

Adverse effects were detected with pneumoperitoneum in rabbits, in some cases more
apparent at higher IAPs. After insufflation to 8 or 12 mm Hg, apnea in all rabbits and increased
ETCO₂ concentration in 5 out of 6 rabbits (50 to 60 mm Hg) necessitated the use of higher
positive-pressure ventilation rates. Increased heart rate (> 200 beats/min versus 160-200 at
baseline) was noted at 8 and 12 mm Hg in one rabbit. One rabbit developed subcutaneous
emphysema as a result of inappropriate cannula placement and was given meloxicam and
buprenorphine for 2 days after the surgery. A second rabbit had signs of gastrointestinal
discomfort after the anesthetic event (i.e., increased intestinal gas, tense response on abdominal
palpation, anxious behavior, and hyperthermia). This animal also received additional meloxicam
and buprenorphine for 2 days. All other rabbits recovered uneventfully from the anesthesia and
abdominal insufflation.
<table>
<thead>
<tr>
<th>IAP (mm Hg)</th>
<th>Volume (L)</th>
<th>Range</th>
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<tr>
<td></td>
<td>Mean ± Standard Deviation</td>
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<tr>
<td>4</td>
<td>0.825 ± 0.157&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.644–1.080</td>
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<tr>
<td>8</td>
<td>0.982 ± 0.168&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.801–1.277</td>
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<td>12</td>
<td>1.050 ± 0.177&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.853–1.341</td>
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<sup>a,b,c</sup> Volumes with different superscript letters differ significantly (\(P < 0.001\)).

**Table 1:** Measurement of laparoscopic working space volume in 6 anesthetized domestic rabbits (*Oryctolagus cuniculus*) with IAPs of 4, 8, and 12 mm Hg established by CO\(_2\) insufflation.
Figure 2: Representative CT images (transverse view obtained at the level of the left kidney) depicting the difference in pneumoperitoneal (laparoscopic working space) volume in a domestic rabbit (*Oryctolagus cuniculus*) at IAPs of 4 mm Hg (A), 8 mm Hg (B), and 12 mm Hg (C); pneumoperitoneal gas isolated from gas within the gastrointestinal tract by use of medical imaging software (D); and a 3-D image of working space volume created by the combination of contiguous transverse CT sections (E) after CO\(_2\) insufflation.
Figure 3: Mean ± standard deviation of abdominal working space volumes achieved in 6 domestic rabbits with IAPs of 4, 8, and 12 mm Hg established by CO₂ insufflation and imaged in various positions. Measurements of working space for anesthetized rabbits in dorsal (circles), left lateral oblique (triangles), and right lateral oblique (squares) recumbency were made from CT and converted into 3-D models with medical imaging software; volumes calculated in cubic millimeters were converted to liters for reporting. The positioning of rabbits did not significantly affect working space volume.
Figure 4: Measurements of mean ± standard deviation of working space for the 6 rabbits in Figure 3 at IAPs of 4, 8, and 12 mm Hg (red circles, green triangles, and blue squares, respectively) as influenced by treatment order (i.e., the sequence in which each IAP was created in a given rabbit). There was a significant ($P < 0.001$) interaction effect between treatment order and IAP.
Figure 5: Representative caudocranial-view (A, B, and C) and craniocaudal-view (D, E, and F) endoscopic images from a subset of the 6 study rabbits, obtained during evaluation of working space achieved by CO₂ insufflation to IAPs of 4 (A and D), 8 (B and E) and 12 (C and F) mm Hg. The diaphragm (black arrowhead), liver (white arrowhead), small intestine (chevron), and cecum (asterisk) are seen. The images provide a subjective assessment of working space available for laparoscopic procedures.
3.4 Discussion

Current recommendations for IAPs to be used during laparoscopic surgeries in species other than rabbits range from 6 to 10 mm Hg. For dogs, an IAP of 8 to 12 mm Hg is considered to have minimal effects on hemodynamic variables. Investigators of a study of induced pneumoperitoneum in cats concluded that pressures of 4 and 8 mm Hg provided adequate visual access for abdominal organs and that an IAP > 8 mm Hg may not yield clinically relevant improvements in working space. Successful laparoscopic ovariectomy was performed in cats when IAPs of 4 mm Hg and 6 mm Hg were created. Based on the authors’ experiences, however, lower IAPs can result in less abdominal wall tension, leading to increased movement and compression of the abdominal wall during certain laparoscopic surgeries. Abdominal wall tension can be considered in future studies together with measurements of working space to aid in the selection of optimal IAP for laparoscopic procedures in rabbits.

Assessment of the effect of IAP on laparoscopic working space volumes in pigs has been evaluated by CT. The working space volume was found to increase by 93% with an increase in IAP from 5 to 10 mm Hg and by 19% with an increase in IAP from 10 to 15 mm Hg in 20-kg adult pigs. In that study, the volume expansion was attributed to a significant increase in the ventrodorsal distance (the maximum diameter from the ventral aspect of the abdominal wall to the ventral aspect of the vertebrae) and the maximal craniocaudal length of the abdomen (from pubis to diaphragm); there was no significant change in the internal width of the abdomen. A similar study in 6-kg juvenile pigs found the same effect, with a linear increase in working space volume as IAP increased from 0 to 8 mm Hg, followed by a decline in abdominal wall compliance with IAPs > 8 mm Hg. Interestingly, it was also found that prestretching of the
abdominal wall, achieved by applying higher IAP and then desufflating the abdomen, allowed for a significant increase in working space volume at each IAP examined.\textsuperscript{20,130} This is an important area for future investigation of pneumoperitoneum in rabbits, as prestretching might allow for greater working space without using a high IAP during the procedure.

The exact working space volume required for a laparoscopic procedure will depend on the organs being accessed and the instruments used; however, there may not be a meaningful increase in working space volume with IAPs > 8 mm Hg. A significantly larger working space was identified for rabbits in the present study at an IAP of 12 mm Hg than at an IAP of 8 mm Hg. Nevertheless, as was described in experiments involving pigs,\textsuperscript{5} the increase in volume with increased IAP in rabbits was not linear, and the percentage difference was much smaller between 8 and 12 mm Hg (6.9\%) than between 4 and 8 mm Hg (19\%). This was likely a result of abdominal wall biomechanical properties, as previously described with the creation of pneumoperitoneum in human patients.\textsuperscript{131} In increasing the IAP from 0 to 12 mm Hg in people, approximately 90\% of the working space volume at 12 mm Hg is achieved by the time the IAP reaches 4 mm Hg. Therefore, it has been suggested that abdominal working space can be optimized at lower IAPs than traditionally used. This may be even more applicable during prolonged laparoscopic procedures when the hemodynamic and respiratory effects of CO\textsubscript{2} insufflation become of greater concern for the patient.

For the present cross-over study, each rabbit had the 3 IAPs applied in a randomly assigned sequence. The order of these treatments had a significant effect on working space volume achieved, especially at 4 mm Hg. This effect may have been attributable to the prestretching phenomenon previously described.\textsuperscript{20} When the IAP of 4 mm Hg was applied first
in the sequence, no prior stretching had occurred. When applied following higher pressures (8 and 12 mm Hg), the working space volume achieved appeared to be greater. Interestingly, the effect differed when assessing the influence of treatment order on working space volume at 8 mm Hg; a significant difference in working space was detected when this IAP was applied second or third in the sequence, compared with that achieved when it was used first, but the working space did not sequentially increase with each pressure as observed at 4 mm Hg. The inability to detect a consistent effect at an IAP of 8 and 12 mm Hg may have been attributable to a lack of power, considering the small number of rabbits used in this study. For each IAP in each treatment order position (first, second, or third), there were only 2 observations from which to draw conclusions. It was also possible that the observed interaction effects resulted from washout (desufflation) periods that were too short. As our design was balanced for carry-over effects, these effects were considered unlikely to confound our results. An interaction between order and IAP existed, and we recommend that future studies on working space randomize and account for the effect of sequence on the entire model. Randomization of cross-over studies requires a balanced Latin square design to control for carry-over effects, which can also be assessed in the statistical model.

As mentioned, a small sample size was the main limitation of this study. To limit the influence of individual animal effects on variability in working space volumes, the rabbits used in the study were of the same breed and similar in size and age, and a cross-over design was used to ensure that each rabbit served as its own control for comparison among IAPs. No significant outliers were found in the assessment of data for individual rabbits. In *a priori* calculations, the sample size was deemed appropriate to identify differences in working space volume associated
with different IAPs; however, evaluating the interaction effect with treatment order may require a larger sample.

The adverse effects observed in these rabbits have been previously reported for other species with pneumoperitoneum created to facilitate laparoscopic procedures.\textsuperscript{6,21} Apnea is expected to be secondary to pressure on the thorax, decreased thoracic compliance, and difficulty in spontaneous ventilation. End-tidal CO\textsubscript{2} concentrations are commonly increased with pneumoperitoneum, due to decreases in venous return and cardiac output, as well as absorption of insufflated CO\textsubscript{2} across the peritoneal wall. Increased heart rate is expected to result from increases in the partial pressure of CO\textsubscript{2} that cause sympathetic stimulation, and this change in heart rate may help to maintain cardiac output. These cardiovascular and respiratory effects did not seem to be a clinically important concern in this population of healthy young rabbits. Nevertheless, adverse effects associated with pneumoperitoneum created by CO\textsubscript{2} insufflation need to be evaluated with further studies in rabbits.

Emphysema and post-anesthetic gastrointestinal effects were each reported for 1 rabbit in the present study. Emphysema is a known adverse effect that can result from inappropriate cannula placement during induction of pneumoperitoneum.\textsuperscript{97,104} Gastrointestinal signs in 1 rabbit may have resulted from effects of anesthesia\textsuperscript{132,133} or changes in splanchnic circulation during pneumoperitoneum, as summarized in a systematic review\textsuperscript{13} that included studies of animals.

Our results suggested that, depending on the visibility needed and instruments required for a specific laparoscopic procedure, it may not be advantageous to use an IAP > 8 mm Hg in rabbits. This information can be used in further research to develop guidelines for pneumoperitoneum use during minimally invasive surgery in rabbits.
3.5 Footnotes

a. Charles River Laboratories, Saint-Constant, Quebec, Canada.
b. Sandoz Canada Inc, Boucherville, Quebec, Canada.
d. BD Canada, Mississauga, Ontario, Canada.
e. Plasmalyte-A, Baxter Healthcare, Deerfield, Illinois, USA.
f. Pharmascience Inc, Montreal, Quebec, Canada.
g. IsoFlo®, Zoetis Canada Inc, Kirkland, Quebec, Canada.
h. Nellcor™️, Covidien Canada, Saint-Laurent, Quebec, Canada.
i. 2500A VET, Nonin Medical Inc, Plymouth, Minnesota, USA.
j. Ultrasonic Doppler 811-B, Parks Medical Electronics Inc, Aloha, Oregon, USA.
l. R, version 3.4.1, R Core Team, R Foundation for Statistical Computing, Vienna, Austria.
m. GE Bright Speed, General Electric Healthcare, Milwaukee, Wisconsin, USA.
n. Stryker, Kalamazoo, Michigan, USA.
o. Karl Storz Endoscopy America Inc, El Segundo, California, USA.
p. PDS® II, Ethicon, Johnson & Johnson Medical Products, Markham, Ontario, Canada.
q. VersaOne™️, Covidien Canada, Saint-Laurent, Quebec, Canada.
r. Metacam® 20 mg/mL Injectable, Boehringer Ingelheim, Burlington, Ontario, Canada.
s. Metacam® 1.5 mg/mL Oral Suspension, Boehringer Ingelheim, Burlington, Ontario, Canada.
t. Materialise Mimics, version 19, Materialise, Leuven, Belgium.

u. 3-matic, version 11, Materialise, Leuven, Belgium.
Chapter 4: Cardiovascular and Respiratory Effects of Carbon Dioxide Pneumoperitoneum in the Domestic Rabbit (*Oryctolagus cuniculus*)

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4.1 Introduction

Laparoscopy is frequently performed in veterinary practice, and it is important that the physiological effects of induced pneumoperitoneum are well-established. Rabbits are common pets and are also used as research and teaching models for pediatric endosurgery. A clear understanding of the cardiovascular and respiratory effects of pneumoperitoneum on this species will help to improve the safety and management of these animals under general anesthesia. Since visualization of abdominal organs and working space are improved as gas is insufflated into the abdomen, it would be appealing to maximize intra-abdominal pressure (IAP); however, this can be detrimental to ventilation, cardiac function, and perfusion. With concurrent consideration of the change in working space with intra-abdominal pressure, it will be possible to make
recommendations on the optimal intra-abdominal pressures for use in rabbit laparoscopic procedures.

Few studies have examined the cardiovascular effects of pneumoperitoneum in rabbits, but it seems that the effects resemble those seen in humans, pigs, and dogs. In general, the IAP produced by insufflation causes decreased venous return due to compression of the vena cava, and simultaneous decreases in cardiac output. This is associated with increases in systemic vascular resistance. Arterial blood pressure does not change in a consistent manner, but is often increased. Heart rate also tends to be increased, which may be partially due to the cardiostimulatory effects of elevated arterial partial pressures of CO2 (PaCO2) during insufflation with carbon dioxide.

Similarly, respiratory parameters are known to be affected by pneumoperitoneum, largely due to reduced diaphragmatic excursion and pulmonary compliance as intra-abdominal pressure increases. Based on previous canine and porcine models, induction of pneumoperitoneum decreases tidal volume or increases peak inspiratory pressure, depending on whether ventilation is spontaneous or controlled. As a result, PaCO2 and end-tidal carbon dioxide (ETCO2) increase. This is due in part to ventilatory changes, but also due to diffusion of carbon dioxide from the abdomen into the bloodstream. The increase in PaCO2 will ultimately lead to changes in blood pH and will negatively affect the arterial partial pressure of O2 (PaO2).

Despite the use of rabbits as research models for laparoscopy in humans, there are limited studies that examine the specific effects of varying pneumoperitoneal pressures on cardiovascular and respiratory parameters in this species. The anatomy of rabbits differs from that of pigs and dogs due to the relatively small thoracic volume and large gastrointestinal
system. This may influence how pneumoperitoneum affects respiration and local perfusion. This study aims to compare cardiovascular and respiratory parameters at 0, 4, and 8 mm Hg IAP in order to improve anesthetic recommendations for rabbits undergoing laparoscopy. These IAPs were selected due to their clinical relevance. Based on our previous research, sufficient laparoscopic working space should be attainable with an IAP of 8 mm Hg; further increases in pressure may be clinically unnecessary.

Based on our current understanding, it was hypothesized that cardiac output would be depressed with increasing IAP as described above, but that the cardiostimulatory effects of increased PaCO₂ might help to maintain heart rate and blood pressure. We also hypothesized that PaCO₂, ETCO₂, and peak inspiratory pressure (PIP) would increase, as has been reported in rabbits and other species. This information will aid in developing guidelines for pneumoperitoneal pressure during laparoscopic procedures in rabbits.

4.2 Materials and Methods

Animals – Six specific-pathogen free 4 to 5-month-old female New Zealand White rabbits were used. The same rabbits were involved in a study on laparoscopic working space prior to this research and were allowed 7 days of recovery from the previous anesthetic event. The rabbits weighed between 3.02-3.53 kg. Physical examinations were performed at the start of the study prior to general anesthesia. The study protocol was reviewed and approved by the Animal Care Committee of the University of Guelph, in accordance with guidelines set by the Canadian Council on Animal Care.
Anesthesia and Instrumentation – Food was withheld from the rabbits for 15-60 minutes, then the rabbits were premedicated with intramuscular midazolam (1 mg/kg) and buprenorphine (0.05 mg/kg). After at least 20 minutes, the right marginal auricular vein was catheterized with a 24 gauge intravenous catheter. The rabbits were induced with intravenous propofol (8-10 mg/kg), intubated blindly in lateral recumbency using a 4 mm uncuffed endotracheal tube, and connected to the anesthetic machine using a circle circuit. The end-tidal isoflurane concentration was maintained at 2.0-2.2%. Isoflurane was provided with an oxygen (100%) flow rate of 2 L/minute using intermittent positive pressure ventilation delivered by an electronically controlled, volume-cycled ventilator, with a rate of 12 breaths per minute and a tidal volume of 15 to 17 mL/kg. An isotonic solution was administered intravenously during the anesthetic procedure at 10 mL/kg/hr. Cefazolin (20-25 mg/kg) was administered intravenously prior to the first skin incision to prevent peri-operative infection.

Rabbits were instrumented in the first 30 minutes of anesthesia for monitoring of electrocardiography, heart rate (HR), direct arterial blood pressure (ABP; including systolic, diastolic, and mean; SBP, DBP, and MBP), peripheral pulse oximetry placed on the digits, esophageal temperature, ETCO2 and end-tidal isoflurane concentrations, and specific spirometry variables (tidal volume, PIP) using a multiparameter monitor.

A 24 gauge catheter was placed in the central auricular artery for measurement of direct arterial blood pressure, and for blood sampling using heparinized syringes to measure PaCO2, PaO2, sodium, and hemoglobin. Cardiac output (CO) measurement was performed using lithium dilution (LiDCO) by attaching a lithium chloride sensor to the side port of a 3-way
valve connected to the arterial catheter. Extension tubing was attached to the 3-way valve, connected to a blood collection bag, and blood passed through a peristaltic pump that produced a blood flow rate of 4 mL/min across the sensor. Lithium chloride (0.006 mmol/kg) was injected into the marginal ear vein catheter and flushed with 2 mL of isotonic saline 8 seconds after starting the injection phase on the LiDCO computer. The arterial hemoglobin and sodium levels required by the LiDCO computer were determined immediately prior to obtaining CO measurements.

**Trocar/Cannula Assembly placement** – The rabbits were placed in dorsal recumbency and the abdomen was clipped and aseptically prepared for surgery. A #15 scalpel blade was used to make a skin incision along ventral midline approximately 1 cm caudal to the umbilicus and 4-0 polydioxanone stay sutures were placed on either side of the linea alba to elevate the body wall and facilitate placement of the trocar/cannula assembly. Initially, a small incision was made with the scalpel and then a 3.5 mm graphite trocar/cannula assembly was inserted into the abdomen. A 2.7 mm 30° angle sheathed rigid endoscope was used to visualize abdominal organs and confirm proper placement of the cannula within the abdomen.

**Experimental procedure** – The design was a cross-over study balanced for carry-over effects using two 3 x 3 latin squares generated by an R statistical package and each rabbit was randomly assigned to a random sequence of three IAPs (0, 4, 8 mm Hg). The pressures used were selected based on previous assessment that 4 and 8 mm Hg provided appropriate laparoscopic working space. Mechanical insufflation with carbon dioxide was maintained using a flow rate of 1 L
per minute at the desired IAP for at least 5 minutes before collecting an arterial blood sample to measure sodium, hemoglobin, PaCO₂, and PaO₂. The remaining variables, including SBP, DBP, MBP, HR, CO, ETCO₂, PIP, and peripheral oxygen saturation (SpO₂), were measured at each IAP after 15 minutes of equilibration. A 5 minute period of zero pneumoperitoneum was applied before the next pressure in the random sequence was established. From these variables, cardiac index (CI) and stroke volume index (SVI) were calculated.

After applying all IAP treatments, the abdomen was purged of CO₂ with manual pressure and the cannula was removed. Abdominal incisions were closed using 4-0 polydioxanone suture in a simple interrupted pattern for the linea alba and 4-0 poliglecaprone 25 suture in a continuous intradermal pattern for apposing the skin.

**Post-anesthetic management** – Meloxicam (1 mg/kg intravenously) and flumazenil (0.025 mg/kg, half intravenously and half subcutaneously) were administered to each rabbit before extubation. All catheters were removed, and the rabbits returned to their pens once they were responsive and sitting upright. For post-operative analgesia, they all received buprenorphine (0.05 mg/kg subcutaneously) 6-8 hours after the initial premedication. Those rabbits that seemed to be more painful post-operatively received additional meloxicam (1 mg/kg orally once daily) or buprenorphine (0.05 mg/kg subcutaneously at least 8 hours apart) as deemed appropriate. Vital parameters were assessed twice daily for three days after anesthesia.

**Statistical Analysis** – The statistical software R was used to fit mixed linear regression models with each cardiovascular and respiratory parameter as an outcome variable. Due to the cross-over
nature of this study, rabbit id was added to the model as a random variable; IAP and order of pressure in the sequence were fixed variables. Cardiovascular and respiratory parameters were assessed for correlation using Pearson correlation coefficients. They were applied as fixed effects in models only if they were not moderately to strongly correlated (-0.65 < r < 0.65) with the outcome measure and if they improved the fit of the model based on the Akaike information criterion (AIC). Assumptions of linearity, homoscedasticity of residuals, and normality of residuals were verified. Residual plots were used to look for outliers. Type III analysis of variance was performed on the fixed effects and Tukey adjustment was applied for post-hoc comparisons. Statistical significance was set at an alpha of 0.05. All figures were created using the “ggplot2” data visualization package of R.129

4.3 Results

A heart murmur was noted in one rabbit. Echocardiographic examination revealed significant tricuspid dysplasia; however, it was deemed to not interfere with the objectives of the study. This rabbit was assessed for any outlying results during data analysis, but all findings were similar to that of the other rabbits and the rabbit was not excluded from the study.

There was no statistically significant difference in HR based on the 3 treatments (IAP of 0, 4, and 8 mm Hg; \( P = 0.5956 \)) or based on the order in which pressures were applied (\( P = 0.5730 \)). There was also no significant difference in SpO2 across IAP (\( P = 0.4096 \)) and order (\( P = 0.4096 \)).
Although there was a positive trend towards increased ABP, the changes for SBP, MBP, and DBP were not significantly affected by IAP (SBP \( P = 0.0611 \); MBP \( P = 0.0732 \); DBP \( P = 0.0816 \)) or order in the sequence of IAP (SBP \( P = 0.3338 \); MBP \( P = 0.2406 \); DBP \( P = 0.2575 \)) (Figure 6). There was a strong positive correlation between SBP and HR (Pearson correlation coefficient, \( r = 0.7176, P = 0.0008 \)) and a moderate correlation between SBP and CO \((r = 0.6831, P = 0.0018)\). There was also a moderate positive correlation between MBP and HR \((r = 0.6501, P = 0.0035)\).

Stroke volume index also decreased at 4 and 8 mm Hg as compared to baseline; however, this effect was not significant \((P = 0.0613)\), and neither was the effect of order in the sequence \((P = 0.7657)\) (Figure 7).

For PaO\(_2\) there was a significant interaction effect between IAP and the order in which pressures were applied in the sequence \((P = 0.0166)\) (Figure 8). This effect could not be interpreted in any meaningful manner but was included in the regression model. IAP did not significantly affect the PaO\(_2\) in this model \((P = 0.1710)\).

There was a significant increase in PaCO\(_2\) at 8 mm Hg as compared to 0 mm Hg \((P = 0.0016)\) and 4 mm Hg \((P = 0.0076)\) (Figure 9). Likewise, ETCO\(_2\) was higher at each subsequent pressure (Figure 10); from IAP of 0 to 4 mm Hg, the mean ETCO\(_2\) increased from 43.8 to 47.3 mm Hg \((P = 0.0424)\) and from IAP of 4 to 8 mm Hg, the mean increased from 47.3 to 52.3 mm Hg \((P = 0.0071)\). ETCO\(_2\) and PaCO\(_2\) were found to be strongly correlated in their outcome measures \((r = 0.7747, P = 0.0002)\).

End-tidal CO\(_2\) and SpO\(_2\) were included in the mixed regression models for CO and CI, since they improved the fit of the models and had only weak correlation with either outcome.
variable. In the final model, CO ($P = 0.0292$) and CI ($P = 0.0317$) were significantly decreased as intra-abdominal pressure was applied. Cardiac output decreased from a mean ($\pm$ standard error) of $436.0 \pm 44.0$ mL/minute without pneumoperitoneum, to $385.3 \pm 30.0$ mL/minute at 4 mm Hg and $386.8 \pm 28.0$ mL/minute at 8 mm Hg (Figure 11). Cardiac output and CI demonstrated moderate positive correlations with heart rate ($r = 0.7324$ for CO, $P = 0.0005$; $r = 0.7222$ for CI, $P = 0.0007$).

Finally, PIP was significantly affected by intra-abdominal pressure ($P = 0.0001$) (Figure 12). PIP increased from $15.3$ mm Hg to $17.2$ mm Hg, when changing from an IAP of 0 to 4 mm Hg ($P = 0.0489$). It subsequently increased from $17.2$ mm Hg to $20.7$ mm Hg as the IAP was increased to 8 mm Hg ($P = 0.0015$).

Mild adverse effects were noted after anesthesia and abdominal insufflation in three rabbits. One rabbit developed dehiscence of the skin layer at the site of cannula placement. The incision was re-sutured and an “Elizabethan collar” was placed until there was complete healing of the skin. This rabbit received additional meloxicam, buprenorphine, and enrofloxacin$^\text{a}$ (10 mg/kg orally twice daily for 7 days). Three rabbits had signs of abdominal discomfort on palpation in the first 24 hours after the procedure. These rabbits received additional doses of meloxicam and buprenorphine and otherwise recovered uneventfully.
Figure 6: The effect of intra-abdominal pressure (in mm Hg) on systolic arterial blood pressure (in mm Hg), displayed in mean and standard error. A similar positive trend in blood pressure was seen for mean and diastolic pressures; this effect was not statistically significant.

Figure 7: The effect of intra-abdominal pressure (in mm Hg) on the stroke volume index (in mL/beat/kg), displayed in mean and standard error. The trend of decreasing SVI when IAP is applied mirrors the trend in cardiac output; however, this effect was not statistically significant.
**Figure 8:** The effect of intra-abdominal pressure (in mm Hg) on arterial partial pressure of oxygen (in mm Hg), displayed in mean and standard error, accounting for the order in which the IAP was applied in the pressure sequence. There was a significant interaction effect between IAP and the order ($P = 0.0166$).

**Figure 9:** The effect of intra-abdominal pressure (in mm Hg) on the arterial partial pressure of carbon dioxide (in mm Hg), displayed in mean and standard error. There was a significant increase in PaCO$_2$ at an IAP of 8 mm Hg ($P \leq 0.0076$).
Figure 10: The effect of intra-abdominal pressure (in mm Hg) on the end-tidal carbon dioxide (in mm Hg), displayed in mean and standard error. There was a significant increase in ETCO$_2$ at each subsequent IAP ($P \leq 0.0424$).

Figure 11: The effect of intra-abdominal pressure (in mm Hg) on the cardiac output (in mL/minute), displayed in mean and standard error. There was a significant decrease in cardiac output as IAP was applied ($P = 0.0292$).
Figure 12: The effect of intra-abdominal pressure (in mm Hg) on the peak inspiratory pressure (in mm Hg), displayed as mean and standard error. There was a significant increase in PIP as IAP was applied ($P = 0.0001$).
4.4 Discussion

Overall, the effect of carbon dioxide insufflation at pressures of 4 and 8 mm Hg was as expected based on studies in humans and other animal models. A decrease in cardiac output and index was observed in this study and is believed to be due to decreased venous return and preload. The resultant effect is a decrease in stroke volume, and this trend was demonstrated in this study for SVI. Stroke volume may also decrease with elevated systemic vascular resistance (SVR); we did not measure SVR and its exact contribution cannot be determined. Nevertheless, there was a positive trend towards increased blood pressure, which may be related to an increase in SVR. In the present study, HR remained unchanged by IAP and in the presence of a decreased SVI with higher IAP, CO or CI could not be maintained as high as for baseline (IAP of 0 mm Hg).

In dogs, it is recommended to use IAPs of less than 12 mm Hg for laparoscopic procedures in order to prevent adverse effects on CO and other hemodynamic parameters. In cats, IAPs of 4, 8, and 15 mm Hg did not cause changes in CI, HR, or SVI. Keeping IAP low may help minimize hemodynamic depression. The change in CO seen with carbon dioxide insufflation is well-tolerated in healthy patients, and in some circumstances, this may be a transient effect. However, patients with cardiovascular compromise or hypovolemia may be more severely affected. It should be noted that the rabbit with tricuspid dysplasia in this study did not appear to have more adverse effects than the other rabbits. Preconditioning, which is the application of pneumoperitoneal pressure for a short period of time prior to the longer pneumoperitoneum needed for a laparoscopic procedure, has also been shown in porcine models to attenuate some of the hemodynamic effects of high intra-abdominal pressures.
A previous study on the effect of IAP on hemodynamic parameters, including CO, in New Zealand White rabbits found an initial increase then decrease in this parameter as the pressure was gradually increased. In that study, IAP was applied by administering saline infusions into the abdomen. As a result, the concurrent cardiostimulatory effects of hypercapnia that occur with carbon dioxide insufflation was not accounted for. Hypercapnia is known to activate the sympathetic nervous system, resulting in increases in ABP, HR, CO, and contractility. Sümpelmann et al. (2006), using rabbits as models for pediatric laparoscopy, also examined the effect of prolonged carbon dioxide pneumoperitoneum at 8 mm Hg on CI and acid-base parameters and demonstrated a lower CI in the insufflated rabbits versus the control group, as corroborated by our current findings. However, in their study, there was an initial increase in CI over the first 30 minutes of insufflation at 8 mm Hg due to an initial shift in blood volume from abdominal to thoracic vessels, with a decline in venous return seen only later.

The cardiostimulatory effect of hypercapnia was not significant enough in this study to result in a concurrent increase in HR and ABP. Although increases in ABP have been reported with pneumoperitoneum, they are not always consistent. The change in ABP is related to release of catecholamines as a result of hypercapnia. A positive trend in blood pressure was seen as IAP increased, even though this effect was not statistically significant.

Maintaining a normal HR and ABP is not sufficient to ensure normal tissue perfusion. Previous studies, including in rabbits, have shown that changes in SVR and abdominal pressure with pneumoperitoneum affect perfusion of abdominal organs, in particular the splanchnic circulation. In addition, the effect of decreased blood pH may contribute to cellular damage of these abdominal tissues. Minimizing the IAP or time with pneumoperitoneum may
help to mitigate these effects in individuals with impaired organ function. In addition to the effects caused by anesthesia and pneumoperitoneum alone (e.g. persistent carbon dioxide in the abdomen and drying of peritoneal tissues), this change in splanchnic perfusion may contribute to post-anaesthetic abdominal discomfort as seen in some of these rabbits.

As hypothesized, we also identified an increase in PaCO\textsubscript{2} and ETCO\textsubscript{2} consistent with previous studies in rabbits and other species.\textsuperscript{31,141} These changes are expected to be the result of carbon dioxide diffusing into the bloodstream, as well as changes in pulmonary compliance and ventilatory pressure due to pneumoperitoneum.\textsuperscript{6,21} The effect of carbon dioxide alone is significant and has been examined in porcine models\textsuperscript{139,140,149} and even a rabbit model\textsuperscript{141} by comparing to insufflation with other gases. Initially, the increase in PaCO\textsubscript{2} may improve cardiovascular function; however, ultimately, this will lead to a decrease in blood pH and affect cellular metabolism. Increased ventilation is recommended to help eliminate carbon dioxide and prevent build up.

The increase in PIP seen in these rabbits reflects how pneumoperitoneum affects ventilation. When using a volume-controlled ventilator, the PIP is expected to increase due to pressure of pneumoperitoneal gas on the diaphragm and decreased lung compliance.\textsuperscript{6} As previously mentioned, the increased thoracic pressure with pneumoperitoneum leads to depression of venous return and decreases in CO.

The order in which IAPs were applied was important in determining the effect on PaO\textsubscript{2}. There was an interaction between IAP and order in the sequence. PaO\textsubscript{2} may be influenced partially by time under anaesthesia and other hemodynamic effects changing over time. The change in PaO\textsubscript{2} seen here may have only minimal clinical significance; however, it highlights the
importance of controlling for or factoring in sequence order in future cross-over studies examining the effects of pneumoperitoneum on cardiovascular or respiratory parameters. It also emphasizes that cross-over designs should be balanced for carry-over effects.

Several closely correlated variables were identified in this study, and that is not unexpected given the close association between the cardiorespiratory parameters and the way they influence one another in vivo. For example, HR, ABP, and CO are all closely linked. Blood pressure is determined by CO and SVR; and CO in turn is determined by HR and SV. Therefore, these variables are expected to fluctuate together. Similarly, ETCO$_2$ and PaCO$_2$ are positively correlated since they reflect an increase in carbon dioxide in the blood and the body’s attempt to eliminate the excess CO$_2$ (respiratory compensation).

The main limitation of the current study is the small sample size. Performing this crossover study on rabbits of similar size, sex, age, and health status allowed for limited variability between individuals. No significant outliers were present in the group. One rabbit was identified as having a heart murmur and tricuspid dysplasia prior to being included in this study. The parameters measured in this rabbit were well within the ranges seen in the remainder of the rabbits; therefore, this rabbit was not deemed to be an outlier and was not excluded from the sample population.

Rabbits are frequent pets, common laboratory animals, and are used as surgical models for pediatric patients; as a result, the use of laparoscopic procedures are becoming more common in this species. The recommended pneumoperitoneal pressure to be used for laparoscopy in rabbits will depend upon the procedure being performed; however, the previous evaluation of working space in rabbits would lead us to recommend IAPs of 4 to 8 mm Hg since
the percent increase in working space is less from 8 to 12 mm Hg. Lower intra-abdominal pressures are particularly important for prolonged procedures or in patients with cardiovascular or respiratory diseases that would compromise their ability to respond to changes under anaesthesia.

4.5 Footnotes

a. Charles River Laboratories, Saint-Constant, Quebec, Canada
b. Sandoz Canada Inc., Boucherville, Quebec, Canada
c. Vetersic®, Sogeval UK Ltd., Sheriff Hutton, York, UK
d. BD Canada, Mississauga, Ontario, Canada
e. Pharmascience Inc., Montreal, Quebec, Canada
f. Datex Ohmeda, DRE Medical, Louisville, Kentucky, USA
g. IsoFlo®, Zoetis Canada Inc., Kirkland, Quebec, Canada
h. S/5 Aespire 7900 ventilator, GE Healthcare, Madison, Wisconsin, USA
i. Plasma-Lyte A, Baxter Healthcare, Deerfield, Illinois, USA
j. Fresenius Kabi Canada Ltd., Toronto, Ontario, Canada
k. S/5 Anesthesia monitor, GE Healthcare, Madison, Wisconsin, USA
l. AirLife™, CareFusion, Yorba Linda, California, USA
m. ABL800 Flex Radiometer, London, Ontario, Canada
n. LiDCOTM Plus, LiDCO, London, UK
o. LiDCOTM Sensor; LiDCO, London, UK
p. LiDCO™ Flow Regulator; LiDCO, London, UK
q. PDS® II, Ethicon, Johnson & Johnson Medical Products, Somerville, New Jersey, USA
r. Karl Storz Endoscopy America, Inc., El Segunda, California, USA
s. R version 3.4.1, R Core Team, R Foundation for Statistical Computing, Vienna, Austria
t. Highflow 40L insufflator, Stryker, Kalamazoo, Michigan, USA
u. Monocryl®, Ethicon, Johnson & Johnson Medical Products, Somerville, New Jersey, USA
v. Metacam® 20 mg/mL Injectable, Boehringer Ingelheim, Burlington, Ontario, Canada
w. Metacam® 1.5 mg/mL Oral Suspension, Boehringer Ingelheim, Burlington, Ontario, Canada
x. Baytril® 50 mg/mL Injectable compounded to dilution of 20 mg/mL, Bayer Animal Health Canada, Mississauga, Ontario, Canada
Chapter 5: A Comparison of Intra- and Post-operative Outcomes of Laparoscopic Versus Open Ovariectomy in the Domestic Rabbit

*(Oryctolagus cuniculus)*

### 5.1 Introduction

Minimally-invasive surgical techniques are being applied with increasing frequency to veterinary species.\(^{60,61}\) There are continuous developments in the complexity of procedures described in the veterinary literature, as well as the availability of novel endoscopic technology for veterinary surgeons.\(^{60}\) Surgeries performed solely with the use of laparoscopy or with laparoscopic assistance are as diverse as adrenalectomies, cholecystectomies, and nephrectomies, with most studies being performed in dogs.\(^{58,59,100,150-154}\) Laparoscopic surgeries have been associated with decreased post-operative morbidity, incidence of infections, and hospitalization periods in humans.\(^{68-71,73,155}\) Despite the frequency with which laparoscopic procedures are currently being described in veterinary species, comparisons with conventional open approaches are still infrequent.

There have been studies comparing the surgical stress and pain in dogs and cats from laparoscopic procedures versus conventional open procedures.\(^{18,89,90,92,156}\) Assessment of post-operative pain and recovery in companion animals usually requires the use of pain scores, generally incorporating a combination of physiologic parameters (such as heart rate and respiratory rate) with behavioral parameters including posture, response to palpation, and activity level.\(^{85,91,157-159}\) Circulating levels of glucose and glucocorticoid have also been used.\(^{52,160,161}\)
Haraguchi et al. (2017) identified significantly lower C-reactive protein and cortisol levels after laparoscopic surgery when compared to open surgery in an experimental gastropexy model in dogs.\(^8^9\) This would suggest differences in post-operative inflammation and surgical stress. However, there were no statistically significant differences between the surgical groups in subjective pain scores, including the visual analog scale and University of Melbourne pain scale. The clinical significance of the physiologic changes needs to be evaluated further. Another prospective trial similarly identified lower C-reactive protein levels in laparoscopic splenectomy as opposed to open splenectomy, in that case also reporting a significant decrease in pain scores.\(^8^8\) In cats with laparoscopic ovariectomy and dogs with laparoscopic ovariohysterectomy, pain scores within 24 hours after surgery were lower than open surgical treatments; however, observers assigning pain scores in these studies were not blinded to the treatments.\(^5^2,8^7,9^0\) Behavioral assessments and pain scores are susceptible to observer bias and require blinding techniques. Post-operative activity, based on accelerometry, has been shown to be increased in dogs after laparoscopic ovariectomy as compared to open ovariectomy.\(^5^1\) Shorter hospitalization times were found in retrospective studies comparing laparoscopic adrenalectomy and laparoscopic-assisted cystotomy to open approaches in dogs.\(^5^7,9^6\) Finally, fewer post-operative complications and adhesions have been reported for canine laparoscopic versus open ovariectomy,\(^7^5,9^4\) however, selection bias may have occurred when assigning patients to surgical treatments in the retrospective observational study. A prospective randomized controlled trial would allow for better evaluation of the differences in these outcome parameters.

The domestic rabbit (Oryctolagus cuniculus) is becoming increasingly popular as a companion animal and is also an important laparoscopic model for pediatric surgeries.\(^6^4-6^6\)
Laparoscopic surgical techniques for rabbits have been described.\(^{106-112}\) Nevertheless, the advantages or disadvantages as compared to conventional open surgeries have not been well explored in this species.\(^{79,93}\) Rabbits are smaller than most dogs and cats, have a small thoracic cavity, and have a large gastrointestinal tract (with a well-developed cecum). Since they are prey species, they also have less apparent pain behaviors than some other animals.\(^{84}\) That being said, rabbits develop gastrointestinal stasis during times of stress and pain, thus laparoscopy may benefit rabbits if it can be shown to minimize post-operative pain and morbidity.\(^{115}\) The unique anatomical and behavioral characteristics of rabbits may influence how pneumoperitoneum and surgery affect this species in the peri-operative and post-operative period, and the outcome of laparoscopic surgery in rabbits needs to be assessed in more detail.

Some authors report subjective advantages in rabbits after laparoscopic surgeries,\(^{112}\) but this has not been formally investigated. The evaluation of pain, discomfort, and stress in a rabbit is complex.\(^{82}\) Facial grimace scales (FGS) are well developed for this species to define facial action units that suggest pain or discomfort.\(^{114}\) Behavioral ethograms are also commonly being combined with physiologic parameters, in order to better evaluate which behaviors are associated with pain and how to interpret an animal’s response to different surgical stressors.\(^{123,124}\)

The purpose of this study was to determine the effect of laparoscopic ovariectomy on several intra-operative and post-operative parameters, including behaviors associated with pain expression, and compare to open ovariectomy in rabbits. It was hypothesized that laparoscopic ovariectomy would decrease post-operative morbidity, as has been described in humans and other domestic animals. Therefore, physiologic and behavioral expressions of pain or discomfort should be alleviated or absent after laparoscopic surgery.
5.2 Materials and Methods

**Animals** – For the pilot study, six 5-month-old intact female New Zealand White rabbits were used at least one week after a previous study on the effects of pneumoperitoneum in rabbits. The most efficient surgical technique for laparoscopic ovariectomy was determined based on surgeries performed in these 6 rabbits.

For the complete study, 12 specific-pathogen free 4 to 5-month-old female New Zealand White rabbits were used, weighing between 2.8-3.3 kg. Physical examinations were performed prior to the start of the study. An animal use protocol was reviewed and approved by the Animal Care Committee of the University of Guelph in accordance with guidelines set by the Canadian Council on Animal Care.

**Housing** – The rabbits were individually housed in floor pens and acclimated to their new environment for 7 days prior to the start of the study period. They were housed in a single room and were able to see and hear neighboring rabbits; however, physical barriers prevented mixing of fecal pellets and food. A 12-hour light cycle was provided. The rabbits were fed a commercial pelleted diet, water from bottles, had access to litterboxes with pine shavings, and each rabbit had enrichment items in their pens (one clear plastic hidebox/hut and toys to chew on). All items in the pen were arranged in the same orientation for each rabbit, with food dishes and huts being in the middle of the pen and litterboxes towards the back.
**Pilot Study** – Laparoscopic ovariectomy was performed on all six rabbits used for the pilot study. The rabbits were fasted for at least 15 minutes, then they were premedicated with hydromorphone\(^b\) (0.2 mg/kg) and midazolam\(^b\) (1 mg/kg) intramuscularly (IM). The lateral auricular vein was catheterized with a 22 gauge catheter\(^c\) to provide intravenous crystalloid fluids\(^d\) (10 mL/kg/hr). Propofol\(^e\) (8 mg/kg with top-up to effect) was administered intravenously (IV) to induce anesthesia. The rabbits were intubated with 4 mm uncuffed endotracheal tubes and were maintained on a circle circuit with 2-3.5% isoflurane\(^f\) delivered in 1.5 L/minute of oxygen by a mechanical volume-cycled ventilator. The rabbits were monitored under anesthesia using a doppler probe, capnography, and pulse oximetry.

A 3-port laparoscopic approach to ovariectomy was performed, similar to previous descriptions in the literature.\(^{108}\) Different port positions were tested for placement of the endoscope, grasping forceps, and Ligasure\(^{TM}\) vessel sealing device\(^g\). The most efficient placement for two surgeons was used for the complete study, and the ovariectomy was performed using a similar technique to that described below. Cefazolin\(^b\) (20 mg/kg IV) was administered peri-operatively. On recovery from anesthesia, flumazenil\(^b\) (0.012 mg/kg IV and 0.012 mg/kg subcutaneously) was provided to each rabbit. The rabbits also received meloxicam (1 mg/kg IV\(^i\) or orally\(^j\) once daily) for 3 days post-operatively, unless surgical complications required additional analgesics (see “Post-operative Complications”).

**Experimental Design** – Twelve rabbits were randomly allocated using a statistical software\(^k\) to either of the two study groups: an open laparotomy approach to ovariectomy (OVE) and a laparoscopic ovariectomy (LapOVE). They were then distributed to pens in the housing room.
such that rabbits from either group were equidistant from the door for the purpose of assessing behavioral responses after surgery.

LapOVE and OVE rabbits were matched such that one rabbit from each group had surgery on the same day and had pre- and post-operative outcomes measured simultaneously. Observation of objective and behavioral outcomes was initiated 24 hours prior to surgery (see “Post-operative Observations” and “Behavioral Observations”).

**Anesthesia** – On the day of surgery, each rabbit was fasted from food for 30-90 minutes prior to premedication. Water was freely available. A physical examination was performed and blood was collected for packed cell volume, measured after centrifugation in a microhematocrit tube¹, and total protein, measured by refractometry, to ensure no abnormalities precluded surgery. The anesthetic procedure for these rabbits was performed as described for the pilot study. The rabbits were induced 25-45 minutes after premedication using IV propofol (8 mg/kg initial dose with top-up if required). The tidal volume during ventilation was maintained at 15-20 mL/kg with a maximum ventilatory pressure of 15 mm Hg and ventilatory rate of 14 breaths per minute. The rabbits were monitored using an esophageal thermometer, electrocardiography, capnography, and pulse oximetry.

**Surgical Approach** – The order in which rabbits had surgery was randomly assigned, and on any given day, one LapOVE rabbit and one OVE rabbit had surgery. Surgeries were performed by an ACVS board-certified surgeon and an ACZM zoological companion animal resident, the former having significant experience with laparoscopic surgery in other companion animal
species and the latter having no prior experience with the exception of the pilot study. The individual assigned to be primary surgeon in any particular procedure was alternated on different days according to a pre-planned randomized sequence. The rabbits were placed in dorsal recumbency and the abdomen was clipped and aseptically prepared.

The laparoscopic ovariectomy was performed using 3 ports based on the results of the pilot study (Figure 13). A skin incision was made approximately 1 cm caudal to the umbilicus using a #15 scalpel blade. The subcutaneous fat was dissected in order to expose the linea alba, and two stay sutures were placed on either side of the linea using 4-0 polydioxanone\textsuperscript{n}. Using a modified Hasson technique, a stab incision was performed with a #15 blade and a 3.9 mm threaded graphite cannula\textsuperscript{o} was inserted into the abdomen. Carbon dioxide insufflation was applied at 8 mm Hg, based on optimal pneumoperitoneal pressures determined for rabbits in earlier studies.\textsuperscript{142,162} A 2.7 mm, 30\textdegree angle rigid endoscope\textsuperscript{n} with its 3.5 mm examination sheath\textsuperscript{o} was used to visualize the abdomen and ensure appropriate cannula placement. A second skin incision was made along midline about 3 cm cranial to the first port. While using the endoscope to visualize the placement, a 3.5 mm threaded graphite cannula with its pyramidal tip metal trocar was inserted into the abdomen after a scalpel stab incision (Figure 14A). This cranial port was used for positioning of fenestrated grasping forceps\textsuperscript{n}. A final skin incision was made about 3 cm caudal to the first port along midline. A 6 mm plastic cannula\textsuperscript{o} with a plastic smooth trocar was placed for positioning of the Ligasure\textsuperscript{TM} device (Figure 14B).

The rabbit’s thorax and abdomen were tilted about 45\textdegree to the right on the horizontal surgical table in order to visualize and access the left ovary (Figure 14C). Standing on the right side of the rabbit, the secondary surgeon controlled the endoscope to provide visualization on a
Figure 13: Placement of ports for laparoscopic ovariectomy in rabbits. (A) A 2.7 mm 30° sheathed rigid endoscope was placed in the initial 3.9 mm subumbilical cannula (b), a 3.5 mm cannula was placed for grasping forceps (a), and a 6 mm plastic cannula was placed for the Ligasure™ device (c). (B) Positioning of the ports in a rabbit patient.
Figure 14: Laparoscopic ovariectionomy in a rabbit using a 3-port approach. Using endoscopic visualization from the subumbilical port, the cranial 3.5 mm graphite cannula (A) and caudal 6 mm plastic cannula (B) were placed along midline. The left ovary was visualized (C), lifted with the grasping forceps, and the ovarian pedicle was sealed and transected using the caudal Ligasure™ device (D). The right ovary was similarly visualized from its position laterodorsal to the cecum (E), and again was grasped (F) prior to sealing and transecting the ovarian pedicle.
monitor, while the primary surgeon manipulated the grasping forceps and Ligasure™ device to seal and transect the ovarian pedicle and oviduct (Figure 14D). The ovary was grasped with the Ligasure™ device then gently removed from the abdomen through the 5 mm caudal incision after retracting the cannula up the shaft of the Ligasure™. The surgeons then moved to the left side of the rabbit and the procedure was repeated for the right ovary using a second slave monitor across from the first monitor for visualization. The right ovary was more difficult to access due to its position under part of the cecum (Figure 14E, F). Once both ovaries were excised, the cannulas were removed and manual compression was applied to release the remaining insufflated gas. The abdominal fascial closure was performed using 4-0 polydioxanone suture material in a simple interrupted pattern and the skin was apposed using 4-0 poliglecaprone 25 in a continuous intradermal pattern.

The open ovariotomy was performed using a standard midline skin incision with a #15 scalpel blade, starting at the umbilicus and extending caudally. Subcutaneous fat was bluntly dissected to reveal the linea alba, and the abdominal opening was made using a scalpel and Metzenbaum scissors being careful to avoid the underlying cecum. The left ovary was identified, grasped with hemostatic forceps, and the ovarian pedicle and oviduct were sealed and transected with the Ligasure™ device. The ovary was removed and the same procedure was repeated on the right side. The fascial closure was again performed using 4-0 polydioxanone suture material in a simple interrupted pattern and the skin closure was performed using 4-0 poliglecaprone 25 in a continuous intradermal pattern. In order to be comparable with the LapOVE, only the ovaries were excised using the Ligasure™ device.
The surgical time for either technique was measured in minutes from the start of the first skin incision until completion of the skin sutures. The anaesthetic time was measured from the time of intubation to the time of extubation. The incision lengths were measured in mm after sutures were placed.

On recovery from anesthesia, flumazenil\(^b\) (0.025 mg/kg IV and 0.025 mg/kg subcutaneously) was administered to partially reverse the sedative effects of midazolam given in the premedication so that rabbits could be returned to the animal facility with decreased supervision. Meloxicam\(^i\) (1 mg/kg IV) was administered to each rabbit on recovery. Post-operatively, the rabbits all received meloxicam\(^i\) (1 mg/kg orally once daily) for an additional 3 days and an additional buprenorphine\(^q\) injection (0.05 mg/kg subcutaneously) 4-5 hours after the premedication dose of hydromorphone.

**Post-operative Observations** – Food consumption was measured by weighing the pellets in grams twice daily (morning and evening) for 24 hours prior to surgery and 7 days after surgery. Fecal production was measured by counting fecal pellets and weighing the feces produced in grams, also twice daily for the same period of time. The same gram scale was used for all food and fecal weights. Rabbits were weighed once daily in kilograms using a consistent scale, starting prior to surgery and for 7 days after. Vital parameters (heart rate, respiratory rate, and rectal temperature) were also determined twice daily over these days.

Blood was collected from each rabbit once daily in the morning from a lateral saphenous vein, starting pre-operatively and for three days post-operatively. Whole blood was used to immediately measure blood glucose levels using a hand-held glucometer\(^r\). The remaining blood
was allowed to clot and separated within 1 hour of collection to obtain serum. Serum was frozen at -20°C until measurements of serum cortisol could be performed by chemiluminescence using a reference laboratory.

Abdominal palpation was performed twice daily for 7 days post-operatively. This was evaluated subjectively for any abnormalities, such as tense muscles, which may indicate discomfort or decreased cecal fill, which may suggest decreased food consumption. The incision sites were also examined twice daily during this time frame and assessed for any complications including incisional infection, dehiscence, or swelling.

**Behavioral Observations** – The rabbits were videotaped for 45-60 minutes twice daily (7 am and 6 pm) with programmed cameras placed approximately 1 metre in front of the pen doors, such that the entire pens could be visualized on the video. People were not allowed access to the room during taping. The first 10 minutes of video footage was removed to allow the rabbits' behaviors to settle. A single blinded observer examined the next 30 minutes of video footage from each rabbit in the morning and in the evening, starting the day prior to surgery and continuing for 3 days after surgery. The observer, who was trained to recognize and score behaviors consistently, was unaware of the surgical approaches assigned to each rabbit and the appearance of surgical incisions could not be identified from the videos. Several behaviors were assessed and measured, either in duration of time that behaviors were displayed or in number of occurrences (Table 2). These behaviors were part of an ethogram previously developed to assess post-operative pain expression in rabbits.\textsuperscript{120,121} Some similar behaviors were combined into composite groups defined in Table 2 based on previous associations,\textsuperscript{120} in order to improve the
frequency and duration of observations and statistical comparisons between surgical groups. For behaviors measured in numbers or counts, the total count was combined from both morning and evening observations and compared daily in order to remove variation associated with time of day and improve statistical power.

The rabbit facial grimace scale\textsuperscript{113,114} was determined at the start and end of every video sequence. This scale is composed of five facial action units: ear position, orbital tightening, cheek flattening, nose shape, and whisker position. The facial action units were assigned scores from 0 to 2, and these were combined to form the total FGS score. The FGS scores from the start and end of both morning and evening video sequences were averaged, and averaged scores were compared across days and between surgical groups so as to remove variability over the course of the day and improve statistical power.

**Statistical Analysis** – Data was analyzed using the R software\textsuperscript{k}. The intra-operative outcomes, surgical time, anaesthetic time, and incision length, were assessed using multiple linear regression with primary surgeon and surgical approach as predictor variables. Assumptions of linear regression were evaluated using qq (quantile-quantile) normality plots and residual plots. Outliers were removed as appropriate. Possible interactions between predictor variables were assessed for significance in the models.

Post-operative outcomes were analyzed for 5 LapOVE rabbits and 6 OVE rabbits. Continuous post-operative outcomes and behavioral observations measured in units of time were evaluated using mixed effects linear regression models. Rabbit identifications were applied as random effects. The outcomes were compared between surgical approaches (laparoscopic versus
open ovariectomy) applied as fixed effects in the models. The time of day (morning versus evening), the time in hours from first video assessment, and the interval relative to surgery (before versus after surgery) were also included as fixed effects when they improved the fit of the model and significantly affected the outcome variables. Model assumptions were evaluated, and outliers were evaluated and removed as appropriate. Variables that were strongly correlated based on Fisher’s exact test, Pearson’s correlation, or analysis of variance were removed from the models in order to provide the best fit. Analysis of variance was performed after linear regression models to identify the significance of each predictor variable in the model. Tukey adjustment was performed for multiple comparisons when assessing interaction between predictor variables.

Count outcome variables (i.e. behaviors measured in numbers of occurrence over the day) were evaluated using Poisson regression in mixed effects models; where overdispersion was present, negative binomial regression models were applied. Again, rabbit id was applied as a random effect. The frequency of behaviors was compared between surgical approaches (laparoscopic versus open ovariectomy). The time from first video assessment and the interval relative to surgery (before versus after surgery) were also applied as fixed effects when they improved the fit of the model and significantly affected the behavior. Assumptions of Poisson and negative binomial regression were confirmed, and outliers were evaluated and removed when appropriate.

Abdominal palpation findings, identified as either normal or abnormal, were analyzed using mixed effects logistic regression, with rabbit id as a random effect and with surgical approach and post-operative day as fixed effects. Averaged FGS scores over 24-hour periods
were assessed using mixed effects ordinal logistic regression, with rabbit id as a random effect and with surgical approach and time relative to surgery as fixed effects.

Effects were considered statistically significant if $p < 0.05$. Figures were created using the “ggplot2” data visualization package of R.\textsuperscript{129}
<table>
<thead>
<tr>
<th>Behavior</th>
<th>Description</th>
<th>Duration (minutes)</th>
<th>Number of incidents</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Activity:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Activity level</td>
<td>Being active and moving around the pen</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Exploring</td>
<td>Exploring environment</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Eating</td>
<td>Eating from food dishes</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Drinking</td>
<td>Drinking from water bottles</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Time at front</td>
<td>Located in front half of pen</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Time at back</td>
<td>Located in back half of pen</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Time in hut</td>
<td>Located in clear plastic hut</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Digging</td>
<td>Digging action on floor</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Jumping</td>
<td>Jumping straight up</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Interacting</td>
<td>Interaction with objects in the pen</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td><strong>Locomotion:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hopping</td>
<td>Hopping around the pen</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Shuffling*</td>
<td>Walking slowly around the pen</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Movement composite*</td>
<td>Combined duration of time spent hopping and/or shuffling</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Hindlimb shuffle*</td>
<td>Slow shuffle of hindlimbs after stretching forelimbs forward</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Partial hop*</td>
<td>Extending forelimbs forward, but not completing hop with hindlimbs</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Stomping</td>
<td>Stomping one foot on the floor</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td><strong>Groom:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grooming</td>
<td>Grooming head, feet, or body</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Grooming abdomen, flank*</td>
<td>Grooming abdomen or flank</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td><strong>Posture:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recumbency</td>
<td>Lying in sternal or lateral recumbency</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Rearing</td>
<td>Rearing or standing on hindlegs</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Stretching</td>
<td>Stretching various parts of the body</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Alert</td>
<td>Stops suddenly with ears erect</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Yawning</td>
<td>Yawning by the rabbit</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Behavior</td>
<td>Description</td>
<td>Duration (minutes)</td>
<td>Number of incidents</td>
</tr>
<tr>
<td>------------------------</td>
<td>-----------------------------------------------------------------------------</td>
<td>--------------------</td>
<td>---------------------</td>
</tr>
<tr>
<td><strong>Inactive Pain</strong></td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Huddling*</td>
<td>Sitting with back arched</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Eyes closed</td>
<td>Eyes closed or semi-closed</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Abdominal pressing*</td>
<td>Arching back and pressing abdomen to the floor</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Stagger*</td>
<td>Falling or loss of balance</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Drawing back*</td>
<td>Wincing or rapid brief drawing back with grimace or blink</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Full-body flex*</td>
<td>Flinching or rapidly flexing body upwards</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Twitching*</td>
<td>Rapid movement of skin or subcutaneous muscles</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Composite of Inactive</td>
<td>Combined counts of twitching, drawing back, stagger, full-body flex,</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Pain Behaviors*</td>
<td>abdominal pressing, and hindlimb shuffle</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Video-recorded behaviors assessed by a blinded observer, expressed as a duration of time (minutes) or a number of incidents. An asterisk indicates behaviors that have previously been associated with pain expression in rabbits.\textsuperscript{120,121}
5.3 Results

Intra-operative Outcomes – There was a significant increase in surgical time for the LapOVE group \( (p = 0.0013) \), but no significant association with who was assigned as the primary surgeon \( (p = 0.2098) \). The mean time (with 95% confidence interval, CI \( +/\) standard deviation, SD) was 43.2 minutes \( (34.9-51.5 +/\ 9.5) \) for the laparoscopic surgery as compared to 21.7 minutes \( (17.0-26.3 +/\ 5.8) \) for the open surgery. The laparoscopic surgery time was divided between time for port placement (mean +/- SD, 10.4 +/- 4.3 minutes), time for the ovariectomy itself (14.2 +/- 2.9 minutes), and time for incision closure (16.4 +/- 3.0 minutes).

Similarly, the anaesthetic time was significantly longer for LapOVE than for OVE \( (p = 0.0057) \) with no significant difference when accounting for primary surgeon in the model. The mean anaesthetic time (95% CI +/- SD) was 76.2 minutes \( (63.4-89.0 +/\ 14.7) \) for LapOVE and 48.8 minutes \( (40.4-57.2 +/\ 10.5) \) for open OVE.

There was also a significant difference in incision length between the surgical approaches \( (p = 0.0011) \), unaffected by surgeon assignment \( (p = 0.7778) \). The mean (with 95% CI +/- SD) of the total incision length was 24.0 mm \( (20.9-27.1 +/\ 3.5) \) for the LapOVE group as compared to 41.5 mm \( (33.0-50.0 +/\ 10.6) \) in the open OVE group. The three laparoscopic incisions had mean lengths of 4.8 mm \( (+/\ 0.4 \text{ SD}) \) for the cranial incision, 12.2 mm \( (+/\ 4.0) \) for the middle incision, and 7.0 mm \( (+/\ 2.0) \) for the caudal incision.

Post-operative Outcomes – The surgical approaches did not result in any statistically significant differences between treatments in objective post-operative outcome variables: weight of food consumed \( (p = 0.2510) \); number and weight of feces produced every 12 hours \( (p \geq 0.8411) \);
change in body weight ($p = 0.3077$); heart rate ($p = 0.2645$); respiratory rate with the exception of a decrease the evening after surgery ($p = 0.6527$); body temperature with the exception of a decrease the evening after surgery ($p = 0.8648$); and glucose measurements ($p = 0.6330$).

Conversely, the time of day (morning versus evening) and time in the study from first evaluation did have some significant effects on these outcomes. With the exception of the 48 hour period after surgery when fecal production was low, there was an interaction effect such that fecal production was decreased during the days as compared to during the nights ($p \leq 0.0032$, Figure 15A). Similarly, food consumption was decreased during the days ($p < 0.0001$, Figure 15B). Glucose measurements were also found to increase slightly over the 4 days that they were evaluated, with the exception of the morning after surgery ($p = 0.0004$, Appendix Supplementary Figure 1).

Blood cortisol measurements were all < 28 nmol/L (approximately 1 μg/dL, the lower limit of the laboratory test) except for a single time point in one rabbit. Therefore, no difference could be assessed statistically.

The subjective assessment of normal versus abnormal abdominal palpation was not significantly different between the two surgical approaches. However, there was a trend towards increased odds of abnormal palpation findings in the open OVE group as compared to the LapOVE group (OR = 2.52, 95% CI: $0.63-10.75$, $p = 0.1250$, Figure 16A). There was a significant effect of day after surgery, such that the odds of a normal palpation outcome increased by 1.48 (95% CI: 1.25-1.79) for every day after surgery ($p < 0.0001$, Figure 16B). Abnormal palpation findings included apparently decreased cecal fill and tense muscles on palpation.
**Post-operative Complications** – The most significant complication in the LapOVE group was small intestinal perforation. Despite the use of stay sutures to elevate the body wall away from the underlying intestines, a loop of bowel was inadvertently grasped with a stay suture. During placement of the initial optical cannula, the small bowel was entered. In this study, the rabbit was euthanized according to our animal use protocol once the abdomen was explored to ensure no anatomic variation or other abnormalities were present. As a result, all other post-operative outcomes were analyzed on a total of 5 rabbits in the LapOVE group and 6 rabbits in the OVE group.

Additional complications in the LapOVE group included subcutaneous emphysema in one rabbit resolving without treatment 4 days after surgery, and mild edema or seroma around the incision for 2 days in one rabbit.

Taking into account both the pilot study and the complete study, incisional dehiscence due to licking or scratching of the wounds was seen in 2 out of 11 LapOVE cases (one from the pilot study and one from the controlled trial) and 2 out of 6 OVE cases (in the controlled trial). The dehiscence was minor in all cases and similar in the two surgical groups, with only minimal to partial opening of the skin incision. These cases were treated with a combination of topical cleaning, topical antimicrobials\(^u\) (2/4 rabbits), E-collar placement (3/4 rabbits), oral enrofloxacin\(^v\) (10 mg/kg twice daily for 10 days in 1/4 rabbits), and placement of skin staples to re-appose skin edges (2/4 rabbits). Additional days of meloxicam treatment was also provided for the 3 rabbits that required E-collars.
**Behavioral Outcomes** – From the behaviors assessed by the blinded observer, those that have previously been associated with post-operative pain expression in rabbits are indicated by asterisks in Table 2. There was no significant difference between the two surgical approaches in the time and frequency with which any behaviors were displayed ($p > 0.30$). Certain behaviors were seen more often in one surgical approach than another, but not significantly so: frequency and duration of abdominal pressing (increased in LapOVE group, $p \geq 0.0607$), frequency of inactive pain composite behaviors (increased in LapOVE group, $p = 0.0725$), time spent eating (increased in OVE group, $p = 0.0914$), and frequency of yawning incidents (increased in LapOVE group, $p = 0.1540$).

On the other hand, time variables did significantly affect the duration or frequency with which behaviors were expressed (Appendix Supplementary Table 1). These variables include time of day (morning versus evening), time in hours from first video assessment (0 to 84 hours), and time interval relative to surgery (before versus after). Overall, rabbits spent more time exploring and displaying the composite movement behaviors (hopping and shuffling) in the morning, and alternatively were recumbent for longer in the evening. The following behaviors were seen significantly less frequently in the 24 to 48 hours following surgery: interacting, hopping, partial hops, rearing, stretching, and alert. On the other hand, the composite inactive pain behaviors were seen more frequently for 24 hours following surgery. Finally, when examining behaviors displayed before versus after surgery, the duration of time spent drinking and displaying the composite movements was decreased, while the duration of time spent huddling and with eyes closed was increased.
Digging and stomping behaviors were not displayed by any rabbits in this study. Twitching behavior was only displayed once by two individual rabbits, and was not compared alone between the surgical groups. However, twitching was included in an analysis as part of the composite inactive pain behaviors.

For facial grimace scale scores, whisker position could not be determined by the observer from most videos; those that could be determined were scored as 0. Therefore, whisker position was excluded when determining the FGS score. Daily FGS scores were not significantly affected by surgical approach (OR = 0.89, 95% CI\text{nt} = 0.30-2.64, p = 0.8290); however, the odds of the average FGS score increasing by 0.25 after surgery is 17.81 as compared to before surgery (95% CI\text{nt} = 4.18-75.84, p < 0.0001, Figure 17).
Figure 15: The effect of time on fecal production (A) and food consumption (B) in grams (displayed as mean with standard deviation error bars). The fecal production and food consumption were decreased over the day, as opposed to overnight. However, over the 48 hour period after surgery (surgery time indicated by the dashed vertical line) there was a consistent decrease in feces and food consumption in both surgical groups.
Figure 16: Abdominal palpation was subjectively assessed as being normal or abnormal. (A) There was an increased odds of abnormal abdominal palpation in OVE as compared to LapOVE (OR = 2.52, 95% CI: 0.63-10.75). The percentage of observations occurring in any given group (surgery and palpation result) is indicated above the bars. (B) The odds of a normal palpation outcome increased by 1.48 (95% CI: 1.25-1.79) for every day after surgery.
**Figure 17**: Averaged daily facial grimace scale scores, with medians, interquartile ranges, and ranges illustrated by the boxplots. Although FGS score did not differ between the surgical treatments ($p = 0.8290$), they were significantly lower before surgery as compared to after ($p = 0.0001$).
5.4 Discussion

The only significant differences seen between surgical approaches in this study were with respect to surgical time, anaesthetic time, and incision length. This is in contrast to other laparoscopic studies that have identified improvements in post-operative activity in dogs;\textsuperscript{51} decreased pain expression and morbidity in both dogs and cats;\textsuperscript{52,87-90,92} decreased incidence of infection or adhesions in dogs, cats, and rabbits;\textsuperscript{76,78-80,94} and decreased hospitalization times in dogs.\textsuperscript{57,96} The lack of differences seen in rabbits may be a result of several factors. First of all, pain expression in rabbits is poorly understood and can be subtle. Changes in facial grimace scales, for example, can be small and require good imaging equipment to confidently differentiate. As a result of subtle signs, a type II error is possible and a larger sample size may improve the ability to detect a difference. On the other hand, it is also possible that any difference that exists between laparoscopic ovariectomy and open ovariectomy is small; therefore, the clinical relevance of such a difference may not be important. The same cannot be said for other laparoscopic surgeries. Ovariectomy is a simple surgical procedure with minimal tissue handling and easy visualization, even without an endoscope. The training time required is minimal, and veterinary medical students can readily be taught to complete laparoscopic ovariectomies with performance similar to that of open ovariectomies.\textsuperscript{163} In the current study, little difference was seen in the post-operative outcomes when comparing surgeries performed primarily by a resident versus those performed primarily by a board-certified surgeon. More complex surgeries or those that benefit from the magnification may result in lower surgical stress, post-operative pain, and better return to function when performed laparoscopically as opposed to using a conventional open approach. This has been verified in canine laparoscopic
procedures, such as adrenalectomy, showing lower complication rates and shorter surgical time with the benefit of laparoscopic visualization.  

Concerning the intra-operative parameters assessed in this study, shorter anaesthetic time with open ovariectomy would be beneficial for rabbit patients. Anesthesia in rabbits has greater potential for complications than is seen in other companion animals.\textsuperscript{133,164} Shortening the time under anesthesia will decrease the risks associated with the procedure, especially for older patients or those with cardiovascular instability. This is of particular importance when providing carbon dioxide insufflation with laparoscopy.\textsuperscript{165} During laparoscopic surgeries, intubation and detailed anesthetic monitoring are important to minimize the physiological impact of pneumoperitoneum.\textsuperscript{162} Open ovariectomy took on average half the time required for laparoscopic ovariectomy. With experience it is expected that a surgeon would improve efficiency with this technique;\textsuperscript{57,166,167} both surgeons involved with this study had limited experience performing laparoscopic ovariectomy specifically in rabbits, with only the pilot animals ovariectomized prior. Approximately 24\% of the LapOVE surgical time (10.4 out of 43.2 minutes) was due to placement of the three ports, which is not required for an open technique. Furthermore, improper placement of the cannulas can result in life-threatening complications including trauma to abdominal organs.\textsuperscript{1,102,167,168} Single port and 2-portal entry techniques have been described for rabbits and other companion animals.\textsuperscript{106,109} These methodologies may lead to differences in rapidity of surgical access and overall surgical times. It is recommended that port placement be a focus of training for practitioners that are new to laparoscopic surgery, since proficiency in this area has the potential to significantly decrease surgical time and complications.
Another important consideration during port placement is the positioning of surgical instruments. Unless surgeons are ambidextrous, it could be a hindrance to restrict the surgeon’s approach using asymmetric cannula placement. With a large cannula placed caudally for the Ligasure™ device, as in this study, the surgeons were unable to use that instrument in the same hand when alternating sides during ovarian pedicle sealing and transection. This could affect surgical dexterity, and thereby influence surgical times or induced trauma. Although evidence to support this supposition could not be determined from the current study data, the use of equally-sized cannulas cranially and caudally is recommended for future studies.

It can be challenging to objectively assess post-operative pain and recovery in companion animals. In this study, we assessed normal body functions (i.e. appetite, defecation), physiologic parameters (i.e. vital parameters, glucose, and cortisol), and behavioural ethograms. There was no significant difference between surgical approaches in the objective parameters measured post-operatively in these rabbits, including food consumption, defecation, vital parameters, weight maintenance, and blood glucose measurements. These parameters were selected to reflect the recovery and comfort of the rabbits post-operatively; changes in these parameters could be considered indicative of infection or pain. The lack of difference between surgical groups could be due to provision of appropriate post-operative analgesia, although the analgesic protocol was fairly conservative: 12 hours of opioid analgesia and 3 days of non-steroidal anti-inflammatory treatment. Furthermore, if analgesics had been sufficient to mask the differences in pain experienced by the rabbits in the two surgical groups, we should not have seen a difference in parameters measured immediately post-operatively as compared to pre-operatively or several days later. A decrease was seen in food consumption, fecal production, and glucose
measurements over the first 48 hours after surgery. Certainly, these changes could have been reflective of changes in gastrointestinal motility as a result of anesthesia or analgesia, but we cannot disregard a possible association with surgical intervention or pain. Pneumoperitoneum with elevated intra-abdominal pressures and CO2 flow rates in rabbits has also been associated with desiccation and adhesions of abdominal organs in rabbits, potentially contributing to post-operative gastrointestinal discomfort after laparoscopy. Interestingly, no intervention was required (ex. promotility agents, additional analgesics, supportive feeding) for these rabbits and food consumption and fecal production returned to normal after two days.

The possibility of a type II error for post-operative outcomes as a result of small sample size was considered. However, post hoc power analyses from the current data (α = 0.05; β = 0.2) determined that sample sizes of greater than 88 rabbits per treatment group would be necessary to identify a statistically significant difference in food consumption (Cohen’s d = 0.189), fecal production (Cohen’s d < 0.041), vital parameters (Cohen’s d < 0.303), and weight change (Cohen’s d < 0.424) providing other variables and factors were unaltered (e.g. surgical technique, surgeon skill). Such a large sample size would not be easily achievable for a study in rabbits; furthermore, the effect is still very small and the difference of means between treatment groups may not be clinically significant.

Another interesting effect that was observed in this study, although unrelated to the ovariectomy, is the effect of time of day on observations. Fecal production and food consumption were significantly reduced during the day as opposed to during the nights. We know that rabbits are crepuscular or nocturnal species, and likely to show increased activity and digestion at the end of the day or early in the morning. There were no windows to indicate
dawn and dusk, but these rabbits were acclimated to 12-hour light cycles. This effect needs to be considered for any future studies on behavioral expression of pain. It is important to consistently monitor rabbits at the same time of day for behavioral outcomes, as was done in this study.

Previous reports on post-operative pain in rabbits have looked at the use of glucocorticoid hormones to assess stimulation of the hypothalamic-pituitary-adrenal axis as a response to stress. In fact, lower cortisol, as well as epinephrine and TNF-α levels, were identified within 24 hours of laparoscopic ovariohysterectomy in rabbits when compared to open surgery. This may be an indicator of lower surgical stress with the laparoscopic approach; however, evaluation beyond 24 hours was not performed in that study. Unfortunately, cortisol levels could not be applied in the current study as a measure of post-operative stress or pain due to inability to appropriately measure the levels. Nonetheless, the levels in all rabbits were consistently below 28 nmol/L (approximately 1 μg/dL), which is lower than cortisol levels reported for 48 hours post-operatively in another rabbit surgical study. This would suggest that neither surgical group in this study encountered significant post-operative stress.

Rabbits are known to excrete both cortisol and corticosterone in response to stress; in this case, cortisol was the most readily available test to perform. Nevertheless, it may be beneficial for future studies to measure corticosterone as a comparative test together with cortisol. Both glucocorticoids are known to respond to circadian rhythms, with peak levels occurring in the afternoons and nadirs in the early morning hours. Higher cortisol levels may have been obtained if the blood samples in this study had been collected in the afternoon. An alternative approach for assessing stress responses post-operatively would be to measure fecal or urine cortisol levels. These samples would have less potential to be affected by daily fluctuations and
acute stress from handling of the animal. It is also important to note that cortisol levels can be elevated in a transient manner; one study found that cortisol levels after ovariohysterectomy in dogs returned to normal within 24 hours after surgery.

Similarly, there are limitations to blood glucose monitoring. Blood glucose levels can change rapidly in response to stress, and the measurements obtained during collection with manual restraint may not be reflective of the usual blood glucose levels at rest. Blood samples were collected in the morning prior to surgery as baseline parameters. The post-operative levels were higher than baseline, with the exception of the morning after surgery. The initial decrease may be a result of decreased appetite over the previous 12 to 24 hours and did not differ between surgical treatments. The subsequent increases in blood glucose could have been a result of post-operative pain or a reflection of anticipated stress with handling for rabbits that were experiencing their third and fourth venipunctures. Future studies could consider indwelling catheters for blood collection with minimal restraint requirements. Despite the limitations of using physiologic blood parameters for assessment of pain, glucose and glucocorticoids continue to be valuable objective measures for metabolic and systemic stress responses after surgery.

While some studies have found associations between glucose or glucocorticoids and pain, other studies have been inconsistent; surgical trauma, inflammation, and post-operative restraint may concurrently influence measured glucose and glucocorticoid levels. More subjective evaluations, including behavioral ethograms, are important fields for future investigation. Abdominal palpation findings were used as a subjective assessment of post-operative well-being in these rabbits. Rabbits that tensed their abdominal muscles were presumed to be uncomfortable or stressed. Rabbits that had apparently less digesta in the cecum
were presumed to be eating less or experiencing changes in intestinal motility. Although there was no statistically significant difference between the surgical treatments, more rabbits in the open ovariectomy group had abnormal palpation findings; however, it must be emphasized that the evaluator was not blinded to the treatment group. Again, *post-hoc* power analyses based on the current data ($\alpha = 0.05; \beta = 0.2; \text{Cohen’s } h = 0.353$) determined that the sample size required to identify a statistically significant effect would be at least 126 rabbits for each treatment group. The effect size is small, and it would be difficult to achieve these numbers using laboratory or pet rabbits. In this study, rabbits with open OVE had longer surgical incisions and more tissue manipulation associated with the surgery, which may contribute to depressed gastrointestinal motility and increased pain post-operatively. Concurrently, the use of post-operative analgesic medications, such as buprenorphine, may promote gastrointestinal discomfort or side effects.\textsuperscript{124,173-175} Abdominal palpation abnormalities were present transiently and normalized over the week of observation after surgery. No additional intervention was required to manage these findings, and changes in abdominal palpation should be expected by surgeons after abdominal procedures in rabbits.

Facial grimace scales have been developed for rabbits to help evaluate pain by assessing facial action units.\textsuperscript{114,176} FGS scores are useful when evaluating individual rabbits in a clinical setting; however, it can be difficult to evaluate these facial action units from a distance or on video monitors. There is a risk that some important aspects of the FGS may be missed. Behavioral ethograms have also recently been used to assess changes that occur with post-operative pain in rabbits.\textsuperscript{114,120,121,123,124} FGS scores and ethograms are both susceptible to observer bias, and may be influenced by the use of sedative or analgesic drugs. Therefore,
behaviors need to be clearly described and assessed by blinded observers that evaluate each animal consistently. It is important to ensure that rabbits are undisturbed by people, that the environment is unchanged, that each pen is similar in layout, and that the rabbits are assessed at the same time of day.\textsuperscript{120,124} Time of day significantly affects the frequency and duration with which some behaviors are expressed. Leach \textit{et al.} previously showed that activity level overall was decreased in the morning relative to the afternoon.\textsuperscript{120} In contrast, rabbits in this study displayed more exploratory behavior in the morning and more time being recumbent in the evenings (Appendix Supplementary Table 1).

Some behaviors have previously been associated (increased or decreased) with surgical pain in rabbits. However, the relative importance of each behavior in the expression of pain is uncertain, so no scores have been developed yet to combine this information. Post-operatively, rabbits have been shown to display decreased activity, rearing behavior, and frolicking (hopping and jumping), increased time sitting, huddling, abdominal pressing, grooming abdomen and flank, and increased incidence of wincing and flinching behaviors, and shuffling or truncated gaits (see Table 2).\textsuperscript{120,121,123,124} Composite behaviors, including duration of movements (i.e. hopping, shuffling) and frequency of inactive pain behaviors (ex. twitching, drawing back, stagger, full-body flex, abdominal pressing, and hindlimb shuffle), are also important indicators of post-operative pain in rabbits.\textsuperscript{120} Despite there being no significant difference between surgical treatments in the expression of behaviors, it is interesting to note that abdominal pressing and inactive pain behaviors were slightly increased in the laparoscopic ovariection group. These behaviors have previously been associated with pain expression, so one could speculate that the laparoscopic ovariection cases showed slight increases in discomfort possibly
associated with morbidities including subcutaneous emphysema, incisional swelling, and dehiscence. Most of these measured variables, while evaluated using standardized methods and through blinded distant observers, are more difficult to assess than objective numerical variables. Thus, a type II error should not be discounted.

Another limitation of this study is the use of only one rabbit breed, the New Zealand White. This breed is commonly used in research and there is previous literature on the post-operative response of these rabbits to surgical stress. However, New Zealand White rabbits are larger than the average companion rabbit breed; laparoscopic access and visualization is expected to be easier when compared to smaller rabbit breeds. As a result, surgical times and the selection of surgical instruments may vary for other breeds; for example, a 5 mm vessel sealing device (ex. Ligasure™) may require replacement with smaller devices or cautery forceps. The expression of post-operative stress or pain may also differ as a result of anatomical conformation (ex. FGS scores), behavioural differences, and socialization with humans. These factors need to be taken into account when applying the current knowledge to clinical practice. A randomized controlled clinical trial is recommended to further evaluate the advantages and disadvantages of laparoscopic ovariecotomy in pet rabbits.

Incisional dehiscence is a common surgical complication that was seen for both open and laparoscopic ovariecotomy in this study. These rabbits only opened the skin layers, presumably due to discomfort from sutures. This could occur with any surgery, regardless of complexity, and has been reported in other companion species as well. However, one would expect that the smaller incision size with a laparoscopic approach would decrease the incidence or extent of dehiscence or incisional infection, as has been shown in other veterinary species and
In a retrospective study on post-operative complications in dogs after laparoscopic ovariectomy, 5% (7/132) opened their skin incisions. Similarly, another study comparing laparoscopic to open ovariectomy found superficial surgical site infections in 3.2% versus 4.7% of dogs, respectively, and no cases of dehiscence in the laparoscopic group versus dehiscence in 3.7% of open surgeries. The rabbits in the current study were assessed twice daily, but not monitored more closely for the remainder of the day. In an owned animal, this complication could easily be avoided by having increased monitoring for overgrooming of the abdomen and maintaining cleaner surroundings to prevent infection. As a result of dehiscence, one LapOVE rabbit and one open OVE rabbit being video recorded required E-collars and an additional day of meloxicam treatment. These animals were not removed from the behavior analyses; however, it was confirmed that they were not outliers in their expression of behaviors, since it is presumed that an E-collar or additional analgesia could influence certain behaviors.

The complications that are unique to laparoscopic surgeries are subcutaneous emphysema and organ trauma during abdominal entry. Emphysema has been reported to occur as a result of improper cannula placement and elevated intra-abdominal pressures during carbon dioxide insufflation. Reported incidence rates in dogs are approximately 6-12%. It is a source of potential discomfort post-operatively, but is otherwise a minor concern with resolution occurring over a few days. Organ trauma, on the other hand, can be serious. In this study, perforation of the small intestines during placement of the first cannula resulted in euthanasia of one rabbit. Trauma to intestines, including the cecum, is a significant risk in rabbits due to the position of these organs under the initial abdominal incision. This is a serious complication which could result in development of peritonitis. In dogs, the greatest risk of trauma is to the spleen, with an
incidence of 7-18% reported by several studies.\textsuperscript{54,87,100-102,105} If organ trauma is suspected during laparoscopic surgery, it may be necessary to convert to an open technique to improve access and resolve the problem. In dogs having laparoscopic and laparoscopic-assisted gastropexies, access-related complications including splenic laceration and bladder rupture have been described in 5/49\textsuperscript{177} and 2/30\textsuperscript{105} dogs. In the former group, three of the dogs required conversion to an open technique. For laparoscopic nephrectomy and ovariohysterectomy, about 18\% of dogs had mild, self-limiting splenic hemorrhage during placement of the first cannula.\textsuperscript{87,100} Likewise, splenic laceration has occurred in single and two-port laparoscopic ovarioectomy, but without a need to convert to an open approach to manage the hemorrhage.\textsuperscript{54,101} A Hasson technique for abdominal entry is recommended to decrease these risks, although they are still reported to occur.

In young healthy rabbits for elective procedures or procedures that require better visualization, laparoscopy is a good option. In this case, ovariectomy was used as a model for an elective procedure that is commonly performed in young rabbits. Due to the high incidence of uterine pathology and adenocarcinoma in rabbits, ovariectomy without hysterectomy is only recommended in animals that are up to 6 months of age.\textsuperscript{116,119} Laparoscopic surgical complications can be minimized by using a Hasson technique and visualizing the entry of cannulas. However, the increased cost of laparoscopic surgeries and requirement for specialized equipment may deter some practitioners from providing these services.\textsuperscript{96,178,179} Based on this study, laparoscopic ovariectomy did not provide added advantages over standard open ovariectomy, other than decreasing incision length. Open surgical techniques are still advised for patients in which anesthetic time needs to be minimized and the effects of carbon dioxide insufflation for laparoscopy may be deleterious. Even for routine laparoscopic surgeries, it is
always important to reserve the option to convert to an open technique if complications such as organ trauma occur.

### 5.5 Footnotes

a. Charles River Laboratories, Saint-Constant, Québec, Canada  

b. Sanofi Canada Inc., Boucherville, Québec, Canada  

c. BD Canada, Mississauga, Ontario, Canada  

d. Plasmalyte-A, Baxter Healthcare, Deerfield, Illinois, USA  

e. Pharmascience Inc., Montreal, Québec, Canada  

f. IsoFlo®, Zoetis Canada Inc., Kirkland, Québec, Canada  

g. Medtronic (Covidien) Canada, Saint-Laurent, Québec, Canada  

h. Fresenius Kabi Canada Ltd., Toronto, Ontario, Canada  

i. Metacam® 20 mg/mL Injectable, Boehringer Ingelheim, Burlington, Ontario, Canada;  

j. Metacam® 1.5 mg/mL Oral Suspension, Boehringer Ingelheim, Burlington, Ontario, Canada  

k. R version 3.5.1, R Core Team, R Foundation for Statistical Computing, Vienna, Austria  

l. Fisherbrand™, Fisher Scientific, Ottawa, Ontario, Canada  

m. PDS® II, Ethicon, Johnson & Johnson Medical Products, Somerville, New Jersey, USA  

n. Karl Storz Endoscopy America, Inc., El Segunda, California, USA  

o. VersaOne™, Covidien Canada, Saint-Laurent, Québec  

p. Monocryl®, Ethicon, Johnson & Johnson Medical Products, Somerville, New Jersey, USA  

q. Vetersetic®, Sogeval UK Ltd., Sheriff Hutton, York, UK
r. Contour® glucometer, Bayer Inc., Mississauga, Ontario, Canada

s. Animal Health Laboratory, University of Guelph, Guelph, Ontario, Canada


u. Flamazine, 1% silver sulfadiazine, Smith and Nephew Inc., Mississauga, Ontario, Canada

v. Compounded by Ontario Veterinary College Pharmacy, Guelph, Ontario, Canada
Chapter 6: Conclusion and Future Directions

6.1 Conclusion

Carbon dioxide pneumoperitoneum has advantages and disadvantages, as previously discussed. Increased intra-abdominal pressures can affect visceral perfusion, cardiovascular parameters including cardiac output, and ventilation due to thoracic compression. However, pneumoperitoneum is important for visualization and surgical manipulation during laparoscopy. One objective of this thesis was to measure working space in rabbits using pneumoperitoneal volumes from CT measurements at intra-abdominal pressures of 4, 8, and 12 mm Hg. Based on these volumes, it was found that the increase in working space dependent on IAP is not linear. From 4 to 8 mm Hg, there was a 19% increase in working space, and from 8 to 12 mm Hg there was an increase of only 6.9%. Abdominal working space in rabbits can be optimized at lower pneumoperitoneal pressures than traditionally used. This is particularly relevant during laparoscopic surgeries with prolonged anaesthetic times, or in patients with pre-existing cardiovascular or ventilatory compromise. Based on this working space evaluation in rabbits, it is recommended that IAPs should be maintained at 8 mm Hg or less.

Another objective of this thesis was to quantify and evaluate the cardiovascular and respiratory effects of different intra-abdominal pressures in rabbits. Carbon dioxide insufflation was applied at pressures of 0, 4, and 8 mm Hg. Heart rates, peripheral oxygen saturation, and arterial blood pressures were not significantly affected by these pressures. As previously reported in other companion animals, PaCO₂ and ETCO₂ both increased at higher IAP as a result of carbon dioxide diffusion from the abdomen into the bloodstream. The abdominal pressure also
caused thoracic compression and an increase in peak inspiratory pressure, as tidal volume remained constant. Finally, pneumoperitoneum also caused significant decreases in cardiac output and cardiac index. Although these changes may not be clinically significant for a healthy patient, it is important for an anaesthetist to be able to monitor for and manage these effects during abdominal insufflation for laparoscopy. In patients with any underlying cardiovascular or pulmonary disease, these changes may cause significant concerns. It is important, therefore, to minimize the IAP used. Although there were significant differences in outcome variables as IAP increased from 4 to 8 mm Hg, the effects were still clinically tolerable up to 8 mm Hg. However, IAPs greater than 8 mm Hg are not recommended in rabbits, because some cardiorespiratory parameters will be difficult to maintain within an acceptable level.

In dogs, cats, and humans, laparoscopic approaches have been associated with decreased pain scores, increased post-operative activity, shortened hospitalization stays, and lower incidences of infection or adhesions. Using ovariectomy as a surgical model, a final aim of this thesis was to objectively determine the value of laparoscopy for rabbits. To this end, intra-operative and post-operative outcomes were compared in rabbits undergoing laparoscopic versus conventional open ovariectomy. It was found that laparoscopic surgical and anaesthetic times were longer and incision lengths were shorter when compared to open OVE. However, there were no significant differences identified in post-operative outcomes, including pain expression, activity level, and appetite. Surgical complications were encountered in both groups, including mild incisional dehiscence. However, complications unique to laparoscopy and pneumoperitoneum were subcutaneous emphysema and intestinal perforation.
It must be noted, however, that this thesis is based on research in New Zealand White rabbits specifically. This breed is large relative to other companion rabbit breeds, so the achievable working space volumes and laparoscopic visualization may vary between rabbits of different ages or sizes. Furthermore, breed-specific differences in behavioural expression of post-operative stress and pain may occur, in particular when using laboratory rabbits that are less often socialized by humans from a young age. Future studies are recommended to apply the findings from this research to a larger cohort of pet rabbit breeds in a randomized clinical trial. For this purpose one must recall that differentiation between pain and stress may be challenging when using different rabbit breeds, surgical procedures, and post-operative environments. Due to the difficulty of assessing pain in rabbits, behavioural ethograms should be applied in addition to objective measures of physiologic function.

Overall, evidence for post-operative pain reduction or faster return to baseline physiology with LapOVE was not found. In contrast to what has been described in other species, laparoscopy may not provide as marked of an advantage in rabbits when compared to a conventional open approach. However, differences between the surgical groups may not have been detected due to limitations in sample size. Furthermore, surgeries that are more complex than ovariectomy and that benefit from the magnification of endoscopy may still have better outcomes using laparoscopy rather than laparotomy. Further evaluation of minimally-invasive surgery is warranted in this species.
6.2 Maximizing Working Space without High Intra-abdominal Pressures

6.2.1 Pre-stretching and Pre-conditioning

Pre-stretching involves application of higher intra-abdominal pressures prior to lowering the IAP for the remainder of the procedure. By pre-stretching the abdomen during insufflation, the ultimate working space achieved is greater for any given IAP. As a result, it is possible to minimize the adverse effects of higher IAPs while maximizing working space. Pre-stretching simultaneously allows for ischemic pre-conditioning of the tissues. Pre-conditioning can minimize the ischemia-and-reperfusion effects of decreased visceral perfusion due to pneumoperitoneum. This results in less oxidative stress to the tissues.

Given the significant influence of pre-stretching on working space, hemodynamic parameters, and tissue perfusion, any future cross-over studies involving pneumoperitoneum should consider the carry-over effects of previous insufflation pressures. Chapter 3 of this thesis identified a significant interaction effect between IAP and the order of pressures used in the rabbits. Therefore, working space studies need to consider these effects during experimental design and statistical analysis of the data.

An interaction effect was also seen between IAP and pressure sequence for PaO₂ measurements in Chapter 4. PaO₂ may be influenced by changes in blood perfusion and hemodynamic parameters. Simultaneously, time under anaesthesia will contribute to the changes in PaO₂. Yet again this emphasizes the importance of accounting for sequence order in future investigations of pneumoperitoneum.

For studies that utilize pre-stretching, it may also be beneficial to assess the abdominal wall tension associated with different insufflation pressures. Abdominal wall tension, in
combination with working space, may provide data about the potential for surgical manipulation; abdominal wall tension and distensibility are suspected to vary between animal species.

6.2.2 Lift Laparoscopy

Lift laparoscopy is an area for further research. Lift laparoscopy does not require gas insufflation; it instead creates working space by passively introducing air through the ports while lifting the abdominal wall. As a result, it eliminates the adverse effects associated with pneumoperitoneum for laparoscopic surgeries. Recent studies in humans and veterinary species have been contradictory in their findings on the surgical utility and laparoscopic visualization during abdominal wall lifting. \textsuperscript{7,9,10,180-182} Lift laparoscopy may provide lower working space volumes requiring longer surgical times than traditional pneumoperitoneum. Therefore, further studies should investigate the effects of lift laparoscopy in a variety of companion animal species, and objectively determine their advantages and disadvantages over pneumoperitoneum.

In summary, a randomized controlled clinical trial to compare lift laparoscopy, abdominal pre-stretching, and standard pneumoperitoneum is necessary to identify the optimal laparoscopic approaches for rabbits. The current recommendation to use insufflation pressures of 8 mm Hg is based on standard pneumoperitoneal approaches. With pre-stretching, this pressure may be decreased further, and with abdominal wall lifting, the need for pneumoperitoneum may be completely eliminated.
6.3 Other Laparoscopic Surgical Procedures

Future investigations should consider larger sample sizes when comparing post-operative outcomes between surgical groups. The effect sizes may be small and require greater power to identify in species, like rabbits, that are subtle in their expression of pain or post-operative morbidity. The study of different rabbit breeds may also permit the findings to be more applicable to the pet rabbit population.

Additional studies comparing laparoscopic procedures to open approaches should also examine different surgical techniques. Laparoscopy can be performed using single ports, as has been described in rabbit ovariohysterectomy,\textsuperscript{106} or multiple ports in different configurations.\textsuperscript{109} In this thesis, the Ligasure\textsuperscript{TM} device was used for vessel sealing and transection. Radiosurgery and laser devices also provide cautery and warrant further investigation when vessel sealing instruments are unavailable or inappropriate for a small patient.\textsuperscript{62}

Finally, in this thesis, ovariectomy is used as a model for comparing laparoscopy to laparotomy in rabbits. However, ovariectomy is a relatively simple surgical procedure, with respect to visualization of the organ and manipulation of the vascular structures required to excise the ovaries. Other surgical procedures, including those performed on smaller abdominal structures like adrenal glands and lymph nodes, are more complex and require better access and visualization. Midline incisions may need to be extended for laparotomy to access deeper tissues. Similarly, the magnified view obtained with laparoscopy may provide a significant benefit over traditional open approaches for certain surgical procedures. Therefore, future comparisons between laparoscopic or laparoscopic-assisted surgeries and open laparotomy should be considered for procedures other than ovariectomy in rabbits.
References


### Appendices

#### Supplementary Tables

<table>
<thead>
<tr>
<th>Behavior</th>
<th>Time of Day</th>
<th>Time from 1st Assessment</th>
<th>Time Relative to Surgery</th>
<th>Other Variables</th>
<th>Interacting Variables</th>
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<td>Activity level</td>
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<td>Increased together with eating $p = 0.0088$ (Figure 4)</td>
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<td>Time at front</td>
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<td></td>
<td>Increased when resident was primary surgeon $p = 0.0031$</td>
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</tr>
<tr>
<td>Time at back</td>
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<td>Time from 1st Assessment</td>
<td>Time Relative to Surgery</td>
<td>Other Variables</td>
<td>Interacting Variables</td>
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<td>Interact</td>
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<tr>
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<td>Increased with activity level $p &lt; 0.0001$</td>
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<td>Movement composite</td>
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<td>Decreased after surgery $p = 0.0025$</td>
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<tr>
<td>(hopping, shuffling)</td>
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<td>Frequency decreased in first 24 hours after surgery $p = 0.0110$</td>
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</table>
| Alert                    |             | Frequency decreased in first 24 hours after surgery  
|                          |             |  
|                          |             | $p = 0.0121$             |              |                      |
| Huddling                 |             | Increased after surgery  
|                          |             |  
|                          |             | $p = 0.0487$             |              |                      |
| Eyes closed              |             | Increased after surgery  
|                          |             |  
|                          |             | $p = 0.0001$             |              |                      |
| Inactive Pain Behaviors  |             | Frequency increased in first 24 hours after surgery  
| (twitch, draw back,     |             |  
| stagger, full-body flex,|             | $p = 0.0198$             |              |                      |
| abdominal press,         |             |  
| hindlimb shuffle)       |             |                         |              |                      |
|                          |             |                         |              |                      |

**Table 1**: Effect of variables, other than surgical treatment, on frequency or duration of time that various behaviours were expressed by rabbits. Variables that had significant interaction effects in generalized linear models are also indicated. Figures illustrating these effects are provided below.
Supplementary Figures

Figure 1: The effect of time on blood glucose measurements (mean +/- standard deviation error bars). Glucose was measured before surgery (12 hours from start of assessment), the morning after surgery (36 hours), and once daily for the next two days. Significant differences in glucose measurements over time are identified by asterisks.
**Figure 2**: The interaction effect of time of day and time relative to surgery (i.e. before versus after surgery) on activity level, displayed as means and standard deviations (error bars). Activity was increased in the morning, with this difference being especially pronounced prior to surgery.

**Figure 3**: The effect of activity level and time of day on exploring behaviour. Data points are plotted together with a robust linear estimation and 95% confidence interval. Time spent exploring was greater in the morning and was positively correlated with activity level.
Figure 4: There is a positive correlation between time spent eating and drinking (in minutes). Data points are plotted together with a linear estimation and 95% confidence interval.

Figure 5: There is a positive correlation between time spent recumbent and being in the back of the pen (in minutes). Data points are plotted together with a linear estimation and 95% confidence interval.
Figure 6: Frequency of interaction events displayed by rabbits relative to surgical day, with Day 0 indicating the 24 hours prior to surgery and the following days being after surgery. Interaction events decreased significantly in the first two days after surgery (indicated by asterisks).

Figure 7: Frequency of hopping relative to surgical day, with Day 0 indicating the 24 hours prior to surgery and the following days being after surgery. Hopping decreased significantly for the first day post-operatively (indicated by an asterisk).
Figure 8: The effect of time of day and time relative to surgery (i.e. before versus after) on the time spent performing movement behaviors (hopping and shuffling), displayed as means and standard deviation (error bars). Movement was greater in the morning and prior to surgery.

Figure 9: Frequency of rearing incidents relative to surgical day, with Day 0 indicating the 24 hours prior to surgery and the following days being after surgery. Rearing frequency decreased significantly for the first two days post-operatively (indicated by asterisks).
**Figure 10**: The effect of time spent recumbent and time relative to surgery (before versus after) on time spent with eyes closed. Data points are plotted together with a robust linear estimation and 95% confidence interval. The amount of time spent with eyes closed was greater after surgery and positively correlated with recumbency.

**Figure 11**: Frequency of inactive pain behaviors relative to surgical day, with Day 0 indicating the 24 hours prior to surgery and the following days being after surgery. Inactive pain behaviors increased significantly for the first day post-operatively (indicated by an asterisk).