

Minerva Access is the Institutional Repository of The University of Melbourne

Author/s:

Mccracken, Blaine David

Title:

Laparoscopic & Laparoscopically-Assisted Surgery in Rabbits: Comparison of Isobaric and Insufflated Laparoscopic Techniques to Open Laparotomy

Date:

2019

Persistent Link:

<https://hdl.handle.net/11343/242013>

Terms and Conditions:

Terms and Conditions: Copyright in works deposited in Minerva Access is retained by the copyright owner. The work may not be altered without permission from the copyright owner. Readers may only download, print and save electronic copies of whole works for their own personal non-commercial use. Any use that exceeds these limits requires permission from the copyright owner. Attribution is essential when quoting or paraphrasing from these works.

Laparoscopic & Laparoscopically-Assisted Surgery in Rabbits: Comparison of Isobaric and Insufflated Laparoscopic Techniques to Open Laparotomy.

Master of Veterinary Science Thesis

Blaine David McCracken

ORCID: 0000-0002-8341-211X

MASTER OF VETERINARY SCIENCE

Submitted: August 2020

University of Melbourne Veterinary Hospital

Faculty of Veterinary and Agricultural Science

250 Princes HWY, Werribee Victoria 3030

This thesis is submitted in partial fulfilment of the degree of Master of Veterinary Science, along with coursework.

Abstract

The use of laparoscopic surgery for routine procedures such as ovariectomy has been well described for dogs and is common in humans. Rabbits have been previously used as models for human laparoscopic surgery and training models for paediatric surgery, however reported use of clinical laparoscopy in rabbits is rare. There are concerns for use of laparoscopic surgery in rabbits due to the effects of the insufflation on ventilation and the risk of increased morbidity from the insufflation contributing to gastrointestinal stasis, a common and life-threatening complication of any surgery in rabbits.

This study is designed to quantify and characterise the changes in the postoperative morbidity between open, insufflated and isobaric laparoscopy in healthy adult rabbits. The hypotheses were that use of isobaric laparoscopy will decrease the morbidity of ovariectomy procedures compared to open and insufflated ovariectomy at the expense of increased surgical time. Various investigations were performed over the research project, including a technical viability cadaveric study, a study describing the effects of isobaric and insufflated pneumoperitoneum on ventilatory capability and abdominal dimensions, a study describing the clinical implementation of a Rabbit Grimace Pain score and Behavioural Pain Score in the detection of postoperative pain, and a clinical trial assessing the effects of both laparoscopy methods and comparing them with open laparotomy for ovariectomy.

The overall findings of the study support the implementation of isobaric laparoscopy in the rabbit, and the use of laparoscopy in general as a method of reducing postoperative morbidity compared with equivalent laparotomy approaches.

Declarations

- i. The thesis comprises only original work towards the Master of Veterinary Science, except where indicated in the preface
- ii. Due acknowledgement has been made in the text to all other materials used
- iii. The thesis is fewer than the maximum word limit in length, exclusive of tables, maps, bibliographies and appendices.

Preface

- i. This thesis is the original work of the author, except where otherwise cited.
- ii. This work in this thesis does not contribute to any other higher-education qualifications. A publication of part of the results did contribute to the minimum publication requirement of credentialing towards the Australian and New Zealand College of Veterinary Scientists Fellowship program.
- iii. No part of this thesis was completed prior to enrolment in the degree.
- iv. No third-party editorial assistance was provided in preparation of the thesis other than the candidate and supervisors.
- v. No contributions were made to any of the work except where noted in the acknowledgements.
- vi. The publication status of the chapters are as follows:
 1. Chapter one is unpublished material
 2. Chapter two is partially published by Journal of Exotic Pet Medicine in January, 2019
 - Some revisions to the statistical analysis have been performed after publication
 - Some of the figures and tables have been updated to reflect that statistical revision
 - The article is attached as an appendix for reference
 3. Chapter three is unpublished material
 4. Chapter four is unpublished material
 5. Chapter five is unpublished material
- vii. The work was performed under the University of Melbourne Veterinary Residency Training Program, which is a dual Masters' degree program. The candidate received a stipend from the Faculty of Veterinary and Agricultural Sciences to complete the program.

Acknowledgements

Project Supervisor:

Dr Stewart Ryan BVSc (hons) MS MANZCVS DipACVS founding fellow ACVS (Surgical oncology)

Secondary Investigators:

A/Prof Thierry Beths DVM PhD CVPP

Dr Sasha Herbert BVSc (hons) MANZCVS (M&S Unusual Pets)

Dr Jaclyn Gatt BVSc MVS

The contents of the thesis were undertaken by the candidate under guidance of the project supervisor and with detailed consultation with the primary supervisor and secondary investigators. A/Prof Thierry Beths was a member of the Masters program supervision team and assisted with the anaesthetic protocols, and Drs Sasha Herbert and Jaclyn Gatt contributed to the conceptualisation and operation of the research, acting as assistant surgeons where required.

Other Acknowledgements

- Dr Brenton Chambers, University of Melbourne/CARE- Assistance in conceptualisation of the study
- Dr Stephen Divers, University of Georgia- discussed unpublished details of published study to aide in the study construction
- Dr Matthew Leach, Newcastle University- provided documentation related to their published grimace and behavioural pain scores including training documents
- Dr Glynnis Lam & Staff, BEAC- use of facilities and contribution to morbidity studies.
- Dr John Moody, Seqirus- sourcing cadavers for study 1.
- Dr Gerry Skinner, Melbourne Rabbit Vets- discussion of study protocols and contact for rabbit carers.
- Prof Ted Whitem, University of Melbourne- Research program guidance
- Dr Manuel Boller, University of Melbourne- Non-supervisory mentor

Table of Contents

Cover page.....	i
Abstract.....	ii
Declarations	iii
Preface	iv
Acknowledgements.....	v
Tables, Figures and Illustrations	ix
Tables	ix
Figures.....	ix
Appendices.....	x
Chapter 1: Introduction & Literature review	1
Introduction	1
Uterine disease in the Rabbit.....	2
Ovariohysterectomy & anaesthesia in the rabbit.....	2
Gastrointestinal stasis in rabbits.....	4
Laparoscopy and laparoscopy-assisted surgery.....	4
Rabbits in laparoscopic surgery research and training	6
Laparoscopic ovariectomy in rabbits	6
Vessel sealing devices & electrocautery	7
Hypotheses	9
Clinical importance	9
Chapter 2: Study one- Isobaric laparoscopic technique.....	11
Introduction	11
Study 1: Part 1	14
Materials and Methods: Part 1	14
Cadavers.....	14
Establishing insufflated pneumoperitoneum	14
Establishing isobaric pneumoperitoneum	16
Surgical Technique	16
Subjective assessment	17
Results: Part 1	17
Discussion: Part 1	20
Study One: Part 2.....	22

Materials and Methods: Part 2	22
Specimen preparation:.....	22
Image acquisition:	23
Outcome measures:.....	23
Statistics: Part 2	24
Results: Part 2	25
Discussion: Part 2	28
Publications.....	31
Chapter 3: Study two- Evaluation of morbidity assessments.....	32
Introduction	32
Materials and Methods.....	34
Statistical analysis	37
Results.....	38
Discussion.....	40
Chapter 4: Clinical Trial	45
Introduction	45
Materials and Methods.....	46
Inclusion criteria:.....	46
Exclusion criteria:	46
Outcome variables assessed:	47
Blinding	47
Ethics	47
Anaesthesia.....	47
Surgical Technique: Isobaric variant	48
Surgical Technique: Insufflated variant	48
Statistical Analysis.....	49
Results.....	49
Discussion.....	56
Conflict of interest	60
Chapter 5: Conclusion	61
References.....	64
Appendices.....	69
Appendix A: Routine anaesthetic protocol	69
Pre-anaesthetic assessment	69

Anaesthetic protocol.....	69
Anaesthetic monitoring	70
Local anaesthesia and postoperative analgesia.....	70
Perioperative supportive care.....	71
Appendix B: Rabbit Grimace-score	72
Appendix C: Intervention Criteria	75
Appendix D: Publications from the thesis.....	81

Tables, Figures and Illustrations

Tables

1. Results of Study 1: Part 1, subjective analogue scale(1-5) describing the surgeons' responses to the described insufflated and isobaric laparoscopic ovariohysterectomy techniques and a brief summary of the interpretation of the score. Colour coded with working space rated 1 (red), 2 (orange), 3 (yellow), 4 (olive), 5 (green).
2. Results of Study 1: Part 1, Mean difference (mm³/kg) in thoracic volume standardised by bodyweight between baseline, insufflated & isobaric groups with and without positive inspiratory pressure (PIP). n=7. Comparisons and p-values made by ANOVA. Duplicate values ('-') have been omitted for clarity
3. Pain scores associated with the use of different opioids during Study 2
4. Clinical trial signalment and preoperative data, opioid was hydromorphone in all but two cases. *fentanyl was given at a 0.06x dose scaling factor.
5. Clinical Trial, outcome variables by surgery method *rabbit received rescue analgesia, which may have artificially lowered the behavioural score due to sedative effects.
6. Clinical Trial, Kruskal-Wallis testing of for bias in treatment groups, Bonferroni adjusted. n=7 per group
7. Clinical Trial Kruskal-Wallis and Dunn's test for outcome variables between treatment groups, bonferroni adjusted. n=7 per group

Figures

1. Gross anatomy of the rabbit reproductive tract demonstrating the paired cervixes (black arrowheads), uterine horns (white arrowheads) uterine (white arrow) and ovarian vessels (black arrow). The ovary is marked with a black star
2. Diagram showing placement of portals and sutures during isobaric laparoscopy. Upper image: X= Xiphoid, U= Umbilical scar, 1-3= portals, P= pubic rim, 4-5= location of stay sutures. Lower images: 1-2= stay sutures, *= exteriorised/ligated uterine stump
3. Example of measurement of maximum abdominal height (MAH), space (MAS) and width at 25%(MAW25), 50%(MAW50) and 75%(MAW75) abdominal depth, as calculated from the computed tomography images of the rabbit abdomen under baseline and pneumoperitoneum conditions.
4. Box & Whisker plot: Comparison of order of procedures, standardised by bodyweight: centre-line indicates mean value, whiskers indicate standard error and box indicates 1st/3rd

quartile values. There is no significant difference between order of procedure for PIP ($p=0.70$) or non-PIP volumes ($p=0.95$)

5. Comparison ($n=7$) of the mean abdominal height (mm), abdominal space or pneumoperitoneum (mm) & visceral height (mm) between different groups. Error bars represent standard error of the mean.

There was no significant difference in visceral height between baseline (a) and groups 'c' or 'd'.

a- statistically different ($p<0.044$) from groups 'b'.

c- statistically different ($p<0.01$) from 'd'

6. Chart demonstrating mean abdominal width (mm) at 25%, 50% and 75% abdominal height for insufflated and isobaric groups with and without positive inspiratory pressure (PIP). Error bars represent standard error of the mean. $n=7$
7. Box & Whisker plot: Difference ($n=7$) between PIP and non-PIP thoracic volumes, standardised by bodyweight: centre-line indicates mean value, whiskers indicate range, and box indicates 1st and 3rd quartile values. The difference between the isobaric and insufflated groups was statistically significant ($p=0.006$)
8. Diagram showing the changes from the baseline cross-section (A) under insufflation (B) and abdominal wall lift in a relatively rigid (C) and relatively pliable (D) abdominal wall. Visceral space is shown in grey and pneumoperitoneum space in white. Note this figure is exaggerated to emphasise the changes that were noted subjectively during the study.
9. Percentage of rabbits from each treatment group that were eating (left) or had defaecated (right) by each timepoint after extubation. $n=7$ per group.

Appendices

Appendix A: Routine anaesthetic protocol

Appendix B: Rabbit Grimace-Score

- This contains material reproduced from the Rabbit Grimace Score Manual, with permission from Dr Matt Leach, University of Newcastle.

Appendix C: Study Intervention Criteria

Appendix D: Publication from Chapter 2

Chapter 1: Introduction & Literature review

Introduction

The Common, or European, rabbit (*Oryctolagus Cuniculus*) is a member of the order lagomorpha, which includes rabbits, hares and pikas. Lagomorphs are characterised by a second, shorter, pair of incisor teeth behind a pair of long central incisors, the latter being a characteristic often associated with rodents (order rodentia). Members of this mammalian order are terrestrial, hindgut-fermenting herbivores.

Multiple breeds of the Common rabbit exist, domesticated for use in fur fibre production or to act as pets¹. In the wild, rabbits live in burrows underground and have diurnal behaviours, with peak activity at evening and morning. Between these times, the wild rabbit retreats to their burrow for safety from predation.¹

Due to their role as a prey species, rabbits tend to flee if threatened and freeze if cornered rather than fight. Their primary method of self-defence is to strike with their powerful and agile hindlegs, which also enable them to feint and leap to evade a would-be predator. When in pain or unwell, rabbits tend to hide their clinical signs and instead become withdrawn. It is therefore difficult to assess pain in rabbits as they instinctually act normally when they perceive that they are being observed.^{1,2}

Ownership of the domestic rabbit has become widespread globally, with rabbits being owned by 3% of UK households (accounting for approximately 1.2million rabbits)³, and 1% of US households (approximately 3.2 million rabbits)⁴. Rabbits are owned by 3% of households in New Zealand (0.1 million rabbits)⁵ with similar ownership rates through Europe⁶. They are still a minor contribution to total pet numbers, compared with cat and dog ownership each accounting for between 25-45% of households in these regions³⁻⁶.

Rabbits are an expanding subsection of domestic animals according to surveys in Australia, however the rabbit ownership rates in Australia are not specifically tracked in census/survey data, as they are combined with “other pets” in national surveys⁷. The ownership of rabbits as pets is banned in the state of Queensland⁸ and rabbits were traditionally seen as a pest species in Australia through much of the 20th century since their introduction during settlement and subsequent importation from 1859⁹. The outbreak of myxoma virus to control feral rabbit populations in the 1940’s was allowed to proceed unimpeded⁹, including the banning of vaccinations against the disease in pet rabbits. Given this traditionally antagonistic attitude of the Australian governments to the domestic rabbit, it is understandable that rabbit ownership in Australia is lower than similar and neighbouring nations.

Uterine disease in the Rabbit

Rabbits have a high rate of uterine adenocarcinoma compared with similar domestic species, with up to 12-17% of entire female^{10,11} rabbits developing uterine adenocarcinoma specifically, and a 50% risk of developing uterine and mammary neoplasia over the age of 5 years. In a retrospective series of full post-mortem examinations in pet rabbits¹¹, uterine pathology was present in 26.8% of female rabbits. In one study¹² of 60 rabbits assessed retrospectively with uterine disorders, 42.5% had endometrial hyperplasia and 49.3% had uterine adenocarcinoma, as the two most common forms of uterine disease. Of rabbits in that study with uterine adenocarcinoma, 26.2% also had granulosa cell tumours, squamous cell carcinomas or mammary adenocarcinomas present, which is higher than the rate of these tumours seen in the general population, suggesting that all of these diseases are associated with intact reproductive status.

Ovariohysterectomy, the removal of the ovaries and uterus, has been performed in rabbits as a method of surgical neutering and treatment of uterine neoplasia. Neutering can be performed to eliminate the production of unwanted litters, reduce undesirable behaviours and the prevention of these uterine and mammary tumours.^{1,13} Despite the utility of routine ovariohysterectomy in the prevention of these diseases, approximately 54% of rabbits in the state of Victoria, Australia are reproductively entire.¹⁴

While it has been suggested that early removal of the ovarian tissue and subsequent hormonal priming of the uterus should prevent the incidence of uterine neoplasia in the rabbit¹⁵, it has been reported that a uterine carcinoma has developed on a uterine stump post desexing¹⁶.

Ovariohysterectomy & anaesthesia in the rabbit

Routine (open, laparotomy) ovariohysterectomy technique is performed through a 1-2cm midline incision approximately halfway between the umbilicus and pubic symphysis. At this location, the major obstruction to exploration of the abdominal cavity is the gas-filled caecum. The caecum is delicate and prone to trauma with excessive manipulation. Penetration of the caecum could result in leakage of its contents into the abdominal space. As the caecum contents is essentially faeces, this would lead to a high bacterial burden resulting in septic peritonitis- infection in the abdomen- which could potentially be fatal.

There is no uterine body in the rabbit, as each uterine horn terminates in a separate (bicornuate) cervix (see Figure 1). The fallopian tube is long, coiled and friable, with mesovarium and mesometrium often filled with fat which may obscure direct visualisation of the ovary. The ovarian artery lies in the broad ligament, and the uterine vessels often require separate ligation.

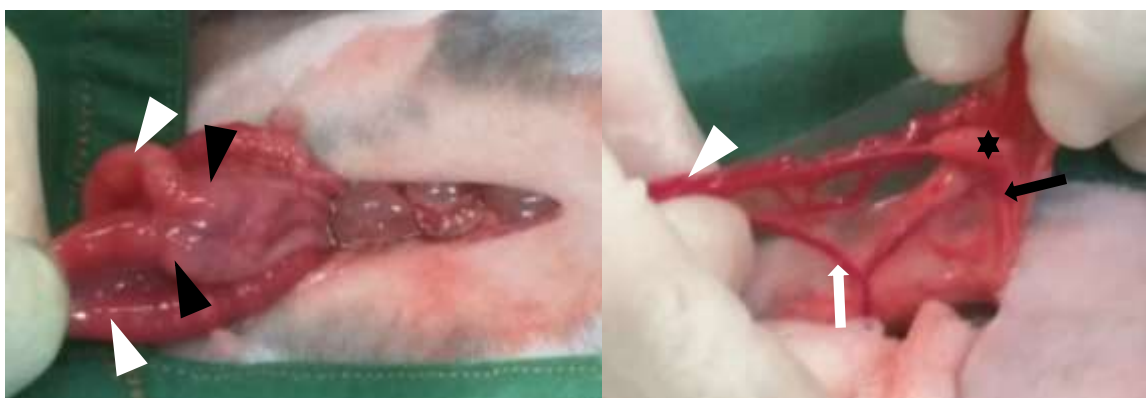


Figure 1: Gross anatomy of the rabbit reproductive tract demonstrating the paired cervixes (black arrowheads), uterine horns (white arrowheads) uterine (white arrow) and ovarian vessels (black arrow). The ovary is marked with a black star

Each ovarian pedicle is ligated prior to division, and the uterine stump/vasculature can be ligated with encircling and transfixing ligatures at or caudal to the cervixes. The sutures in this region must be secure as the vagina dilates with urine during micturition. Cervical ligatures must be caudal enough to ensure complete removal of the uteri; otherwise there is a risk of residual uterine tissue forming an adenocarcinoma.^{1,13,16}

Compared with routine ovariohysterectomy in the dog or cat, there are significant inherent risks associated with routine surgeries in rabbits.

Regarding the perioperative complications, a previous multi-institutional study in the UK of cases in 2002-2004 showed that in ASA (American Society of Anaesthesiologists) grade 1-2 patients (i.e. without severe systemic disease), the risk of mortality within 24 hours of surgery in rabbits was 0.73% (approximately one in 137), compared with 0.11% and 0.05% in cats and dogs respectively. In ASA 3+ patients, perioperative mortality in rabbits was 7.37%, or one in fifteen cases, compared to 1.33-1.40% in dogs and cats.¹⁷

The increased risk of anaesthesia-associated mortality in the rabbit is likely multifactorial. Rabbits have a high likelihood of stress on induction and handling; have a high surface-area to volume ratio (increased risk of hypothermia) and an increased risk of respiratory and digestive disease as well as being sensitive to fluid balance disturbance. Both venous catheterisation and endotracheal intubation are more technically difficult, and they have a low respiratory reserve volume, making them particularly sensitive to respiratory depression.^{1,17,18}

Some medications (especially opioids) commonly used during anaesthesia and for analgesia (e.g. buprenorphine) may have substantial effects on the rabbit physiology including inappetence¹⁹ which may decrease faecal output for up to three days¹⁹, and affect the rabbit's gut biome and increase the risk of gastrointestinal stasis.

The propensity for rabbits to develop hypotension under general anaesthesia also commonly requires intervention with vasopressors, these dose rates are often high due and therefore may increase the susceptibility of rabbits to the adverse effects of these drugs.¹

Overall, this increased risk of general anaesthetic mortality underlines the importance of any methods of improving surgical and/or anaesthetic safety for routine procedures, especially in healthy patients. The ten-fold risk of anaesthetic mortality in sick rabbits also highlights the vital role of prophylactic, rather than therapeutic, surgery.

Gastrointestinal stasis in rabbits

The caecal flora in the rabbit is critical to its overall health. As a hindgut fermenter, the rabbit's caecal flora is responsible for processing otherwise indigestible cellulose into caecotrophs- nutrient-rich faeces which is passed and reingested.²⁰ Stress and pain in the postoperative phase can lead to alterations in the microflora and subsequent gastrointestinal stasis, a disease of gastrointestinal hypomotility.^{2,21}

In carnivores, postoperative ileus is relatively benign, whereas stasis in rabbits leads to enteritis, enterotoxaemia, dehydration and hepatic lipidosis. Gastrointestinal stasis not only inhibits the clearance of faecal waste (as it does in carnivores) but also the rabbit's primary source of volatile fatty acids and energy. Without treatment, gastrointestinal stasis is commonly fatal.^{2,20}

Treatment of gastrointestinal stasis is centred around maintaining hydration and analgesia to encourage return to eating, which naturally stimulates gastrointestinal motility. The links between stress/pain and gastrointestinal stasis emphasise the importance of minimising the morbidity of surgery in this species. While it is uncommon for routine surgery to lead to gastrointestinal stasis, it is a risk higher in severity and frequency than the equivalent standard postoperative risks faced by other species during routine neutering surgery.

Laparoscopy and laparoscopy-assisted surgery

Laparoscopic surgery is a form of minimally invasive surgery, whereby a rigid endoscope is introduced into the abdomen via a narrow cannula. The cannula maintains a "portal" which is a small "keyhole" incision. The endoscope then displays a real-time video picture to a monitor viewed by the surgeon. Further cannulas are then placed to enable introduction of instruments into the abdomen to allow for the surgery to be performed while the abdomen is maintained as a single closed system.

To enable visualisation and "working space" for the instruments, positive-pressure insufflation of the abdomen is standard. This takes advantage of the closed nature of the system to actively push out the body wall.

In contrast to laparoscopic surgery, laparoscopy-assisted surgery is surgery performed using laparoscopic equipment, but the closed nature of the abdomen is not maintained through the procedure. This may be performed to locate a mobile organ using laparoscopy and then withdraw the organ through a small extended incision to perform surgery. Alternatively, laparoscopy-assisted surgery could involve placement of the endoscope into an “open” surgery in order to visualise an otherwise obscured organ or surface, or to utilise the benefit of magnification that is a beneficial consequence of endoscopy.

The demand for laparoscopic procedures in companion animals has been expanding in dogs and cats in recent years following success and acceptance in humans, with interest building in other species expected to follow.¹⁵ The most common utilities for laparoscopic surgery in veterinary medicine is for routine intra-abdominal surgeries, such as ovariectomy, cryptorchid castration and gastropexy, though techniques have been described for less common techniques such as cholecystectomy and adrenalectomy.²²⁻²⁵

Insufflation with carbon dioxide (CO₂) has been studied at length in human and canine literature, with suggestion that it may increase the morbidity in the perioperative period. While it has previously been suggested that the adhesions are due to local hypoxia due to compression of the tissues due to the expanding abdomen against the restriction of the abdominal wall, one study in a rabbit model suggests that pressure is not related to adhesion formation²⁶ and instead may be due to the carbon dioxide effects on the tissues. It has been suggested that replacing CO₂ with air, mixture of CO₂/O₂ or saline may reduce hypoxic injury/adhesion formation for this reason.²⁷⁻²⁹

The other undesirable effect of insufflation of the abdomen is that it transmits this pressure to the diaphragm, potentially increasing resistance to ventilation. Rabbits have a low respiratory reserve, meaning that there is little redundancy in their respiratory capacity. This means that decreased ventilation, such as decreased end-tidal lung volume could increase the risk of hypoxia and subsequent anaesthesia-associated mortality risk. Insufflation with pressure as low as 8mmHg with CO₂ has been shown in the rabbit to increase the partial pressure of CO₂ and depress cardiac function, though it is uncertain whether this is primarily due to the CO₂ itself, the mechanical pressure on the ventilation or both that is responsible for these effects.³⁰

The use of gasless laparoscopy, which enables the use of normal instruments in an isobaric abdomen and eliminates the negative complications associated with positive pressure CO₂ pneumoperitoneum has been described in human literature.^{24,31-36}

Gasless laparoscopic surgery has been found to be feasible in a rabbit model for paediatric surgery given the additional risks of insufflation pressure on the depression of respiratory capacity in smaller patients.³⁴

The advantages of gasless laparoscopy in the veterinary patients have been studied primarily in dogs^{23,37,38} and found to be feasible; however there are no studies into how this technique may translate to a smaller abdomen, where working space is already limited. It is not reported whether the thinner and more elastic abdominal wall of rabbits will affect the achievable working space, or whether the gas-filled caecum would influence the working space in this species.

Rabbits in laparoscopic surgery research and training

There is a wide range of literature implementing rabbits as an experimental and teaching model for paediatric laparoscopic surgery³⁹⁻⁴⁵ though limited studies into clinical use of laparoscopy in rabbits.¹⁵

The use of rabbits particularly in these studies are generally due to the availability of purpose-bred laboratory animals within research institutions, and either their size in regards to simulating a paediatric abdomen or their similarity to humans in regards to their development of adhesions postoperatively.³⁹⁻⁴⁵

The development of gastrointestinal stasis in rabbits is multifactorial, including patient stress, breed, dehydration, opioid use, diet, inappetence and general health^{20,21}. Despite significant morbidity and mortality related to gastrointestinal stasis²⁰, and suggestion that stress/morbidity may contribute to gastrointestinal stasis or strictures^{2,21,46}, there have been no studies published that assess the risk of minimally invasive surgery on the risk of gastrointestinal stasis/obstruction.

This is due to the fact that the outcome measure of interest in rabbit models for human disease are the formation of adhesions, the effect of which on humans adhesions is well known, so rabbit models are used primarily to determine if a given modality or product causes an increased or decreased amount of adhesions, and not the effect of these adhesions on the rabbits³⁹⁻⁴⁵. It goes without saying that there is no need to model gastrointestinal stasis for humans, as humans are not hindgut fermenters.

What these rabbit model studies do reveal, is that the rabbits undergoing minimally invasive surgery tend to have lower degrees of serosal irritation which leads to less development of adhesions, and this combined with anecdotal and extrapolated reports of decreased perioperative morbidity may suggest that there would be a decreased risk of gastrointestinal stasis in minimally invasive surgery. A study directly assessing and confirming the perioperative morbidity differences in the rabbit may therefore provide evidence towards the assumption that the reduced surgical morbidity of minimally invasive surgery is clinically relevant as a means of reducing the incidence of gastrointestinal stasis.

Laparoscopic ovariectomy in rabbits

There is an article of primary literature describing the technique of laparoscopic ovariectomy by S. J. Divers¹⁵. In this article, the technique is described with the use of a 2.7mm rigid endoscope, 3.5-

3.9mm plastic/graphite cannulas, 3mm arthroscopic instruments including bipolar electrocautery or 3.8-4.0MHz radiosurgery capabilities.

Insufflation with CO₂ was described in this paper, to levels of 8-10mmHg pressure. Only ovariectomy was described within the article, as ovariohysterectomy would require additional ligation/sealing of the uterine stump, and it is seen as unnecessary compared to ovariectomy alone due to the aetiology of uterine neoplasia being linked with reproductive hormones and therefore that, anecdotally, ovariectomy appears to be “protective against uterine neoplasia if performed... before or soon after reaching sexual maturity”¹⁵.

It is noted in the article that this technique is only appropriate for use in juvenile (less than 6 months of age) rabbits, or rabbits without uterine pathology, as it is possible that undetected uterine carcinomas could be responsible for the incidence of ovariectomised uterine carcinomas or stump carcinomas.

While the article is a descriptive study of the technique, Divers reports that they are implementing the technique in clinical patients with great success and are currently recruiting rabbits into a prospective study⁴⁷. Divers’ anecdotal opinion suggests decreased morbidity associated with this technique, though there are yet to be any results published in peer-reviewed journals to support this aside from extrapolation from other species.

A case series of 33 rabbits was published by M. S. Al-Badrany⁴⁸ which reported feasibility of the technique with extracorporeal ligation, electrocautery or haemoclip ligation. Three rabbits died perioperatively and there was no long term follow up or assessment of morbidity, though the authors reported that use of haemoclip was superior and the methodology overall was considered feasible.

Other suggested methods of reducing surgical morbidity further beyond standard 3-port laparoscopic surgery are reducing the number of incisions. Articles have been published describing two-portal⁴⁹ and single portal (single-incision laparoscopic surgery; SILS) in the rabbit.⁵⁰ Both of these studies are descriptive studies, and the actual effect on postoperative morbidity has only been extrapolated from other species and is still anecdotal.

Vessel sealing devices & electrocautery

Vessels sealing devices, electrocautery, ligatures and endoscopic clips have been compared in numerous studies.⁵¹⁻⁵⁶ Of these techniques, vessel sealing devices have been shown to be one of the most reliable methods of vessel and viscera closure. The complicating feature of vessel sealing devices is that they rely on the intrinsic properties of the tissue- elastin/collagen ratio- to form a

seal⁵⁷ and as a result of this, extrapolation of sealing strength cannot be made across tissues or species.^{53,56}

Electrocautery as a modality functions unlike vessel sealing devices by using the inherent electrical resistance to generate thermal energy which coagulates the tissues. This process is independent of the collagen/elastin ratio and therefore the results are reliable between species. Cautery has been shown to be effective and low-morbidity in rabbits for the management of ovarian pedicles.^{15,48} The greatest limitation in the use of electrocautery is the inadequacy of long-term seal in viscus, due to wider spread thermal necrosis.

Previous studies in VSD have shown no significant difference in postoperative morbidity between VSD and suture ligation^{51,52}. Use of VSD, if shown to be appropriate for the uteri, would likely reduce the surgery time difference between the open and laparoscopic ovariectomies and therefore mitigate the effect of this difference on the postoperative morbidity

To date no research has been performed into the applicability of vessel sealing devices to rabbits. While vessel sealing devices have been reported for the sealing of reproductive tracts in dogs^{54,58} and pigs,⁵⁷ the strength of the resultant seal strength is determined by the elastin/collagen composition,^{52-54,57} thus sealing strength cannot be extrapolated between species.

Sealing strength is of relevance in the rabbit, due to normal urine distension of the vestibule.

Ligation above the cervixes would result in retention of a small amount of uterine tissue, which could act as a source for adenocarcinoma.^{1,13,59-62}

Hypotheses

1. As assessed using surgeon responses and subjective qualitative feedback, isobaric laparoscopy will be seen a viable method of performing laparoscopically-assisted ovariohysterectomy in the rabbit.
2. The use of abdominal wall-lift will provide equivalent working space to insufflated pneumoperitoneum in the rabbit abdomen to allow for completion of a laparoscopically-assisted ovariohysterectomy.
3. The establishment of an isobaric pneumoperitoneum will allow for a greater minimum and maximum thoracic volume and tidal volume, as measured by total intrathoracic volume at simulated inspiratory and expiratory phases during positive pressure ventilation.
4. As assessed by behavioural and grimace pain scores, perioperative morbidity of isobaric laparoscopically-assisted ovariohysterectomy will be significantly less than of open ovariohysterectomy in the rabbit.
5. Laparoscopic/Laparoscopy-assisted surgery time from first incision to closure will be significantly greater than open surgery time for ovariohysterectomy in the rabbit.
6. There will be no statistical difference in the rate of conversion from laparoscopic to open surgery in the rabbit compared with historical data in other species for equivalent procedures.
 - Historical conversion rates range from 7-21% in veterinary laparoscopic surgery in general⁶³, though in ovariectomy in dogs in novices the conversion rate was zero⁶⁴ so in a limited clinical trial scenario, it may not be possible to show this statistically unless there is a very high conversion rate in this study.

There are three parts to this study, two experimental studies and a clinical trial.

The experimental parts will focus primarily on developing and evaluating the technical feasibility of the laparoscopic modalities that are to be implemented in the third part of the study, and the morbidity measures that will become the outcome variables of the third part of the study.

Clinical importance

Low stress, postoperative pain, and early return to normal behaviours (e.g. eating, defaecating) have a particularly strong relevance in the rabbit above and beyond the standard desire for the veterinary patient to be free of pain. Since stress, inappetence and pain from surgical procedures is a significant contributor to the development of gut stasis, these factors could lead to a particularly costly emergency treatment, which is undesirable particularly in the case of a “routine” surgery performed on (generally) healthy rabbits. If laparoscopy, especially gasless/isobaric laparoscopy, can achieve a reliable decrease in morbidity for an equivalent procedure, it would lessen the need for

postoperative analgesic intervention.

In addition to this, it is particularly difficult to assess morbidity in the rabbit due to its disposition as a prey species, and therefore it is likely that current standards of practice may underestimate the discomfort being experienced by these rabbits postoperatively. Use of multiple pain scoring systems in this study aim to assess the postoperative pain more precisely in an ovariohysterectomised rabbit in a typical clinical scenario. As some of these methods (e.g. behavioural pain score) may not be relevant to the clinical scenario, it is likely that outside of a prospective clinical study such as this, data for this scoring methodology may not be available for comparison.

The use of isobaric laparoscopy has not been reported in rabbits for use in clinical patients. The methods of achieving this lift may be more or less applicable to rabbits than the human/canine patients it has previously been assessed in due to the increased pliability of the abdominal wall in rabbits compared to these species, and with the complicating effects of the large gas-filled caecum which may inhibit working space.

The central purpose of the project is to determine whether rabbit laparoscopic ovariohysterectomy in general, and isobaric laparoscopically-assisted ovariohysterectomy in particular, has the potential to improve morbidity outcomes in the clinical patient. A secondary aim of the project is to further develop the identification and quantification of postoperative morbidity in the rabbit, and factors that may be associated with reduction to this morbidity.

Chapter 2: Study one- Isobaric laparoscopic technique

Introduction

Laparoscopic surgery, as discussed in Chapter 1, is a method of performing surgery designed to decrease surgical morbidity, recovery times and size of scar. Often referred to colloquially as “keyhole surgery”, the small ports and the use of the rigid telescope for light and visualisation minimises the surgical trauma to the abdominal wall and overlying skin.

In humans this is reported to decrease postoperative pain compared to open surgical techniques.⁶⁵

The main challenge to laparoscopy is that the peritoneal space is a potential space, meaning that viscera fill the abdominal space and defines the distension of the abdominal wall. In order to function within the abdomen in any meaningful way, the abdominal wall needs to be retracted away from the abdominal organs to gain space for the instruments to move around within a visual field, so-called “working space”.

The method by which this is traditionally achieved is by inflating the abdomen with a gas, typically air or CO₂, in order to expand the potential space and at once lightly compress the abdominal organs. The main drawbacks of this “insufflation” are threefold.

Firstly, for the abdomen to reach sufficient pressure to maintain the working space, the abdominal wall must be air-tight with no leakage around the surgical ports. This necessitates the use of specialised cannulas with seals designed to house the specific diameter of the instruments which will pass through them. If this seal is lost, for example- if a port needs to be moved or the incision needs to be enlarged to fit something through the port, then the technique may need to be abandoned and the procedure “converted” to an open approach if the seal cannot be restored.

Secondly, as well as expanding the abdominal wall itself, the insufflation also applies pressure to all other surfaces within the abdomen. Pressure on the diaphragm inhibits functional ventilation of the patient and pressure on vasculature interferes with intravascular blood pressure and visceral perfusion as well as venous return especially via caudal vena cava and portal vein.²⁹ Increased peritoneal pressure can, in extreme cases, lead to respiratory, cardiac and renal failure and death.⁶⁶

Finally; the use of carbon dioxide to insufflate the abdomen is typical as it cannot be ignited in the presence of electrocautery, and since it is highly soluble, it has minimal deleterious effects if absorbed into the blood stream (e.g. embolic disease).²⁹ It is assumed that absorption across the peritoneum occurs and that this additional carbon dioxide is cleared via respiration, however with decreased ventilation due to anaesthesia, increased diaphragmatic pressure and use of analgesic agents such as opioids, it may be more challenging to clear this additional burden of carbon dioxide in the system.³³ In a study by Kim et al⁶⁷ in rabbits undergoing mechanical ventilation at a peak

inspiratory pressure of 11mmHg under anaesthesia, induction of carbon dioxide pneumoperitoneum increased partial arterial pressure of CO₂ by a factor 2.38 (mean 82.1mmHg PaCO₂) and was only reduced to 1.37 times the baseline after increasing peak inspiratory pressure to 18mmHg. Human patients report chest pains following carbon dioxide insufflation and this is expected to be due to hypercarbia and acidosis. This change is expected to normalise over the procedure and into recovery as the body equilibrates, though chest pain has been reported to persist for days to weeks after surgery.³² Two recent papers were published by Kabakchiev et al, in one, it was found that maximal working space benefit was seen in increasing intra-abdominal pressure to 8mmHg, but that increasing to 12 mmHg yielded decreasing returns in working space.⁶⁸ In the second paper they found that increase of abdominal insufflation to 8mmHg pressure significantly increased PaCO₂, ETCO₂ and peak inspiratory pressure, while decreasing cardiac output and cardiac index.³⁰

Additionally, it has been suggested that the presence of unusual peritoneal concentrations of CO₂ can lead to local acidosis of the peritoneal surfaces which may have effects on local immune suppression and peritoneal metastasis⁶⁹⁻⁷². Peritoneal immune suppression in the face of bowel injury persists for up to 3 days after laparoscopy, with depression in peritoneal lymphocytic and monocytic populations, which may mask signs of septic peritonitis⁷³, but may also prolong survival of patients with septic peritonitis by inhibiting the deleterious effects of local (and subsequent systemic) inflammation.⁷⁴

To obviate the need for insufflation altogether, the concept of isobaric pneumoperitoneum was developed. Isobaric (sometimes referred to as gasless) laparoscopy achieves pneumoperitoneum with the pressure of the air in the abdomen the same as the outside air. Rather than driving pressurised air into the abdomen to raise the abdominal wall away from the viscera, the abdominal wall is instead raised with traction devices so that the air can flow passively into the abdomen.

First described in the early 1990's, isobaric techniques were developed to allow the implementation of laparoscopic techniques without needing to maintain a positive pressure environment. This was seen as a way to perform minimally invasive surgery in patients that would otherwise be at risk of the potential complications of insufflation (e.g. cardiopulmonary disease, infant paediatrics, trauma/shock patients).^{24,34-36,60,75} A secondary benefit of the isobaric technique is that it allows the surgeon to use conventional instruments without need for airtight cannulas, it allows freedom to transition between laparoscopic, laparoscopic-assisted or limited approach techniques without need to re-establish abdominal seal, or in cases of abdominal wall trauma where a seal is not possible. It is also easier to aspirate and lavage the abdomen without losing visualisation.^{24,31-33,35,36}

A technical challenge to the implementation of isobaric laparoscopy is achieving adequate working space without interrupting the surgical field. Many varied lift devices have been implemented for this purpose^{33-35,60}. In isobaric techniques as the force is directed against gravity focused on a single or few points, the abdominal wall will tend to “tent”, or form a triangular cross-section, with the apex at the region of the lift device, compared to the rounded cross-section of an insufflated abdomen. This then means that the working space in areas not directly below the lift-point is generally less than that available in an insufflated abdomen. Compounding this problem is the fact that without positive pressure pneumoperitoneum, there is nothing compressing the gas- and fluid-filled viscera of the abdomen, and there is a tendency for these viscera to dilate and expand into the visual field. This is particularly relevant in the rabbit, where the gas filled caecum occupies a large proportion of the abdominal space. In order to address this problem, additional retraction of abdominal viscera beyond what is normally required in an insufflated abdomen may be required.^{23,33-35,37,38}

One solution to the issue of abdominal tenting was the use of a flat spiral lifting device that can be inserted like a screw into the abdomen. The use of such a device in veterinary patients was reported by Fransson et al²³ in a series of dogs. In their reported cases, the use of a custom-designed lifting device was used to achieve abdominal wall lift, as the spiral enabled placement of a large-diameter profile through a narrow incision.

In follow-up studies from the same institution Watkins et al³⁸ defined the effects of insufflation and lift laparoscopy on the thoracic and abdominal spaces. Their findings were that positive pressure pneumoperitoneum achieved a higher generalised abdominal space, though it also decreased the thoracic volume due to pressure on the diaphragm. Kennedy et al³⁷ from the same team also published on the effects of varying lift tension and varying/multiple lift locations. They found that higher tensions yielded higher working space in the region of the lift point and that more caudal lift points tended to yield less space overall, but when used in combination with standard lifting point, could achieve a visualisation that was equivalent to the insufflated abdomen for assessing caudal abdominal structures.

The technique is yet to be described on smaller creatures with more pliable abdominal walls, however, and it is unknown how differences in abdominal wall elasticity may affect the degree of “tenting” and the overall working space in these species.

The specific aims of Study One are to address Hypotheses 1-3.

Hypothesis 1 “as assessed using surgeon responses and subjective qualitative feedback, isobaric laparoscopy will be seen a viable method of performing laparoscopically-assisted ovariohysterectomy in the rabbit” requires definition of the specific techniques that will be used for achieving abdominal lift for isobaric laparoscopy in rabbits and to determine the feasibility of these techniques in comparison to insufflated laparoscopic techniques. The feasibility of the techniques that will be defined, will then be assessed subjectively in cadavers for subjective viability of the techniques prior to proceeding further with a trial in live rabbits. This specifically is addressed in Part One of the study.

As part of assessing the effect of the isobaric techniques on pneumoperitoneum as per Hypothesis 2, “the use of abdominal wall-lift will provide equivalent working space to insufflated pneumoperitoneum in the rabbit abdomen to allow for completion of a laparoscopically-assisted ovariohysterectomy”, both subjective assessment in the cadaveric mock procedures (Part 1) as well as assessment of abdominal height profile (Part 2) will be assessed in this study.

To address Hypothesis 3, “the establishment of an isobaric pneumoperitoneum will allow for a greater minimum and maximum thoracic volume and tidal volume, as measured by total intrathoracic volume at simulated inspiratory and expiratory phases during positive pressure ventilation”, the thoracic volume will be assessed at room air pressure and simulated breath-hold positive pressure ventilation in the insufflated and isobaric pneumoperitoneum states. This is assessed during Part 2 of the study.

Study 1: Part 1

Materials and Methods: Part 1

Cadavers

Eleven New Zealand White rabbit cadavers were sourced from a research facility after having been humanely euthanised as part of an unrelated study (AEC approval: QA 7470). The cadavers were collected within 10 minutes of euthanasia and transported in a thermally insulated container to be frozen within 2 hours of euthanasia.

Establishing insufflated pneumoperitoneum

A described method for insufflated laparoscopic ovariectomy¹⁵, was modified and utilised as a control case for comparison with the isobaric ovariohysterectomy techniques during the clinical trial (see description below). The cadaver study was required to evaluate the feasibility of converting this laparoscopic ovariectomy technique to a laparoscopy-assisted ovariohysterectomy technique.

Four rabbit cadavers were used for the initial part of the study, the cadavers were thawed for 36-48 hours at 2-8 degrees Celsius prior to any interventions. All cadavers were clipped over their abdomens and a spot on the rear of the rabbit was clipped for electrocautery return. The cadavers

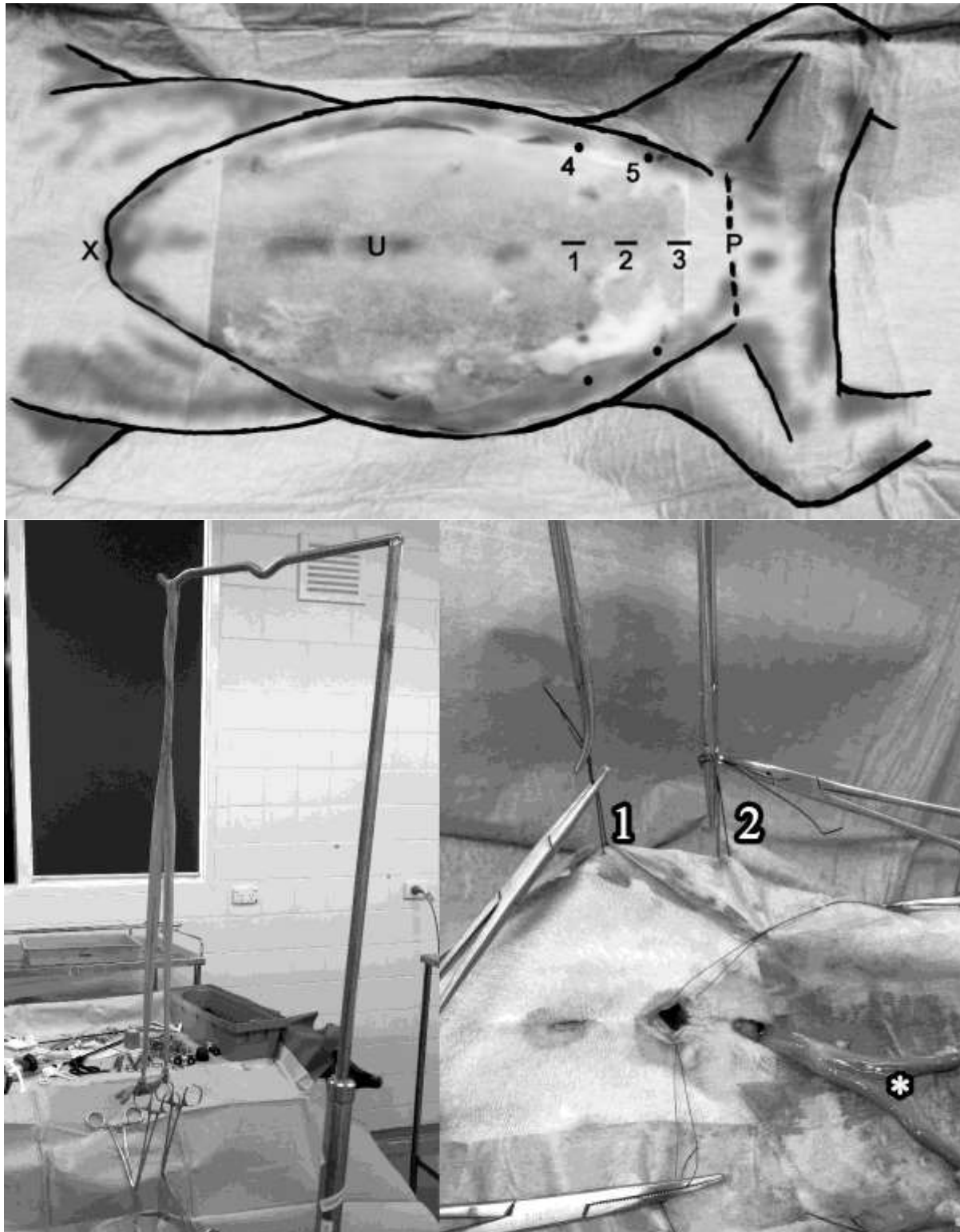


Figure 2: Diagram showing placement of portals and sutures during isobaric laparoscopy. Upper image: X= Xiphoid, U= Umbilical scar, 1-3= portals, P= pubic rim, 4-5= location of stay sutures. Lower images: 1-2= stay sutures, *= exteriorised/ligated uterine stump

were then placed in a tilting cradle, a specialised table that enables rotation of the rabbit between dorsal and lateral recumbencies intraoperatively.

5mm diameter laparoscopic cannulas (Covidien Versaport™ 5mm bladeless optical trocars with universal cannulas and/or Karl Storz Ternamian EndoTIP 5mm cannulas & trocars) were used during this study. The 3.5 - 3.9mm cannulas recommended in the previously-described study¹⁵ were not available for the current study. A xenon light-source was used for illumination, CO₂ insufflation and images were captured with either the laparoscope camera (Stryker 1088, 1/3" progressive scan CCD/HD, 60Hz) or an external camera (Sony IMX214, 13 megapixel, f/2.0 aperture lens with optical image stabilization, dual-LED flash ring).

Triple midline-port techniques were used with the cranial port placed at or just cranial to the midpoint between the umbilicus and the pubis, with the middle and caudal port placed at one-third and two-thirds distance between the cranial port and the pubis.

A modified-Hasson technique^{76,77} was used to place the first port/cannula in the cranial location. This port was used to insert the 5mm 30° fore-oblique (HOPKINS®, Karl Storz) telescope, and the CO₂ insufflation was set to a default 1L/min flow rate to a 10mmHg pressure-limited insufflation. The middle and caudal ports were made under laparoscopic visualisation once the insufflation had been established.

The 5mm cannulas were placed to enable maintenance of the insufflation pressure.

Establishing isobaric pneumoperitoneum

The isobaric technique followed similar preparation and the same camera/scopes were used. The ports were also established using modified-Hasson technique, though no cannulas were inserted.

Establishment of the first portal allowed passage of the telescope to directly visualise the placement of the lift-points. Once the lift points were established, additional incisions were made under visualisation for the middle & caudal ports.

Various configurations of lift points were assessed (see Results) to assess for optimal visualisation with fewest lift points. A simple suspended stay-suture model was used to achieve lift using 2/0 or 0 suture material on a half-round taper needle. For improved visual field, two stay sutures were used per side in later cases (see Figure 2).

Surgical Technique

The tilting cradle was tilted into near-lateral recumbency on the side opposing the side of interest. For consistency of technique, the left side was performed prior to the right side in all cases. The caecum was identified and retracted or avoided, the bladder was

visualised by directing the telescope caudally. The cervixes could then be directly visualised dorsal to the bladder and the left uterus traced to the level of the uterine horn. The ovary was visualised, and electrocautery and laparoscopic scissors used to transect the mesovarium, mesometrium and suspensory ligament.

The cadaver was then tilted to the opposite side (left recumbency) and the right side was performed similarly to the left side.

Both uterine horns with attached ovary were exteriorised through the caudal port and an extracorporeal encircling ligature of 3/0 polydioxanone was placed caudal to the cervixes.

A single simple-interrupted or cruciate suture were required to close the body wall using 3/0 polydioxanone.

In all cases which were not already converted, the abdomen was opened to inspect for damage to abdominal viscera (see results).

Subjective assessment

The techniques were assessed subjectively using a simple analogue scale with categorisations such as “working space”, “ease of port placement”, “ease of exteriorising cervixes” and “ease of accessing ovary”. These are reported in Table 1 (see below).

Results: Part 1

Case 1:

This case was used to trial the insufflated technique; however, there was difficulty in maintaining the cannulas in this specimen, as they continually slid out of the ports. This may have been due to the defrosting process being incomplete in this specimen. The abdominal lift technique was then trialled on the specimen using the previously incised ports. In this initial specimen, a towel-clamp was used to suspend the body wall. This allowed a complete right ovariectomy to be performed, however the left uterus and ovary was insufficiently defrosted to allow further progress.

The surgery was converted to an open laparotomy to inspect the condition of the abdomen and identify any obstacles that may prevent further use of rabbits in this study. The uterine and ovarian region was closely assessed, and was decided that even open, ovariectomy/ovariectomy would be impossible due to the frozen visceral fat.

Case 2:

The insufflated method was attempted again in this case, with cannulas anchored in with purse-string/finger-trap sutures. It is unclear whether this will be necessary in live rabbits due to differences in compliance post-mortem. The described technique¹⁵ for insufflated ovariectomy was emulated successfully, with modification to ovariohysterectomy by breakdown of the broad ligament and exteriorisation of the uteri through a 5-10mm caudal incision prior to ligation. The abdomen was then opened to confirm complete removal of ovarian/uterine tissue and assess the security of the cervical ligature.

Table 1: Results of Study 1: Part 1, subjective analogue scale(1-5) describing the surgeons' responses to the described insufflated and isobaric laparoscopic ovariohysterectomy techniques and a brief summary of the interpretation of the score. Colour coded with working space rated 1 (red), 2 (orange), 3 (yellow), 4 (olive), 5 (green).

Case	Insufflated or Isobaric	Working Space	Ease of port placement	Ease of exteriorising cervixes	Ease of accessing ovary
1	Insufflated	1: unable to maintain insufflation	1: unable to maintain insufflation	1: unable to access	1: unable to access either ovary
	Isobaric	3: able to visualise one side but not the other	5: simple	5: able to exteriorise easily	3: able to access one side easily, unable to access other side
2	Insufflated	4: Able to visualise all structures, but some difficulty manipulating instruments	5: simple	5: able to exteriorise easily	4: Able to access both ovaries, with at least one side slightly challenging to manipulate
3	Isobaric	5: Able to visualise both sides and manipulate without difficulty	5: simple	5: able to exteriorise easily	4: Able to access both ovaries with at least one side slightly challenging to manipulate
4	Isobaric	5: Able to visualise both sides and manipulate without difficulty	5: simple	5: able to exteriorise easily	5: Able to access and manipulate easily

Case 3:

The 3rd case was done with assistance and the full lift technique was used successfully. A stay suture placed through the abdominal wall under direct visualisation was clamped with haemostats and suspended from a drip stand with sterile bandage material. The major finding was that the port sites need to be more caudal than initially thought; the middle port should be midway between pubis and umbilical scar to maximise access to the ovarian region. The caudal port should be halfway between the middle port and the pubis. It was considered whether all ports should be collinear or if the cranial ports should be off midline to enable fan retractors to be placed in the dependent port, though this would require a separate instrument port to have a camera and two instrument ports. The alternative is to place the retractor through the middle camera port alongside the camera, since there are no cannulas, multiple diverging instruments can be placed through the same port. Once the ovarian and uterine attachments had been cleared to the level of the cervixes, the caudal port can be widened enough to exteriorise them and a ligature can be placed around the proximal cervixes.

After attempting with a single lift-point, it was determined that the optimal location for lift points were directly over the ovarian region (about in line with the central port) and as caudally as possible. The sutures can be released from the haemostats to repeat the process on the other side.

Using a tilt table was especially useful to enable gravity-dependent retraction of the viscera. The table was tilted to about 45 degrees off midline (rabbit into lateral) in each direction.

Overall, the visualisation was excellent, and the procedure was not any more difficult than the insufflated version. The procedure took a total of 100mins, including the time it took to demonstrate the procedure and acquire images. After the surgery, the abdomen was opened and fully inspected, including the location of the lift points for evidence of trauma from the stay sutures. No concerns were noted.

Case 4:

The fourth and final case for this study was used to optimise lift points and sizes. Images were acquired to compare the working space between insufflated and lift techniques. When using the laparoscopic instruments, the minimum instrument port size is smaller than the cannulas, as the diameter of the 5mm instruments is 3.5mm. Retraction with a closed pair of curved Rochester-Carmalt haemostats placed through the camera port was effective for clearing the caecum from the visual field.

The location of the lift point midway between the umbilical scar and pubis and laterally to the level

of the inguinum was optimal for visualisation of all but the most caudal structures for ovariohysterectomy. A secondary lift point (stay suture) in the inguinum grants optimal visualisation space for the whole procedure. The procedure can be done without an assistant; however it is more difficult to manipulate the instruments without an assistant. This procedure took a total of 60 minutes to complete without an assistant. As with previous cases, the abdomen was opened and investigated to assess the success of the surgery and any injury caused by the stay sutures, the surgery appeared to have been completed successfully with no evident gross trauma to the abdominal wall associated with the stay sutures.

Discussion: Part 1

The isobaric laparoscopy-assisted ovariohysterectomy technique developed was feasible and was subjectively assessed as easier to perform compared to the insufflated laparoscopy-assisted technique in rabbit cadavers. Some difficulties were encountered during this part of the study due to uneven thawing of the cadaver and friability of the specimens, however these were accounted for in the subjective assessments and affected both techniques to a similar degree. The working spaces achieved in both techniques were subjectively equivalent.

The finding that the procedure was subjectively equivalent or easier to complete under isobaric pneumoperitoneum conditions compared with isobaric conditions was considered important in the decision to proceed with later clinical trials of the technique, as it was considered that the potential benefits to the rabbits of the isobaric technique would likely be difficult to distinguish clinically with achievable case numbers. If the technique was considered significantly more difficult (and time-consuming) than the insufflated technique, pursuing further development of the technique would be more challenging to justify.

In designing the stay-suture method of achieving isobaric pneumoperitoneum, some consideration was given to utilizing skills and equipment to which most veterinary clinics have access. This is of relevance to rabbits and small mammals, as many surgical procedures and neutering procedures in particular are performed by primary-accession veterinarians, or referral small-mammal/exotic pet veterinarians which have less access to laparoscopic training and equipment.

In contrast to the previous reports in other species, the abdominal wall tenting was not seen as a significant negative factor in the provision of working space in this study, and in fact, the surgeons described the working space as superior in the isobaric cases. The superiority of the working space in these cases may have been due to the difficulty the surgeons experienced in maintaining an airtight seal around the laparoscopic cannulas in the insufflation cases, or potentially the smaller surface

area and increased pliability of the abdominal wall, allowing for more efficient lifting of the abdomen with two lift points compared to dogs and humans.

While a second lift point is recommended in the study design, this was more desirable than essential to easily access the caudal abdomen. A similar effect could also be achieved with downward tilting of the cranial end of the table (Trendelenberg position). It should be considered that this positioning would also increase pressure on the diaphragm and lungs by the weight of the abdominal organs.⁷⁸ The use of two lift points also decreases the local strain on the abdominal wall at each point compared to if the same lifting force were applied to a single point, this could also be achieved with a spiral/fan-shaped lifting device as published previously.³⁸

The insufflated technique utilised in this study is a modification of previously reported techniques^{15,48} which are entirely laparoscopic ovariectomy techniques. In this study, an ovariohysterectomy technique was reported to describe a method of completely resecting uterine tissues and enable comparison with the current standard technique.

To allow for ovariohysterectomy, extension of the caudal incision was required to exteriorise both cervixes prior to ligation due to the thickness of the reproductive tract in the rabbit. Sealing the reproductive tract cannot be done with electrocautery alone in the same manner as ovariectomy. While alternative methods of sealing the vestibular tract are possible, ligation was the most analogous to that performed in the open laparotomy ovariohysterectomy. Alternatives for this approach include intracorporeal ligation and use of a vessel-sealing device. Using either of these techniques, retrieval of the uteri through the portals without the need to extend them would require morselization which complicates and increases the expense of the procedure.

A major difficulty in the implementation of the insufflated technique in rabbits is the thin and pliable abdominal muscles. There was difficulty in maintaining an airtight seal in the abdomen and the cannulas were prone to stretching the muscle and sliding loose. The depth of the abdomen and working space also limits the intra-abdominal length of the cannula. As a result, it is recommended to use deep-threaded cannulas with threads that continue to the tip of the cannula (e.g. Ternamian cannulas) to maximise engagement. It was also considered that the weight of the cannulas used may have been a factor in the difficulties observed. Use of a 3mm cannula is reported to decrease this effect¹⁵; however, 3.5/3.9mm cannulas and a 2.7mm LigaSure handpieces were not available for this project. Use of a purse-string suture was necessary in this study, though this may have been due to autolysis and is not expected to be necessary in a live rabbit. The ability to eschew cannulas and use

conventional instruments in a laparoscopy-assisted manner is a major advantage of isobaric laparoscopy.^{60,61,79,80}

Study One: Part 2

Materials and Methods: Part 2

The second part of this study is an assessment of available working space in a more objective fashion, as per previous studies in the dog³⁷.

Seven of the previously mentioned eleven female New Zealand White rabbit cadavers were sourced from an unrelated study (see Part 1 materials and methods).

Each rabbit was randomly assigned a first procedure by coin toss, with at least one rabbit in each first-procedure.

Each rabbit cadaver had computed tomography (CT) scans of the abdomen and thorax acquired in a standardised manner as described below, under the following five conditions:

baseline with no instrumentation, isobaric technique with and without positive inspiratory pressure, and insufflated with and without positive inspiratory pressure (PIP).

Specimen preparation:

The specimens were prepared by placement of a laparoscopy cannula via a modified Hasson technique at a point 1cm caudal to the umbilicus. A ventral midline approach was made to the trachea, which was transected between either the 4th-5th or 5th-6th tracheal rings. A cuffed endotracheal tube was placed into the transected trachea and, if necessary, a Rummel tourniquet was placed cranial to the inflated cuff to prevent inadvertent removal of the endotracheal tube. Five simulated breaths were made using intermittent positive pressure ventilation to an end-inspiratory pressure of 20mmHg to identify any leaking of the endotracheal tube cuff and to dilate the airways prior to the baseline images being acquired.

Specimens designated to the insufflated group were insufflated with CO₂ via the cannula to 10mmHg capnoperitoneum. Specimens designated to the isobaric group had their cannulas removed and a single stay suture placed in the inguinal region at the level of the midway point between pelvic rim and umbilicus and were suspended by the stay suture from a radiolucent support jig custom assembled to fit within the CT machine aperture. Specimens were suspended by approximately 40% of their bodyweight, as measured by a flat scale placed under them and then replaced with an equal thickness mat.

Image acquisition:

Images were acquired using a 16-slice Siemens CT machine. Transverse and sagittal reconstructions were exported to the PACS (SYNAPSE, FUJIFILM Australia Pty. Ltd., “Fujifilm”) in 0.8mm slice thickness and select representative 3-dimensional segmented reconstructions were created using soft-tissue and bone density windowing.

The specimens were placed in groups of 2 or 3 rabbits on the CT table and a baseline CT scan of the abdomen and thorax of all rabbit cadavers was taken under atmospheric airway pressure and without pneumoperitoneum prior to allocation to treatment groups.

The endotracheal tubes of the specimens were attached to an anaesthetic machine. A positive inspiratory pressure of approximately 11mmHg was applied through the endotracheal tube and the trachea was clamped with a non-traumatic haemostat to prevent leakage and deflation of the lungs and the image acquisition was repeated. The same image acquisition and simulated inspiratory positive pressure procedure was then repeated under the isobaric condition in cases where the insufflation condition was first performed, and vice versa.

Outcome measures:

Commercial medical imaging software (Synapse, Fujifilm) was used to collect measurements from the CT reconstructions in the soft tissue window. The sagittal thoracic reconstructions were assessed by tracing the thoracic space delineated by the internal surface of the bony structures of the thoracic cavity, the diaphragm and a straight line drawn from the 5th-6th intervertebral space to the cranial tip of the manubrium. Each slice was assessed using the region-of-interest tool to calculate cross-sectional area. The slices were summed and multiplied by the slice thickness to yield an approximate volume in cubic millimetres.

The maximum sagittal height of the abdomen from the internal surface of the ventral abdominal wall to the ventral aspect of the relevant vertebral body was measured in millimetres and defined as the “Maximum abdominal height” (MAH). The same measurement was taken along this line from the internal surface of the ventral abdominal wall to the ventral-most visceral surface, and this was defined as “Maximum abdominal space” (MAS). The visceral height was also defined as the MAH minus the MAS and represented translation of the visceral level within the abdomen between techniques.

At the level of L5-L6, a mid-sagittal line was drawn from the internal surface of the ventral abdominal wall to the ventral aspect of the L5-L6 disc space. This line was subdivided into quarters and the width of the abdomen – defined as the distance between the points where a line perpendicular to the sagittal line intersect with the internal surface of the abdominal wall – was measured in millimetres at each of these points. These widths were assigned as “25%, 50% or 75%

maximum abdominal width" (25%/50%/75% MAW) at the deep, middle, and superficial quartile points respectively (see Figure 3).

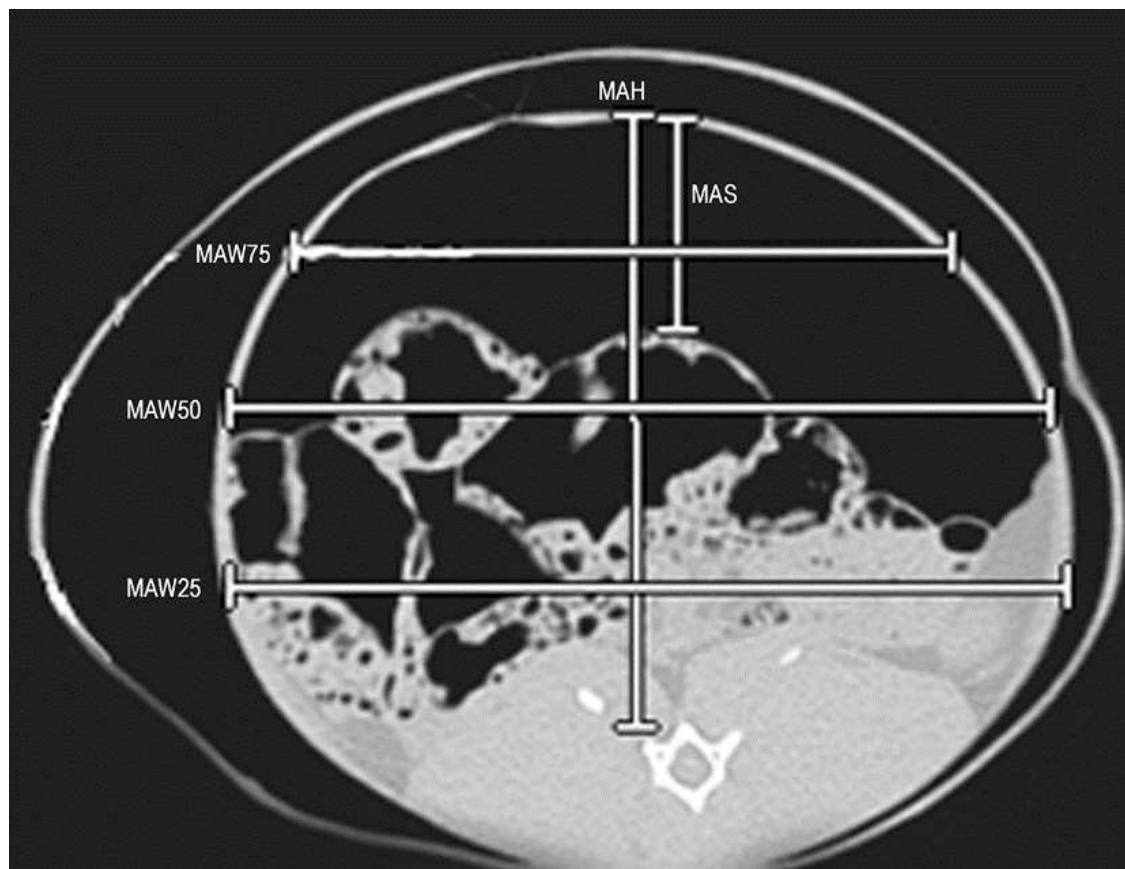


Figure 3: Example of measurement of maximum abdominal height (MAH), space (MAS) and width at 25%(MAW25), 50%(MAW50) and 75%(MAW75) abdominal depth, as calculated from the computed tomography images of the rabbit abdomen under baseline and pneumoperitoneum conditions.

Statistics: Part 2

The sample size of the second study was estimated from preliminary scans on three rabbits, using the resulting calculated means and standard deviations with a power set at 80% and 5% Type I error rate. The difference between the non-PIP and PIP thoracic volumes of the three subjects was used as the primary outcome measure for sample size calculations using two-sample inference, comparing the initial mean difference in volume between the groups ($5,328\text{mm}^3$ and 2792mm^3) to their standard deviation (2101mm^3 , 756mm^3 respectively). A sample size of 7 subjects was calculated to yield a power of 85.2%.

Direct testing of two groups was performed with Shapiro-Wilk testing was performed to assess consistency with normal distributions and subsequently, two-tailed T-tests (if normally distributed). For the comparison of thoracic volumes, an ANOVA was performed with Bonferroni correction. Significance was set at a p-value of <0.05 .

Results: Part 2

The cadavers had a mean bodyweight of 2.41 kg (range=2.06-2.79kg) with no difference between order (Figure 4) of isobaric or insufflated conditions ($p=0.18$). There were no differences between any of the pneumoperitoneum (non-baseline) groups in maximum abdominal height or space and all treatment groups had greater abdominal height than the baseline measurement ($p<0.044$). There was decrease ($p<0.01$) in the maximum visceral height in the insufflated groups compared to the isobaric groups (see Figure 5). There was no difference in visceral height between the baseline and any other groups.

The dorsal/deep abdominal width (25%MAW) at the level of L5-L6 was not different between the pneumoperitoneum groups. In the middle and superficial abdomen, (50% MAW & 75% MAW), the width of the insufflated groups was greater than the isobaric groups (see Figure 6). The PIP groups were not different from the respective non-PIP pressure groups.

In the volumetric assessment (see Table 2), thorax volume in the isobaric PIP group was significantly larger than all other groups; thorax volume in the insufflated PIP group was larger than non-PIP groups though this failed to reach significance. There was no difference between non-PIP and baseline thoracic volumes. The isobaric groups had larger ($p=0.006$) increase in thoracic volumes under PIP than insufflated groups (see Figure 7) with a mean loss of 28% of potential PIP thoracic volume in the insufflated group.

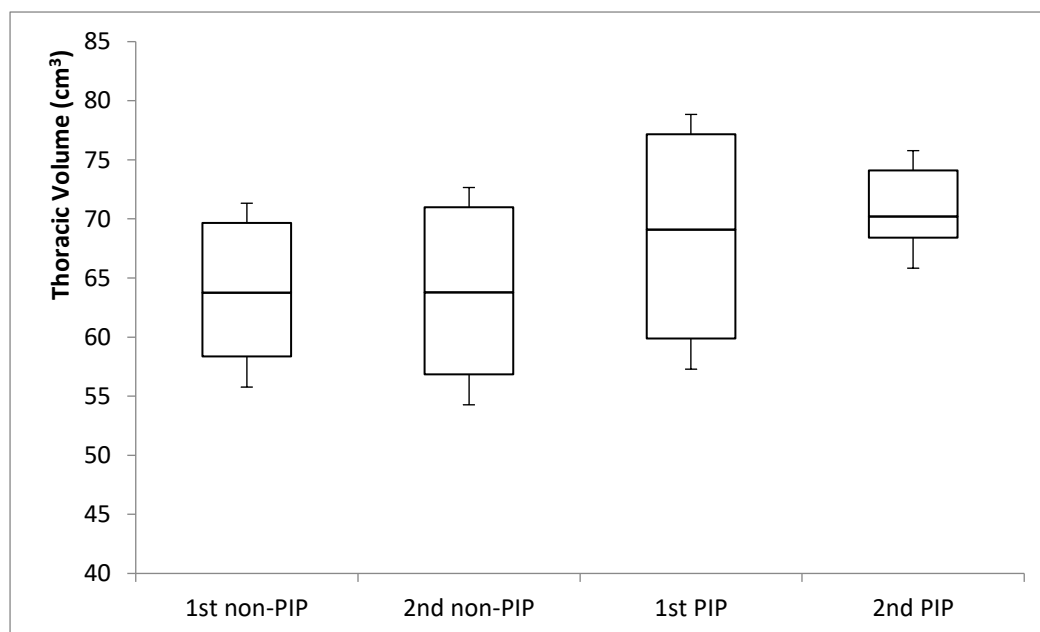


Figure 4: Box & Whisker plot: Comparison of order of procedures, standardised by bodyweight: centre-line indicates mean value, whiskers indicate standard error and box indicates 1st/3rd quartile values. There is no significant difference between order of procedure for PIP ($p=0.70$) or non-PIP volumes ($p=0.95$)

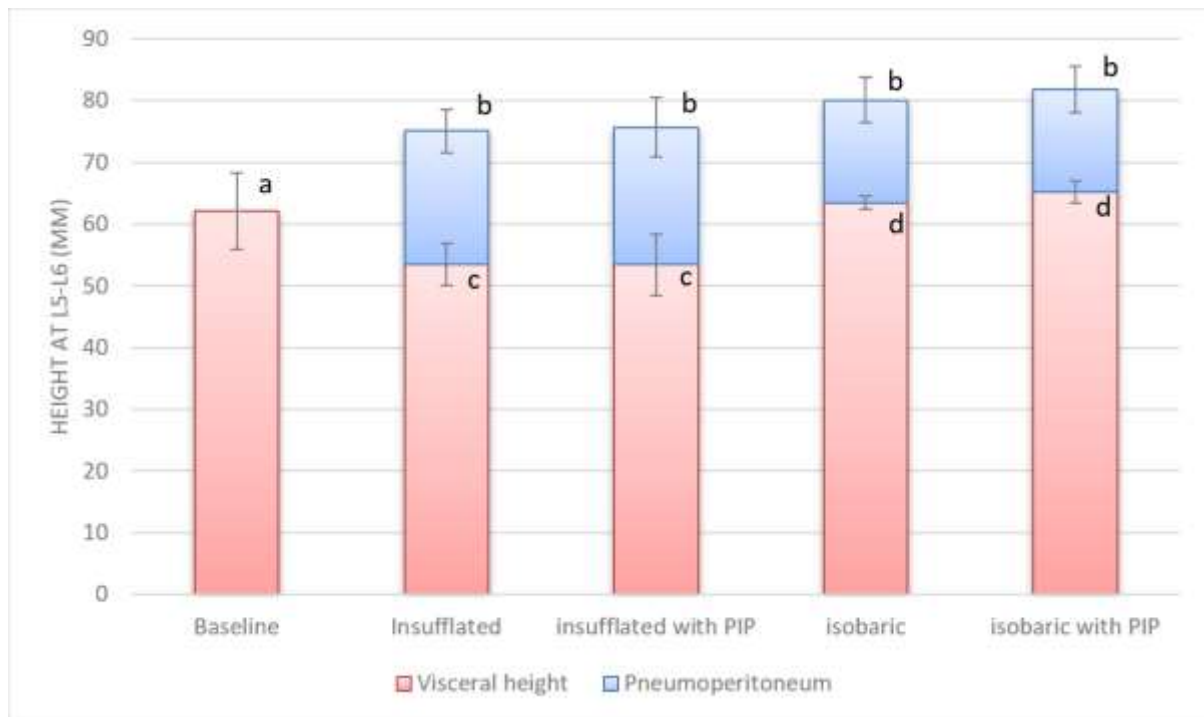


Figure 5: Comparison (n=7) of the mean abdominal height (mm), abdominal space or pneumoperitoneum (mm) & visceral height (mm) between different groups. Error bars represent standard error of the mean. There was no significant difference in visceral height between baseline (a) and groups 'c' or 'd'.
a- statistically different ($p < 0.044$) from groups 'b'.
c- statistically different ($p < 0.01$) from 'd'

Table 2: Mean difference (mm^3/kg) in thoracic volume standardised by bodyweight between baseline, insufflated & isobaric groups with and without positive inspiratory pressure (PIP). n=7. Comparisons and p-values made by ANOVA. Duplicate values ('-') have been omitted for clarity

	Isobaric	(p-value)	Isobaric (PIP)	(p-value)	Insufflated	(p-value)	Insufflated (PIP)	(p-value)
Baseline	1360	0.89	7798	0.00022	92	0.99	2667	0.41
Isobaric	-	-	9158	2.4×10^{-5}	1453	0.87	4028	0.086
Isobaric (PIP)	-	-	-	-	7705	0.00026	5130	0.017
Insufflated	-	-	-	-	-	-	2574	0.44

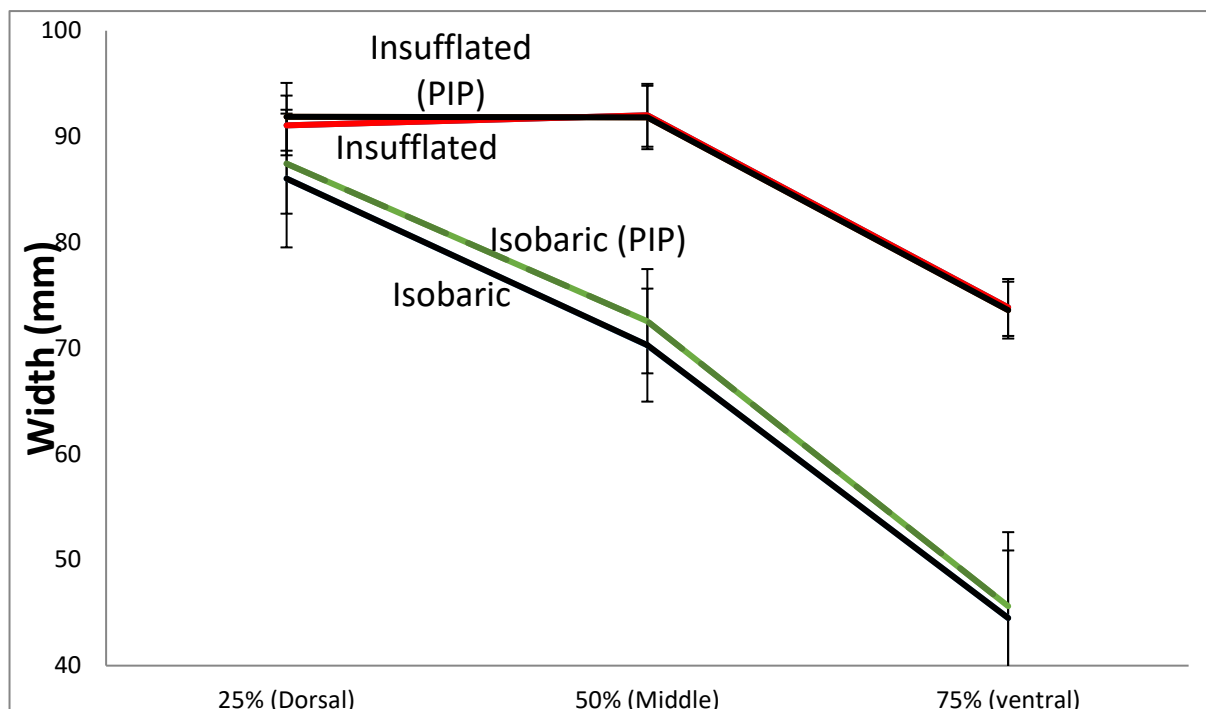


Figure 6: Chart demonstrating mean abdominal width (mm) at 25%, 50% and 75% abdominal height for insufflated and isobaric groups with and without positive inspiratory pressure (PIP). Error bars represent standard error of the mean. n=7

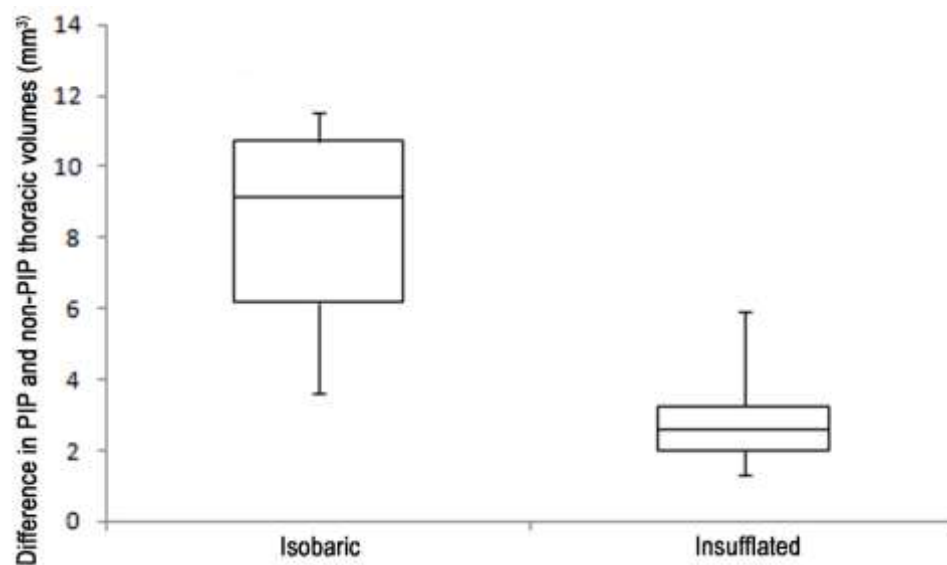


Figure 7: Box & Whisker plot: Difference (n=7) between PIP and non-PIP thoracic volumes, standardised by bodyweight: centre-line indicates mean value, whiskers indicate range, and box indicates 1st and 3rd quartile values. The difference between the isobaric and insufflated groups was statistically significant (p=0.006)

Discussion: Part 2

The simulated thoracic expansion achievable over a specified and typical inspiratory and expiratory pressure range was significantly lower in cadavers where positive pressure insufflation of 10mmHg was utilised to achieve pneumoperitoneum compared to isobaric pneumoperitoneum achieved by abdominal wall lift technique in the rabbit. It should be considered that the ventilatory pressures used in this study are recommended for rabbits regardless of the abdominal pressure.^{1,2,13,81} While an increase in applied positive airway pressure to achieve normal tidal volume would not translate directly to increased risk of lung injury (compared with overdistension), there are currently no guidelines as to what pressure would safely compensate for this in the clinical rabbit.

The decrease in PIP thoracic volume in the insufflated specimens described in this study is potentially clinically significant due to the small lung capacity and tidal volume in rabbits, which places rabbits undergoing anaesthesia at a high risk of hypoventilation. In a study by Kim et al⁶⁷ in rabbits ventilated with 11mmHg positive inspiratory pressure, PaCO₂ increased from a mean of 34.4mmHg to 82.1mmHg and blood pH decreased from 7.45 to 7.16 due to respiratory acidosis with the initiation of 8mmHg capnoperitoneum measured at 30, 60 & 90 minutes post initiation of capnoperitoneum. The positive pressure ventilation values used in the current study were chosen to describe the physical volumetric changes that could reasonably be associated with the physiological changes as described in the Kim et al study. This depression of ventilation was also described in the dog, however both insufflation and ventilation pressures used in canines are significantly higher than that recommended for use in rabbits (8-10 mm Hg as per Divers et al,¹⁵ 6 mm Hg as per Al-Badrany⁴⁸) so it was considered worthwhile completing the study in rabbits rather than attempting to extrapolate from other species. While these data collected at only two peritoneal pressure levels (0- & 10-mm Hg) cannot assess a direct relationship between different insufflation pressures and thoracic space, lower peritoneal pressures may spare the ventilatory capacity at the cost of working space. Further research would be required to quantify this relationship.

A substantial drawback to the isobaric technique that has been reported³⁸ was the effect of abdominal “tenting” on working space. Working space is provided by pneumoperitoneum and enables the surgeon/s to visualise viscera with the camera and operate their instruments. Tenting of the abdomen is caused by the upwards tension of the lifting device. In contrast, during insufflated pneumoperitoneum, the forces on the abdominal wall are radially-directed. The latter force creates a “domed” profile to the working space³⁸ which is postulated to allow easier operation of instruments (see 8, A-C).

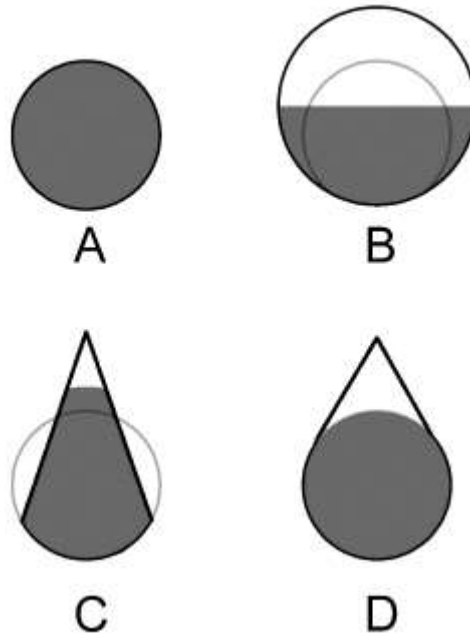


Figure 8: Diagram showing the changes from the baseline cross-section (A) under insufflation (B) and abdominal wall lift in a relatively rigid (C) and relatively pliable (D) abdominal wall. Visceral space is shown in grey and pneumoperitoneum space in white. Note this figure is exaggerated to emphasise the changes that were noted subjectively during the study.

Mitigation of this tenting has previously been suggested⁸² by providing multiple lift points operating in slightly opposing directions to simulate a domed profile, or use of a wide, spiral-shaped, fan or spatula-shaped lifting device.³⁸ Blind placement of a lifting device as previously reported was not favoured as an option by the authors due to the risk of trauma to the prominent and thin-walled cecum in the rabbit.

The working space demonstrated in the second study supported the subjective findings of study 1. Tenting of the abdomen generally leads to a triangular cross-section of the abdomen in species with less flexible abdominal musculature, however in the rabbit, the downward displacement of the viscera due to gravity acts to blunt the abdominal profile into a comma-shaped cross-section, which relatively increased the width of the working space compared to what would be expected in species with less pliable abdominal walls (see Figure 8 A-D). Insufflated groups had significantly more width at middle- and ventral- abdomen levels, suggesting that the working space had increased. The transverse reconstructions demonstrate that effective working space is more concentrated over the region of interest in the isobaric technique. Much of the additional working space in the insufflated abdomen was cranial or contralateral to the region of interest and so was not of benefit to the execution of the surgical procedure in question.

The downward displacement of the viscera in the insufflated group is likely to be of more clinical benefit than the increased abdominal width (See Figure 7). The increased pliability of the abdominal wall in the rabbit allows the viscera to remain settled in the dorsal abdomen under their own weight rather than being pulled ventrally due to abdominal tenting (See Figure 8). The increased pliability of the abdominal wall in this study is also present in cats and similar small species, and so the findings of this study could be extrapolated to those species. Although isobaric laparoscopy has been described in cats previously,⁷⁹ no direct comparison has been made between the propensity of the abdominal wall towards tenting.

An approximate lifting force of 40% body weight (split between two lift points) was used for standardization purposes for the CT study based on previous studies.³⁸ This force could be approximated in a clinical setting by placing a scale underneath the rabbit, however, subjectively, it was considered that lower lifting forces may be required to achieve similar effects due to the pliability of the abdominal wall and achieving a precise force is likely not relevant to the clinical picture. It is unknown what effect the forces used in this study would have on local perfusion in a live rabbit, however at this relatively high tension, no suture broke and there was no abdominal tearing. General stretching of the abdominal wall also occurs during insufflation, so this appears to be a general disadvantage to laparoscopy rather than specific to isobaric procedures^{60,61}.

The measurement of thoracic volume was achieved via an integrated region-of-interest function of the hospital imaging software (SYNAPSE, Fujifilm). Each slice was manually traced to calculate thoracic area. There was inherent inaccuracy in this technique, both in the difficulty of delineating the thoracic space, and the physical inaccuracy of the manual trace. To minimise this effect, the bounds of the thorax were strictly defined to the bony landmarks where sharp contrast was available. The soft tissue was able to be ignored as the specimens were compared to baseline values. It should be noted, that as the measurements are of total thoracic volume rather than lung volume, the change in diameter of the bronchial tree under pressure or compression of soft tissues could also contribute to the lung volumes, however this was considered to be a minor effect and would affect both insufflated and isobaric groups equally.

A limitation of this study is the use of cadavers rather than live rabbits. Cadavers were chosen for this study to develop the isobaric technique and assess if it was feasible for use in live rabbits. While extrapolation to live rabbits is possible, further research utilizing the described isobaric technique in live rabbits is required to assess what impact the isobaric technique may have on reduction in morbidity and mortality of these rabbits in the clinical setting. It is unclear whether the degree of changes seen with abdominal tenting may be different in a live rabbit, it is likely that compliance

would increase in the post-mortem rabbit, however this degree of increase has not been described in cadaver studies in other species^{33,35,36,79,80,82} which leads the authors to surmise that the effect observed is genuine, though may not be as emphasised in live rabbits.

Furthermore, due to the study design, it was impossible to blind the assessor to the insufflation status of the specimen during CT assessment, while standardisation of methodology throughout the study aimed at minimising assessor bias, this cannot be eliminated as a limitation of the methodology.

In conclusion, while both isobaric and insufflated laparoscopic or laparoscopy-assisted techniques are viable for the rabbit, the isobaric techniques can be conveniently achieved without specialised lifting devices in this species. Due to mechanical inhibition of ventilation caused by positive pressure pneumoperitoneum, insufflated cases may require ventilatory control (intermittent positive pressure ventilation) to maintain adequate ventilation. Rabbits with isobaric pneumoperitoneum may not require ventilatory control under similar circumstances. Further in vivo studies are recommended to assess the validity of these findings in clinical rabbits under different insufflation and ventilatory pressures.

Publications

Data from this project was published in the Journal of Exotic Pet Medicine (**see Appendix D**)

Chapter 3: Study two- Evaluation of morbidity assessments

Introduction

The challenge in assessing outcome variables of rabbit morbidity is the need for reproducible quantitative assessment criteria. In humans, pain scores are generally modelled off subjective pain assessments from verbal self-assessment schemes such as the Visual Analogue Scale, Numerical Analogue Scale or Verbal Analogue Scales.^{83,84} In these schemes, there is significant variability in the interpersonal scores that may be reported to a standardised pain stimulus. There is also a bias towards extreme values in these systems.⁸³⁻⁸⁵

In dogs and cats, non-verbal assessment is required, and so visual analogue scales are used, for example the Glasgow⁸⁶ and Melbourne⁸⁷ pain scores. These scores are based on the clinician's interpretation of the patient's response to a particular stimulus, for example touching over a region of known pain. A difficulty in the interpretation of this scoring methodology is the variability in individual animal's amenability to being handled, aggression levels and learned anticipation of pain.

Additionally, pain is a subjective experience which has been shown in studies in humans to be affected by alterations in emotional state,⁸⁸ stress, other pain stimuli⁸⁹ and modification by the central nervous system in response to chronicity^{83,84}. As such it is difficult to standardise a single nociceptive signal and determine the level of pain that an individual will experience in response to such a signal.

Compared with the previously noted species, rabbits are a prey species. As such, their natural inclination when experiencing pain is to mask their pain in the presence of an observer.^{1,2,13,85,90} The evolutionary advantage of masking pain in prey species is to decrease the likelihood of an injured animal being identified by a predator and thus targeted as an easy kill.

This creates even greater difficulty in observational means of applying pain scores to rabbits, so more subtle means of assessing their pain (and hence, post-operative morbidity) must be employed. Previously utilised subjective visual-analogue pain scores employed in other species are only particularly effective when pain is extreme due to this masking behaviour. Therefore, in order to more accurately define subtle pain, a refinement of the visual analogue scale was designed by Flecknell et al⁹¹ which is confined to the facial response to passive pain, i.e. not pain elicited in response to interference by an observer.

This so-named "grimace" pain score identifies several facial changes that rabbits exhibit when painful, including subtle alterations to their eye aperture, nasal and cheek tension, whisker and ear position. Altogether, the presence of multiple signs in a rabbit indicates a rabbit with increased facial

stress similar to a pain grimace expression in a human. This pain score was validated against rabbits receiving a standardised procedure (ovariohysterectomy) under differing analgesia protocols and found to increase under conditions of lower analgesic administration.⁹²

The major advantage of this pain-scoring technique is that it can be done without an observer where remote observation of the rabbits is possible e.g. via closed-circuit television monitoring of the rabbit. This absence of observer may reduce pain-masking behaviours in the rabbits, though the unfamiliar environment may also result in some degree of pain-masking. This is also a single-time-point measure, meaning that a score can be assigned by a trained observer within a minute and therefore rapid response such as rescue analgesia can be made in the case where a painful animal is identified.

The major disadvantage of the grimace pain score is that it remains subjective in nature, requires training in the interpretation of the rabbit's facial features and does not indicate the intensity of the acute pain that an individual rabbit may experience. For example, if a rabbit experiences severe pain when ambulating, but otherwise only mild pain when non-ambulatory, this pain will not be detected by the facial grimace score if the rabbit lies still and refuses to walk. This is particularly of relevance in the presence of an observer, when painful rabbits will generally lie still rather than attempt to flee.

Detection of "active" pain requires a measure of behaviours over time, rather than a single time-point assessment. If left alone in a cage without observers present, a non-painful rabbit will generally move around the cage, browse on greenery, and otherwise hop around unimpeded.

In order to identify rabbits experiencing active pain, a study by Leach et al⁹³ identified that certain behaviours were exhibited more frequently over a set time period in rabbits experiencing post-ovariohysterectomy pain with lower planes of analgesia. Attributed pain-behaviours include the tendency to walk- or shuffle- rather than hop, loss of balance when attempting to move, pressing their abdomen to the ground, wincing, flinching or particularly slow adjustment of position when sitting still. They allow in their scheme for miscellaneous "pain" behaviours where an observer notices a behaviour that appears to be related to pain but doesn't fit into any of the defined categories (e.g. animal not fully weight-bearing on a particular limb).

Each of these values was statistically significantly more likely to occur more frequently in rabbits which were expected to be more painful. As such, a frequency pain score was established using these variables, referred to as a behavioural pain score.

Like the facial grimace score, the main advantage of this technique is the ability to remotely observe the rabbit via closed-circuit television or scheduled video recording with no observers present. Compared with the facial grimace score, the behavioural score is expected to detect more subtle changes in pain, as well as active pain behaviours, capturing the cases where clinical signs of particularly acute pain would otherwise be unassessed in the facial grimace score.

The primary disadvantage of this scoring system relative to the facial grimace score is that as it is a frequency-over-time assessment, the time taken to acquire and then interpret the pain data is in the order of 10-30 minutes, and therefore is not a replacement for grimace score for the purposes of rapid administration of rescue analgesia. As such, it is recommended that any behaviour pain scoring period be immediately preceded by assessment of a facial grimace score and, where indicated, rescue analgesia be administered prior to, or in lieu of the behavioural pain scoring period.

Both pain scores were validated for use in interpreting rabbits and other laboratory animals post-ovariohysterectomy procedures for the purposes of administering analgesics and confirming the utility of specific analgesic protocols. As such, they have been shown to be good measures of post-ovariohysterectomy pain control.^{85,92-95}

While pain scoring is by no means the only method of detecting pain or stress, other methods of detecting chronic pain (e.g. serum cortisol and adrenaline) have been shown to demonstrate an elevated stress response during laparotomy compared with laparoscopy in the rabbit experimentally⁹⁶, however whether this translates to a clinical scenario is uncertain, as the baseline values in a pet rabbit may be elevated compared to laboratory bred rabbits due to the general stress of an unfamiliar environment, and the stress of handling to acquire blood samples, to which a pet rabbit would be less accustomed.

The main aims of this study were to determine the baseline levels of postoperative pain as measured by facial grimace scores and behavioural pain scores exhibited by rabbits undergoing open laparotomy for ovariohysterectomy under existing protocols for prophylactic analgesia. This study is purely a descriptive study, as the study design at this point did not allow for intervention other than recording of pain scores.

Materials and Methods

Animals:

Ten female rabbits were selected from rabbits undergoing routine ovariohysterectomy in the hospital. No interventions were made for the purposes of the study aside from non-invasive monitoring (video recordings and photos of them during recovery). The standardised anaesthesia

protocols used were as per Appendix A: Routine anaesthetic protocol).

Pain scoring assessment:

Each recording was made to assess for the frequency of pain-associated behaviours over a 10 minute period within a 15 minute video taken prior to premedication (baseline frequency) and three hours after extubation (post-operative assessment)⁹². Videos were recorded with a 2.5" 8.0 megapixel CMOS sensor set to record 720P at 120 frames per second (Kaiser Baas® X150 camera), which is able to be set to record video and take images remotely via a remote control or proprietary smartphone app.

The behaviours that were counted during the 10 minute period are "inactive pain" behaviours defined as: twitch, wince, stagger, flinch, press, pain, adjust slow, shuffle^{2,92,93,97} which have been shown to be significantly increased in frequency between baseline and post-ovariohysterectomy rabbits as well as different between rabbits treated with placebo and administration of meloxicam ranging from 0.1-1mg/kg given orally every 24 hours.

The following definitions were used in assessing these behaviours (with video examples provided from the designers of the pain score for reference available for comparison):⁹³

- Twitch: Rapid movement of fur on back (oscillation in the cutaneous trunci muscle)
- Wince: Rapid movement backwards in a rocking motion accompanied by eye closing and swallowing action (jerking backwards with closing and tightening of the eyes and swallowing)
- Stagger: Partial loss of balance when walking/hopping
- Flinch: Body jerks upward for no discernible reason
- Press: Abdomen pushed downwards towards the floor, usually before beginning to walk from rest
- Pain: A behaviour not exhibited in baseline that is believed to be associated with pain but does not fall into another behaviour category
- Adjust slow: Very slow adjustments of entire body posture while stationary
- Shuffle: Walking (i.e. not hopping) slowly with each limb moving sequentially.

This frequency scale was used in a summative fashion to attempt to detect a difference in the "passive" pain in the rabbits without observers present. In this way, this scale was assessed retrospectively to confirm if/when rescue analgesia was given. appropriately/inappropriately. The frequency of each behaviour was compared with baseline values in each behaviour individually, as well as all behaviours pooled. The difference between baseline and postoperative frequencies was

defined as positive if the postoperative frequency is greater than the baseline frequency, and negative if the baseline frequency is greater than the postoperative frequency.

In the acute scenario (during recovery itself), pain status was assessed using the grimace pain-scale. The grimace pain scale was assessed at 20, 40, 60 and 180 minutes after extubation, and immediately and 10 minutes following administration of rescue analgesia. Images were acquired at these intervals and assessed retrospectively by the primary investigator using the grimace pain scale. The grimace pain-scale assesses for facial coding units (“FCUs”) of “orbital tightening”, “whisker position”, “cheek flattening”, “ear position” and “nose shape” (see **Appendix B: Rabbit Grimace-score for the scoring rubric**).^{92,94}

These FCUs are defined from still images taken at these set time-points and are defined as follows:

- **Orbital tightening:** The eyelid is partially or completely closed. The globes themselves may also be drawn in toward the head so that they protrude less. If the eye is partially closed, and/or the globe less pronounced, this FCU is scored as “1”. If the eye closure reduces the visibility of the eye by more than half, it is scored as “2”. Otherwise, this FCU is scored as “0”.
- **Cheek flattening:** The muzzle is contracted so that the whisker pads are pressed against the side of the face. The side contour of the face and nose is angular, and the cheeks are not of a rounded appearance. If cheeks are slightly flattened, or not obviously rounded, this FCU is scored as “1”. If the cheeks are obviously flattened or concave, it is scored as “2”. Otherwise, this FCU is scored as “0”.
- **Pointed nose:** The nares (nostrils) which are usually present in a “U” shape are instead drawn vertically creating a “V”-shaped configuration. The tip of the nose may also be tucked under, which may exaggerate this change. If there is mild angulation in the nares forming a “Tall U-shape” or “pointed U-shape”, this FCU is scored as “1”. If the nares are sharply angulated in a “V-shape”, it is scored as “2”. Otherwise, this FCU is scored as “0”.
- **Whisker position:** The whiskers are straightened and extended horizontally or pulled back towards the cheeks instead of having a normal downward curve. If there is loss of the normal gentle neutral downward curving of the whiskers, this FCU is scored as “1”. If the whiskers are fully straightened and pulled-back, it is scored as “2”. Otherwise, this FCU is scored as “0”.
- **Ear position:** The ears are normally held roughly perpendicular to the angle of the top of the head and facing forward or to the side, away from the back and sides of the body with open/loosely curled shape. If the ears are held sloped towards the body, slightly curled and rotated backwards, this FCU is scored as “1”. If the ears are held tightly folded, rotated

towards the body and against the back/shoulders, it is scored as “2”. Otherwise, this FCU is scored as “0”.

The final facial grimace score was recorded as the sum of the FCU values (total value out of 8).

Rescue analgesia was administered if any subject scores a total pain score greater than 3/8 or if the subject is scored as “2” in any single FCU. If, for any reason, an individual FCU is unable to be assessed in a particular animal (e.g. the ears cannot be assessed due to catheters obscuring both ears), that FCU was excluded and the score was given out of six (the same minimum scores will trigger rescue analgesia). If two or more FCUs are unable to be assessed for multiple time-points, the rabbit was otherwise subjectively assessed for the need for rescue analgesia (e.g. heart rate, responsiveness) and data on facial grimace scores and rescue analgesia administration was excluded from statistical analysis.

Rescue analgesia was buprenorphine administered 0.03mg/kg intravenously or 0.05mg/kg subcutaneously up to every 6-8 hours. If rescue analgesia is administered, the grimace score was reassessed 10 minutes after administration to verify that the grimace score has reduced.

The grimace scores will also be assessed retrospectively from a still image taken during the observation phase of the video recording for the behavioural assessment. Observation was made from a still image from the video cropped to the face, or a digital image of the face. The pre- and post-operative images/videos were combined and randomised prior to assessment to blind the observer to the treatment.

Time to eating and stool production^{2,85} was recorded and was defined as the time (in hours or part thereof) between extubation and eating or first defecation. If the rabbit is found to be eating/having defecated after a period of no observation, the time-point was recorded as a range between last negative observation and the time at which the subject is observed to be eating/have defecated. If the rabbit has not been observed to eat voluntarily within three hours of extubation, rescue analgesia was administered as described above.

Indices tracked by carers were compared to assessments of pain scores in the immediate postoperative period to assess the correlation of these tests.

Any episodes of gastrointestinal stasis or other complications will also be recorded on the treatment sheet.

Statistical analysis

The mean values for passive pain behaviour frequencies (individual variables and pooled total behaviour frequency) were calculated. The behaviour scores were described in mean/standard deviation both in the postoperative total score and the difference between the preop- and postop-scores.

The facial grimace scores were described in the median/range for: the maximum FCU score (in the 0-2 scale), the maximum total score for a single timepoint (i.e. the highest sum of the FCU scores at a single timepoint for each rabbit), and the total score for each rabbit (i.e. the sum of all total scores across the observation period).

As only one animal required rescue analgesia, no meaningful statistical inferences could be made from the single datapoint, but the data was described both including and excluding the rescue analgesia patient to assess if there was a meaningful difference.

Results

Animals:

Ten cases were recruited into the study. One case was censored as she died during induction after premedication with fentanyl, so nine cases were available with complete video recordings and follow up information regarding post-operative analgesia requirements.

The carer of the rabbit that died reported that she had a history of sensitivity to drugs and the primary clinician was concerned that she may have had latent renal insufficiency, though no pre-anaesthetic blood tests were performed.

Table 3: Pain scores associated with the use of different opioids during Study 2

Opioid	Mean postop behaviour score	Std dev	Mean difference from baseline	Std dev	Median max FCU score	Range	Median max total grimace score	Range	Median cumulative grimace score	Range
Any	3.78	6.36	3.22	6.5	1	9	2	23	2	23
Any (-Rescue)	1.75	1.98	1.13	1.73	1	9	2	23	2	23
Buprenorphine	1.33	1.53	0.33	0.58	1	0	2	1	2	3
Fentanyl	8.33	9.46	8.33	9.46	0	9	0	23	0	23
Fent. (-Rescue)	2.5	2.89	2.5	3.54	0	0	0	0	0	0
Hydromorphone	1.62	2.08	1	1	1	1	2	2	2	5

Pain Scores:

One patient (premedicated with fentanyl) required rescue analgesia due to elevation of the facial grimace score. The postoperative behavioural score of 20 observed in the rabbit administered rescue analgesia was significantly higher than the sample population ($p < 0.0001$). This was the only rabbit that qualified for rescue analgesia due to facial grimace scores and was the one rabbit to

score significantly high on the behavioural pain score. The means (behavioural) and medians (FCU) were recorded (see Table 3) with both the rescue analgesia rabbit included and excluded.

The most common FCU to score was orbital tightening, and this is difficult to confirm in rabbits that are sedate/drowsy, as they tend to have their eyes closed. All rabbits who were scored >0 in cheek flattening were premedicated with buprenorphine, and the rabbit that was administered rescue analgesia scored 2 in pointed-nose FCU and a total grimace score of 4 prior to being administered analgesia. The other rabbits to score positively in pointed-nose FCU were all administered buprenorphine premedication.

Ear position was difficult to assess in rabbits with lop ears, which is a potential limitation of the grimace score, which was created for the New Zealand white rabbit breed (non-lops) which are generally used in laboratory populations. Additionally, the weight of the ear vein catheter often obscured the ear position. It is assumed that this leads to an underestimation of the ear position FCU.

Opioids:

Fentanyl had largest variance of the different opioids used (see Table 3), and had the only case that required rescue analgesia. Fentanyl still had the highest behavioural variance even when the case requiring rescue-analgesia was excluded. Fentanyl was also associated with the rabbit with the highest mean elevation in postop behavioural score, even when rescue analgesia case was excluded, likely due to short half-life, meaning analgesia wears off by the time the behavioural score was taken (see Table 3). Aside from the rabbit with breakthrough pain, no rabbits administered fentanyl analgesia scored >0 on any FCUs.

Hydromorphone and buprenorphine had a low overall mean and variance in both scores, suggesting a reliable analgesia. However, at multiple timepoints and in some cases into the behavioural monitoring period, it was noted that patients administered buprenorphine premedication were sleeping/sedated so the pain scores may not necessarily be accurate given the hydromorphone-administered patients were generally awake and eating.

The difference between pre- and post-op behavioural scores was not large in magnitude (0.087, or 0.053 when excluding the rescue analgesia case). The purpose of the study, however, was to estimate the mean and standard deviation behavioural and grimace pain scores using the proposed opioid analgesic agents.

Discussion

This study used facial grimace scores and behavioural pain scores to detect postoperative pain in rabbits undergoing routine ovariohysterectomy. Implementation of the facial grimace and behavioural pain scores for the detection of postoperative pain in ovariohysterectomy rabbits appeared to be successful in the test environments, with observers reporting that the facial grimace was not particularly difficult to implement.

All facial grimace score assessments were accompanied by a photograph of the rabbit's face and were later independently assessed by the primary author and a second score was given and compared to the initial score. All behavioural scores were assessed retrospectively by the primary author. rabbits that were generally identified as painful under one score were also identified as painful under the other score, however concordance could not be assessed statistically, as the scores were each implemented over differing time periods.

Due to the lack of widespread regulations into the pain-scoring of rabbits, there is a paucity of information as to what should be identified as a "cut-off" value for a painful rabbit. As such, relatively arbitrary values were selected as the level at which rescue analgesia would be administered. These values were determined somewhat by reference to the historical values reported in the original literature for the techniques; however, it was also subjectively assessed compared to the attending veterinarian's subjective assessment of the case. If a rabbit with a low pain score were assessed as being subjectively painful by the clinician, they were instructed to use their clinical judgement and err on the side of administration of the rescue analgesia.

On retrospective assessment, there were no situations where rescue analgesia was administered to a rabbit without an elevated grimace or behavioural pain score, indicating that the rabbits identified as painful subjectively were generally assessed as painful under the scoring criteria. In some circumstances, the rescue analgesia was required prior to acquisition of the behavioural pain score video and this may have decreased the sensitivity of this scoring criteria.

Due to the complimentary nature of the two scoring methods, it is recommended that both be implemented in any studies assessing the postoperative pain from ovariohysterectomy in the rabbit.

In the case of the later studies in this thesis document, it was the intention of the authors to confirm that the pain scores were able to be reliably implemented in the particular clinical setting of the veterinary hospitals in question, by staff previously naïve to the scoring methodology, and that it had the potential to detect inadequate pain control in specific rabbits.

The identification of the baseline of pain levels in open ovariohysterectomy as routinely performed in the clinical setting was essential for the studies to follow for two reasons. The first reason was to ensure that later data would be relatively consistent under similar anaesthesia and analgesia protocols. The second reason was to ensure that standard anaesthesia and analgesia protocols did not yield flat zero pain scores, as this would make statistical analysis impossible.

If the measured pain scores resulted in null values under the anaesthesia and analgesia protocol, it could indicate a multitude of failures, most concerning that the scoring failed to detect pain features in the rabbits, which would result in under-analgesia of the rabbits. It was expected based on the historical data^{85,92,93} that even with standard analgesia, the scores would be above zero.

While pain scoring is by no means the only method of detecting pain or stress, other methods of detecting chronic pain (e.g. serum cortisol)⁹⁰ are relatively unreliable in rabbits in the acute scenario due to the general stress that all rabbits experience when housed in unfamiliar environments, and the stress of handling to acquire blood samples. In addition, serial measurement of serum cortisol does not reflect the clinical scenario of housed rabbits, and collection of serial blood samples would increase the burden on the rabbits and would likely exceed the ethical allowances. As such, it was decided that this study remain observational-only.

Implementation issues existed in the acquisition of the video for the behavioural scores, including the requirement for exclusion of personnel from the room in which the video recordings were taking place. This requirement resulted in the inability to access other rabbits in the same ward, meaning that the video period had to be performed in times when it was expected that regular rabbit assessment was not due to be performed. In a recovery suite environment, it also meant that there were necessary interruptions to this schedule as it was not always predictable when another rabbit would return from surgery.

The video camera utilised in the recording of the behavioural score videos was capable of remote activation, however it was generally noted that the camera would need to be set up to accommodate the position the rabbit was in postoperatively and that a grimace pain score should be done immediately preceding the video acquisition period. The remote activation also functioned via Wi-Fi and was found to be unreliable, therefore manual activation and deactivation of the camera was performed to acquire the video data. This required extension of the video period – adding 5 minutes before and after the assessment period – in order to allow for any disruption to the pain scoring that may occur due to the investigator entering and exiting the room during the videoed time period.

While all attempts were made to minimally disrupt the workflow in the ward, there were necessarily times when personnel were required to enter the room during video recording, and in one case it is evident on the video that a staff member entered to talk to the rabbit being monitored. Where possible, the time-period for the data acquisition was repeated or extended to exclude such interruptions, however there were also periods of time when personnel entered the room without informing the investigator, and so no repeated video periods were recorded.

It is therefore expected that some of the pain scoring information in this study was affected by the presence of an observer, however it should be noted that the animals that were exposed to personnel interference were neither the highest nor lowest scoring rabbits in the study.

There was some variation in the analgesic protocols implemented in this study that were unavoidable due to the observational-only nature of the study. Due to the observational nature of the study design, the investigator did not have control over the analgesic protocol that was implemented in the individual case.

The variation in administered dose and agent for pre-emptive analgesia was assessed between cases. In the initial stages of the study, rabbits appeared to be over-analysed and were occasionally assessed as being narcotised and unresponsive during the pain-scoring. Following this finding, the clinicians in charge of the cases were recommended to use the standardised anaesthetic protocol (See Appendix A) , as the pain scoring assessment should be capable of capturing any break-out pain and promptly responding with rescue analgesia.

The other concern in this variation of analgesia agent and dose used between cases is that the differing analgesic and sedative properties of the agents could create confounding variables whereby behavioural pain scores could potentially be artificially decreased due to sedation rather than analgesia, i.e. the rabbit could be painful and lethargic rather than comfortable. This was observed in some of the rabbits receiving longer-acting analgesics, where scoring could not be reliably attributed to the rabbit due to the rabbit still being asleep.

Although it is possible that the rabbits were asleep due to sedation and adequately analysed, it cannot be determined via either of the pain scoring methodologies if this was the case. Additionally it is not certain what effect this prolonged sedation would have on the rabbits' morbidity overall, as inhibition of gastrointestinal function, hypotension and hypothermia have all been associated with sedation and are risk factors for postoperative complications including gastrointestinal stasis.^{1,2,13,81}

There were no rabbits assessed within 15 minutes of recovery that were identified as painful under any scoring metrics, and it is therefore suggested that all protocols are sufficient to control pain at

the preceding time points. It is therefore recommended that only a single pain score be taken during this time point to capture the rare case where total failure of analgesia occurs. After this time point, it is recommended that pain scores be repeated regularly to identify breakthrough pain as soon as possible.

In response to these findings of the variation in pain score parameters and the length of time postoperatively that pain scoring is relevant, the analgesia protocol and scoring protocols for later studies in this thesis document have been modified to better accommodate the expected clinical findings.

During the study period, there was a single case where a rabbit died under anaesthetic. This rabbit died for reasons unrelated to the study, as no drugs were specifically given or withheld for the purposes of the study, and the clinicians were instructed prior to the study that should a rabbit experience any adverse events under anaesthetic that the rabbit would be immediately unenrolled from the study for the purposes of animal welfare. As a result, there is an additional baseline video to those reported without corresponding postoperative scores.

As per standard protocol with resuscitation, the involved clinicians were debriefed following the procedure to attempt to identify if there were any risk factors in that case that could be used to identify future at-risk cases. Features identified by the clinicians in charge of the case included a low body weight (due to the rabbit being a dwarf-breed and being a runt of its litter), and advanced age. Due to the small size of the rabbit, the rabbit was much older than the typical rabbit would be at the time of ovariohysterectomy, as the carer had waited as long as possible for the rabbit to grow to an ideal weight. It was possible that the rabbit had an underlying renal insufficiency or cardiac abnormality, as its immediate response to anaesthesia was noted as being atypical, with higher than normal fluctuations in anaesthetic monitoring parameters.

No alterations were made to the anaesthetic protocol in response to this particular case, as it was assessed by a veterinary anaesthetist that it could not be identified what, if any, modifications to the anaesthetic protocol may have decreased the mortality risk in this and subsequent cases.

The observational nature of the study was the major limitation in the study methodology. Though the study was prospective insofar as the data to be acquired was selected in advance of the case presentation, the investigators did not control the general case management.

The decision to use this methodology was twofold: the intention of the study was to assess the implementation of the pain scoring systems in the existing clinical environment/s; and the involvement of the investigators into case management may have led to unforeseen negative

impacts on the clinical cases, and it was the principle direction of the study design that the study should have no or negligible impact on the rabbits enrolled. All rabbits enrolled in the study were already presented to the hospital/s for ovariohysterectomy and aside from postoperative observation; no deviation in standard protocol was made because of enrolment.

The owners of all rabbits enrolled in the study were rabbit shelter carers, who gave consent for the observations to be made and the images retained for use in publication. No financial incentives were offered to these owners specifically to entice enrolment in the study. Though the hospital/s may typically offer discounts on neutering procedures to shelter carers, this was left at the discretion of the clinician in charge of the case and was not affected in any way by enrolment in the study.

Further to this, the only guidance given by the investigators during the study were the recommendations to adhere as closely as possible to the existing clinic protocols, and that if departure from the protocols was necessary, it would be noted if minor, or the rabbit would be immediately unenrolled from the study if major, so as not to impact on the ability of the clinician in charge of the case to make immediate clinical decisions in the best interest of the rabbit. Pain scoring undertaken by investigators postoperatively was more than the postoperative monitoring usually performed in the clinic and was done in such a way as to minimally disrupt both the rabbits being monitored and other animals in the ward.

The investigator was blinded to the opioid used in each case when taking any pain scores, though this was not strictly necessary in an observational study where statistical comparison was not made between the groups.

In summary, based on the findings in this study, it can be concluded that the introduction of both the grimace facial pain score and behavioural pain scores investigated can be readily utilised in a clinical scenario, and should yield consistent data points to enable comparison of postoperative pain. It is recommended to use both methods to assess pain in these rabbits, as they can be considered complimentary in the cases they can identify.

Chapter 4: Clinical Trial

Introduction

Thus far, we have shown that the isobaric technique for OHE is feasible in rabbit cadavers and that thoracic compromise is less than with insufflation. We have also demonstrated that techniques for evaluation of postoperative pain are feasible. We now turn to evaluation of isobaric techniques for ovariectomy in rabbits in living patients and the postoperative morbidity associated with this technique compared to traditional laparoscopy.

The objectives of this study were to determine if the technique for isobaric laparoscopic ovariectomy were feasible in the rabbit and to determine if there was a difference in postoperative pain between isobaric and laparoscopic assisted OHE. Human literature has reported increased surgical time with abdominal lift isobaric procedures, but decreased respiratory compromise, postoperative morbidity and hospital stay length, suggesting a lower overall morbidity.⁷⁵ There have been no reports of increased postoperative pain in non-human patients following pneumoperitoneum.^{23,38}

It was therefore decided that a study comparing the novel isobaric technique and open laparotomy technique should be performed to confirm if there is a real and clinically significant benefit to the isobaric technique. The other consideration is that to suggest a benefit of the novel technique compared to the insufflated laparoscopic technique, the investigators would need to compare the results of any clinical trial to historical morbidity data. As there is currently no such data available, the study was expanded to include an insufflated laparoscopy group.

Additionally, the insufflated laparoscopy group would need to be lap-assisted to allow for ovariectomy, to mitigate confounding variables associated with any potential morbidity that may be caused or spared by performing an ovariectomy only and leaving the uteri in situ. This modification to the currently described ovariectomy technique has not been reported previously in the literature in rabbits, though it is similar to described techniques used in other species.⁹⁸

After the successful introduction of the morbidity assessment for postoperative rabbits in the clinic during the study reported in

Chapter 3: Study two- Evaluation of morbidity assessments, an approximate mean pain score was determined for rabbits undergoing open ovariohysterectomy under the proscribed anaesthesia protocol. This data was then used to construct a prospective power analysis for the clinical trial.

The clinical trial is designed to confirm if there is a real difference in postoperative pain between open and laparoscopic ovariohysterectomy, and also to assess if there is a difference between the insufflated and isobaric laparoscopic techniques. The clinical trial is also designed to confirm the viability of the lift laparoscopy on live rabbits in clinical practice.

The hypotheses that the clinical trial addresses are hypotheses 4-6:

4. As assessed by behavioural and grimace pain scores, perioperative morbidity of isobaric laparoscopically-assisted ovariohysterectomy will be significantly less than of open ovariohysterectomy in the rabbit.
5. Laparoscopic/Laparoscopy-assisted surgery time from first incision to closure will be significantly greater than open surgery time for ovariohysterectomy in the rabbit.
6. There will be no statistical difference in the rate of conversion from laparoscopic to open surgery in the rabbit compared with historical data in other species for equivalent procedures.

Materials and Methods

22 female entire rabbits were recruited for the study, with seven recruited into the isobaric treatment group & eight into the insufflated treatment group. An additional rabbit was included in the insufflated group due to open conversion of one rabbit. The remaining seven rabbits were recruited into the control (open laparotomy) group.

Randomization to treatment group was performed by a clinician blinded to the signalment of the individual rabbit. Breed was not used as a selection criterion.

Inclusion criteria:

Healthy female rabbits being presented for routine ovariohysterectomy by private clients or rabbit shelter/rescue program.

Exclusion criteria:

History of diarrhoea or inappetence

Respiratory distress during examination

Preoperative PCV below 30% or over 48%

Total protein below 52 or above 80

Dehydration or lack of gut sounds on gut auscultation

Weight below 0.75kg or 1kg for non-dwarf breeds

Outcome variables assessed:

Morbidity assessed was assessed with a behavioural pain score 3 hours post extubation, which is compared with a baseline behaviour score taken preoperatively (see Chapter 3 for a detailed breakdown of the pain score), and a Rabbit Facial Grimace Pain Score (see Appendix B: Rabbit Grimace-score and Chapter 3 for a detailed description of this pain scoring method) measured at 30 minute intervals until 3 hours post extubation. Time until voluntary eating and first defaecation was also tracked at the same 30-minute intervals until 5.5 hours post extubation.

Other criteria assessed:

- Time to completion of ovariohysterectomy (from first incision to transection of the final pedicle)
- Total surgery time (from first incision to final closure)
- Total anaesthetic time (from induction to extubation)
- Rescue analgesia
- Conversion rate as a percentage of insufflated or isobaric cases which require conversion to an open laparotomy
- Any complications including gut stasis

Blinding

The data, both images for grimace pain scores and behavioural pain scores were repeated independently by the primary investigator at a later date and saved in a file format that allowed blinding of the investigator from the case notes and results of other outcome variables until after the pain scores had been performed.

For the purposes of other outcome variables aside from pain scores (e.g. surgery times, etc...) blinding was not possible.

Ethics

Animal ethics approval (AEC# 1513771.1) was granted by the University of Melbourne Animal Ethics committee for all procedures described in this clinical trial.

Anaesthesia

A standardised anaesthetic protocol was used (see Appendix A: Routine anaesthetic protocol)

Additional doses given beyond the standard protocol were recorded for statistical treatment. These were given at the discretion of the anaesthetist as required, for example if additional sedation was required to enable induction, or if delays had led to excessive time elapsing between premedication and induction.

Surgical Technique: Isobaric variant

1. The rabbit is placed in a tilting cradle in dorsal recumbency, a modified Hasson technique is used to create the first portal midway between the umbilicus and pelvic rim. The cradle is moved into lateral recumbency.
2. The laparoscopic camera is inserted into the incision with a long blunt curved haemostat (e.g. Carmalt) and a stay suture is placed at the lateral-most part of the abdomen directly lateral to the portal under visualisation (see Figure 2, Chapter 2).
3. The blunt haemostat is removed, and the abdominal wall is elevated from the stay suture.
4. A second stay suture is placed as distally as can be visualised in the inguinal region under visualisation of the laparoscopic camera.
5. Both stay sutures are clamped to small haemostats and a sling of sterile bandage material is tied between them and hung from a drip-stand hook over the surgical field to maintain body wall elevation during the procedure.
6. Both remaining portal incisions are then made under visualisation.
7. Following cauterisation and transection of the ovarian pedicle on the uppermost ovary and mesometrium (i.e. the side being elevated with stay sutures) to the level of the cervix, the stay sutures are released from the sling haemostats and the cradle is rotated into contralateral lateral recumbency.
8. The stay sutures are placed under visualisation as per steps 2-4
9. Following cauterisation and transection of the second ovarian pedicle and mesometrium, the uterine horns are exteriorised through the most distal portal and the stump is ligated caudal to the cervix and transected through the caudal cervix.
10. The portal incisions are closed with 3/0 monofilament absorbable suture in a single simple interrupted (or cruciate, if required due to size of incision) in the linea alba. A second subcutaneous/dermal suture is placed to approximate the skin and the skin is closed with methylmethacrylate tissue glue.

Surgical Technique: Insufflated variant

In cases where insufflated laparoscopy is performed for this study, the placement of the initial camera portal in step 2 above is replaced with a camera canula, 8-10 mm Hg of CO₂ insufflation is applied through the canula, and both instrument portals are made simultaneously.

Otherwise the technique is the same as per the isobaric technique in regards to tilting of the cradle and the order of pedicle ligations.

Ligation and transection of the cervical stump extracorporeally requires removal of the caudal-most

canula, and therefore insufflation is discontinued at that point and the remainder of the procedure is performed as per the isobaric technique.

Statistical Analysis

A sample size of 7 per treatment group and 7 control rabbits is proposed for the clinical study, as this will yield a 91% statistical power to achieve a confidence of 95% in a difference of one category of the scale with a standard deviation of 0.8 based on previous studies which assessed the standard deviation of the grimace pain score^{92,93,97} and was consistent with the variances noted in study 2. Continuous variables were assessed with Shapiro-Wilk test to assess for normal distribution, and for homogeneity with Levene's test. As the data were not normally distributed and homogeneous, Kruskal-Wallis testing was utilised with an alpha of 0.05 and Bonferroni correction. Where a significant difference was noted via Kruskal-Wallis Test, Dunn's Post-hoc Test was used to further define the inter-group variance.

Results

The preoperative factors (See Table 4) of signalment, pre-anaesthetic examination and anaesthesia doses were tested to assess for selection bias. There was no significant difference (See Table 6) between the rabbits in the insufflated, isobaric or open groups based on age, weight, body condition score, PCV, TP, heart rate, respiratory rate, opioid dose, ketamine dose, medetomidine dose or alfaxalone dose.

The outcome variables were similarly assessed (See Table 5) and there was no significant difference (see Table 7) between the three treatment groups in regards to total surgery time, maximum FCU grimace scores, total grimace scores or time to first faeces.

Time to completion of ovariohysterectomy was a median of 20 minutes, which was significantly shorter than for isobaric (50 minutes, $p=0.0080$) and insufflated (57 minutes, $p=0.011$) laparoscopically-assisted techniques. Isobaric and insufflated ovariohysterectomy times were not significantly different from each other ($p=0.91$)

Total anaesthesia time for the insufflated group (median 135 minutes) was significantly longer than for the open group (75 minutes, $p=0.011$) but not significantly different from the isobaric group (110 minutes, $p=0.052$). There was no significant difference between the isobaric and open groups for total anaesthesia time ($p=0.060$).

The behavioural pain score was significantly higher in the open group (median score of 8) than it was in the isobaric group (1, $p=0.017$). There was no significant difference between the insufflated group and the isobaric or open groups ($p=0.57$, $p=0.067$ respectively) for behavioural pain score.

The time to voluntary eating in the isobaric group (median 120 minutes) was significantly shorter than the insufflated group (>300 minutes, $p=0.044$) and the open group (>300 minutes, $p=0.029$). There was no significant difference between the open and insufflated groups ($p=0.86$) in time to voluntary eating.

For the time to voluntary eating and time to defaecation (see Figure 9) the median rabbit had not voluntarily eaten for any groups except for the isobaric group, and the median rabbit had not passed faeces in any group. In order to account for the monitoring period end-point of 5.5 hours post extubation, rabbits which had not eaten voluntarily or defaecated by that point were assigned the next possible value of 300 minutes for statistical treatment, which is why the median value is listed as 300 in the Kruskal-Wallis analysis. This most likely substantially underestimates the true median values, so was reported as >300 minutes in the preceding paragraph.

There was one major complication noted (4.5% major complication rate) in the 22 rabbits. The single major complication was one rabbit of the insufflated group requiring conversion to open laparotomy. The conversion rate of the project was 6.77%, with this one insufflated case requiring conversion to open due to significant uterine pathology (diffuse enlargement, possibly cystic or neoplastic, though histopathology was declined). An additional rabbit was recruited to the insufflated group to balance group numbers and this rabbit's pain outcomes were monitored, but it was not included in the statistical assessment (as it had both an insufflated and open technique performed).

Minor complications were encountered in 7/22 rabbits (31%), including leaking cannulas ($n=2$), friable tissues ($n=2$), mild haemorrhage ($n=1$), excessive peritoneal fluid making visualisation difficult ($n=1$), hypotension requiring positive inotropes ($n=1$), difficulty intubating ($n=1$).

Some subjective assessments in regards to the postoperative period were that the anaesthetists and nurses reported a very rapid anaesthetic recovery for the isobaric group (7/7 described as having a "rapid recovery" and 3/7 described as having an "unexpectedly rapid recovery"). All rabbits in the open group had a "rapid recovery". Subjectively the recovery of the insufflated group was slower than the open group, with 4/7 rabbits subjectively graded as having a "slow or prolonged recovery".

One patient (open group) required rescue analgesia in the postoperative period. This rabbit was given hydromorphone premedication and was given rescue analgesia after two sequential scores of "2" for facial grimace score (it scored 1 in the "orbital tightening" FCU and 1 in "cheek flattening" FCU over two sequential assessments). While this on its own did not meet the intervention criteria, the anaesthetist recommended rescue analgesia on subjective assessment and the investigator

agreed. This may have had an effect on the behavioural pain score, as it was given 0.03mg/kg buprenorphine for its rescue analgesia, which may have contributed to sedation.

Table 4: Clinical trial signalment and preoperative data, opioid was hydromorphone in all but two cases. *fentanyl was given at a 0.06x dose scaling factor.

Surgery Method	Age (months)	Weight (kg)	BCS (/5)	PCV (L/L)	TP (g/L)	HR (bpm)	RR (bpm)	Opioid (mg/kg)	Medetomidine (mg/kg)	Ketamine (mg/kg)	Alfaxalone (mg/kg)
Insufflated	7	1.77	2.5	44	64	250	250	0.2	0.04	10	0.3
	6	1.6	2.5	-	-	240	200	0.2	0.02	5	2
	36	2.15	4	36	56	260	240	0.15	0.02	5	1
	11	1.98	2.5	35	68	210	250	0.15	0.02	5	2
	12	2.45	3	44	62	160	80	0.2	0.04	10	0.83
	10	1.3	2	-	-	260	200	0.15	0.02	7	3.8
	6	1.56	3	44	60	220	140	0.2	0.02	10	2
Insufflated (converted)	48	2.6	3	35	65	220	200	0.2	0.02	10	5.6
Isobaric	24	2.3	3.5	39	80	250	250	0.17	0.02	5	1
	24	2.15	3.5	39	65	224	48	0.15	0.02	5	1
	24	2.3	3.5	40	65	240	200	0.2	0.02	5	2.17
	10	1.8	3	-	-	240	200	0.15	0.02	7	1
	7	1.5	2.5	-	-	260	240	0.25	0.05	10	2.05
	9	2.65	3	-	-	220	140	0.2	0.02	7	1.5
	4.5	1.3	2.5	-	-	240	140	0.2	0.02	5	2.7
Open	6.5	1.65	2.5	40	54	240	30	0.2*	0.03	8	1
	7	1.71	3	-	-	236	-	0.2*	0.03	8.5	1
	6	1.36	3	38	62	240	120	0.15	0.02	5	1
	6.5	1.55	3	38	72	180	120	0.15	0.02	5	1
	12	3.5	5	34	58	240	200	0.15	0.02	5	1
	36	2.77	3	44	72	200	140	0.2	0.02	5	1.6
	9	0.75	3	40	60	240	200	0.15	0.04	10	5

Table 5: Clinical Trial, outcome variables by surgery method *rabbit received rescue analgesia, which may have artificially lowered the behavioural score due to sedative effects.

Surgery Method	Time to OVH completion (mins)	Surgery time (mins)	Anaesthesia time (mins)	First voluntary eating (mins)	First faeces production (mins)	Rescue analgesia given	Behavioural Pain score	Facial grimace Score	
								Maximum FCU	Total cumulative score
Insufflated	90	95	150	next day	next day	N	0	1	1
	60	70	135	next day	next day	N	0	1	3
	57	60	155	240	240	N	0	1	1
	45	53	100	next day	next day	N	5	1	2
	35	45	105	next day	next day	N	3	1	2
	60	65	140	150	next day	N	7	1	1
	50	55	130	150	240	N	2	0	0
Insufflated (converted)	75	85	160	next day	next day	N	2	0	0
Isobaric	88	94	150	60	90	N	1	0	0
	90	105	145	120	next day	N	2	0	0
	50	55	110	next day	next day	N	0	0	0
	70	75	110	120	next day	N	4	0	0
	45	55	100	180	next day	N	2	1	1
	30	35	150	240	next day	N	0	1	1
	20	25	70	120	180	N	0	0	0
Open	40	75	120	next day	next day	N	4	-	-
	41	70	110	next day	next day	N	11	-	-
	21	34	90	270	next day	N	8	0	0
	10	20	75	270	next day	N	9	0	0
	15	30	75	next day	next day	Y	3*	2	4
	7	15	75	next day	next day	N	0	1	1
	20	30	65	120	120	N	9	0	0

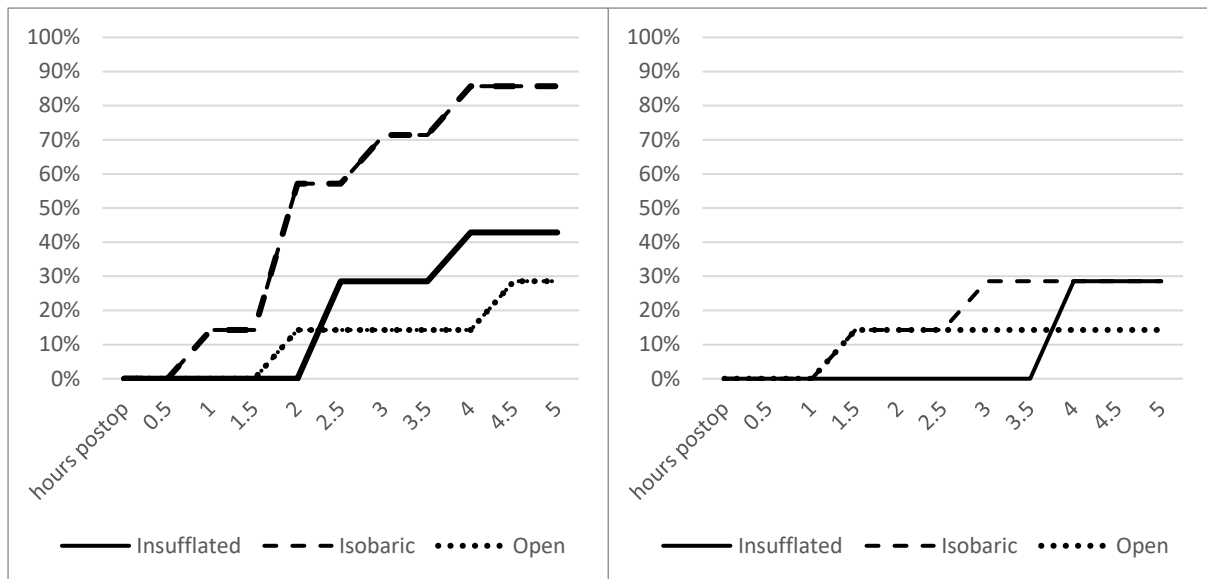


Figure 9: Percentage of rabbits from each treatment group that were eating (left) or had defaecated (right) by each timepoint after extubation. n=7 per group.

Table 6: Kruskal-Wallace testing of for bias in treatment groups, Bonferroni adjusted. n=7 per group

Variable	Kruskal-Wallace			p-Value
	Insufflated	Isobaric	Open	
Age	10	10	7	0.75
Weight	1.77	2.15	1.65	0.79
Condition score	2.5	3	3	0.34
Packed cell volume	44	39	39	0.78
Total Protein	62	65	61	0.26
Heart rate	240	240	240	0.53
Respiratory rate	200	200	130	0.19
Opioid dose	0.2	0.2	0.15	0.61
Ketamine dose	0.02	0.02	0.02	0.72
Medetomidine dose	7	5	5	0.66
Alfaxalone dose	2	1.5	1	0.71

Table 7: Kruskal-Wallis and Dunn's test for outcome variables between treatment groups, bonferroni adjusted. n=7 per group

Variable	Median			p-Value
	Insufflated	Isobaric	Open	
OVH Time	57	50	20	0.0077
Total surgery time	60	55	30	0.15
Anaesthesia time	135	110	75	0.031
Behavioural pain score	2	1	8	0.044
Maximum FCU	1	0	0	0.15
Total grimace score	1	0	0	0.079
Time to voluntary eating	300	120	300	0.0066
Time to first faeces	300	300	300	0.80

Variable	P-value		
	Isobaric - Insufflated	Insufflated - Open	Isobaric - Open
OVH Time	0.91	0.011	0.0080
Anaesthesia Time	0.52	0.011	0.060
Behavioural pain score	0.57	0.067	0.017
Time to voluntary eating	0.044	0.86	0.029

Discussion

This study was a clinical trial designed to assess the difference between open laparotomy, isobaric laparoscopic and insufflated laparoscopic ovariectomy. As the surgery involves extracorporeal ligation, it could technically be classed as laparoscopic-assisted surgery, rather than true laparoscopy, however for the purposes of brevity, the term laparoscopy will be used to refer to the procedures performed in the isobaric and insufflated treatment groups.

The 3 hypotheses addressed by this study were that there would be decreased pain scores and increased surgery times associated with the isobaric and insufflated groups compared with the open group, and that the rate of conversion would be consistent with historical data.

Pain scoring in this study was a combination of the Rabbit Facial Grimace Pain Score and the behavioural pain score⁹²⁻⁹⁴, in this case, there was no significant difference between grimace scores across the different groups. This could be due to genuinely no difference between the pain in the groups, or it could be a lack of power due to low case numbers. Additionally, while the grimace score has been validated for use for this purpose, the studies were generally done in New Zealand White rabbits in a laboratory environment. In particular, lop-eared breeds present a difficulty for the “ear position” FCU, as they do not move their ears in relation to pain. In our study, it was also noted that once the rabbits had their intravenous catheters in place, their ears would droop due to the weight. The lower possible total grimace score maximum may have contributed to a lack of difference detected.

There was a significant difference between groups for behavioural pain score, with the behavioural scores in the isobaric group being significantly lower than the open group. This difference seems to genuinely support a decreased postoperative pain in the isobaric group compared to open, but it does not support a benefit in postoperative pain in the isobaric group compared to the insufflated group. Given the long anaesthetics and slow recoveries in the insufflated group, it should be noted that residual sedation would artificially lower the behavioural score. This suggests that the significant difference in the isobaric group, with the subjectively rapid recoveries was not affected by sedation.

In one case (in an open group) a rabbit was administered rescue analgesia. Buprenorphine was administered approximately 2.5 hours after extubation at the recommendation of the anaesthetist, despite not strictly qualifying for rescue analgesia by the facial grimace score alone. This may have affected the behavioural pain score, which was assessed soon afterwards, due to the sedative effects of the buprenorphine. However, if it did affect it, it would have artificially lowered the pain

score in the open group, so would not have artificially created the observed significant difference between groups.

The other recovery difference that was noted during this study was a shorter time to voluntary eating in the isobaric group. This group had a faster median time to voluntary eating than both other groups. Since many of the patients reached the end of the monitoring period without voluntarily eating or producing faeces, and did not do so until the next day, for statistical analysis purposes, all non-recorded points were assigned “300 minutes”, as this was the next earliest time that the patients could have voluntarily eaten or defaecated and therefore would likely underestimate any differences between groups rather than setting it to later timepoints. The shorter delay to voluntary eating is likely associated with the previously mentioned rapid anaesthetic recovery in this group.

There was no difference between any group for time to first defaecation, however faeces can be delayed up to three days post anaesthesia¹⁹ so the monitoring period was not long enough to detect any difference that may have been there.

Overall the morbidity measures partially supported Hypothesis 4, as there is evidence to suggest a decreased postoperative morbidity in the isobaric group compared to the open group, though not evidence to suggest a decrease in morbidity in the insufflated group compared to the open group. There was also little evidence to suggest a difference between insufflated and isobaric groups.

The surgical procedure itself also had some significant differences between groups. It was expected (in Hypothesis 5) that the open group procedures would be significantly shorter and have shorter anaesthesia time than the other two groups. Again, this hypothesis was partially supported.

Regarding the ovariectomy time, the open procedure was significantly shorter than for the other two groups, however, this was not the case for total surgery time. While the medians seem to be quite significantly different, there was a large variance in the total surgery time, which explains the lack of significant difference in this variable. The difference in time between completion of the spay and completion of the surgery was only an additional five minutes in the laparoscopic groups but was usually closer to 10 minutes in the open group.

Regarding the total anaesthesia time, the very rapid recovery in the isobaric group compensated somewhat for the increased surgical time and set-up time from the laparoscope. Additionally, since the establishment of portals was faster in the isobaric group where cannulas and insufflation were not required, so both pre-operative and post-operative anaesthesia was shorter in the isobaric group compared to the slow-recovering insufflated group. As such, even though anaesthesia time

was shorter in the open group than the other two groups, it was only significantly shorter compared to the insufflated group.

The final hypothesis was in relation to the conversion rate. In this study the conversion rate was 6.77%. Given that a single rabbit was responsible for this, the conversion rate is relatively consistent with historical rates (7-12% in veterinary laparoscopic surgery in general)⁶³. As such the 6th hypothesis was supported in this study.

The technique of laparoscopic-assisted ovariohysterectomy (rather than ovariectomy) has not been previously reported in clinical rabbits in the rabbit and was implemented in this study to eliminate the possible variability of postoperative morbidity that may be associated with removal or retention of the uteri. While it could also have alternately been decided to perform ovariectomy procedures on all rabbits (including open procedures) it was instead decided to describe the modified laparoscopic-assisted procedure based on similar modifications in use in other species, due to the uncertainty of what clinical effects leaving the uterus in situ^{1,10,12,13,16,59} and the fact that ovariohysterectomy is considered the standard procedure for surgical neutering in this species for the country in which the study was undertaken.^{1,13}

In general, it should be emphasised that this study design is not intended to suggest that ovariohysterectomy is advantageous to ovariectomy, but simply that there was some reasoning behind the decision to utilize this technique for the study rather than the more commonly performed ovariectomy. Since the most commonly utilised laparoscopic neutering procedure in rabbits is the ovariectomy, it is a limitation of this study that it likely cannot be easily compared directly with those studies, and extrapolation to ovariohysterectomy techniques should be done cautiously.

Another limitation to this study was that the laparoscopic equipment used in the study were not designed to be used in rabbits. This would have increased the total incision size due to the necessity for larger cannulas, and this would have disproportionately affected the insufflated group.

The primary investigator was the primary surgeon in every procedure in the study, and any other investigator was utilised only as surgical assistants in these cases. All investigators acting in the role of surgeon during the study reported being “proficient” at open laparotomy and ovariohysterectomy in the rabbit prior to the beginning of the study.

The rabbits recruited into this study were offered pro gratis surgical procedures (subsidised by the study budget to cover incidental costs) in order to simplify the process of randomisation by preventing cost differentials from influencing client willingness to undertake the ordinarily more

expensive laparoscopy procedures, and to acknowledge the fact that many of the clients reporting a preference for the less invasive, laparoscopic procedures as one of their reasons for participating in the study, and the need for randomly selected control rabbits meant that these wishes could not be granted under the study design.

The inducement of a pro gratis procedure was balanced by strict inclusion criteria in order to ensure that rabbits that would not have otherwise been submitted for ovariectomy were not enrolled in the study. From an ethical standpoint this was important to ensure that inclusion in the study did not add additional surgical burden to the rabbits, and the rabbits received either the current standard surgery (open laparotomy) or a less-invasive alternative.

In summary, the results of the behavioural pain score and subjective assessments of fast anaesthetic recovery observed in this clinical trial are supportive of pursuing further development of isobaric laparoscopy in the rabbit.

Conflict of interest

No financial incentives were made available to any investigators in the study. The study was funded by the University of Melbourne's graduate research program pursuant to the principal investigator's Master of Veterinary Science qualification, for which this thesis document is produced. No external funding from private or public companies were provided to the study or investigators.

Chapter 5: Conclusion

This thesis reports the results of a multi-part investigation into the utilisation of isobaric laparoscopy-assisted ovariectomy in the rabbit.

In Chapter 1, a literature review summarised the unique factors involved in the decision to neuter rabbits, including for population control and to prevent ovarian and uterine neoplasia. Due to the perception of rabbits as low-cost pets or a pest species, particularly when feral, there is a decreased desire of the general public to pay for what is seen as an additional expenditure such as a neutering procedure. This being the case, there is a global trend towards the adoption of rabbits as pets, with recognition of their role in the family approaching that of dogs and cats. It can be speculated that the demand of the average rabbit owner for advanced and non-invasive surgical modalities is likely to increase, as it has done in the dog and cat in recent years.

Chapter 1 also described the philosophy of minimally-invasive surgery as it relates to laparoscopic and laparoscopy-assisted techniques. Isobaric pneumoperitoneum methods for laparoscopic techniques in rabbits were developed for use where positive pressure pneumoperitoneum is not possible or poses a risk to the rabbit's welfare. In particular, the lack of pressure on the diaphragm of isobaric pneumoperitoneum is relevant to rabbits at risk of ventilatory compromise. Parallels were drawn between the general patient at-risk of ventilatory compromise and the high peri-anaesthetic mortality risks observed in rabbits due in part to hypoxia associated with a proportionately small lung volume.

In Chapter 2, an investigation into the technical viability of isobaric laparoscopy in the rabbit was undertaken, alongside a descriptive study to investigate the assumption that isobaric pneumoperitoneum would mitigate the ventilatory compromise observed in positive-pressure pneumoperitoneum methodologies. The subjective assessments undertaken during this investigation were supportive of pursuing isobaric laparoscopic techniques in rabbits, as the technical difficulty of implementing these methods was comparable or superior to the implementation of insufflated laparoscopic techniques in rabbit cadavers.

The descriptive volumetric study investigating the effects of positive-pressure and isobaric pneumoperitoneum on the simulated ventilatory cycle of rabbit cadavers found significantly larger changes in thoracic volume after the application of a standardised positive ventilation pressure in cases with isobaric pneumoperitoneum compared to positive-pressure pneumoperitoneum. While this finding was not particularly surprising, it was important to verify that a potentially clinically-

relevant difference could be observed between the techniques in order to justify proceeding with a clinical trial to assess the impact of the two procedures in live rabbits.

Chapter 3 reports the results of a pilot study to verify the implementation of behavioural pain scores in postoperative rabbit rabbits with typical clinical analgesic programs implemented. The challenges facing clinicians in assessing rabbit rabbits for postoperative pain are well established, and prior to undertaking this study, it was uncertain if behavioural pain scores were relevant to the clinical setting, as previously they have been implemented primarily in laboratory animals. The results of this study were consistent with the complimentary use of the Rabbit Grimace Score and the Behavioural Pain Score, with the former most useful for acute cage-side assessment and implementation of rescue analgesia and the latter potentially more sensitive for the detection of occult or dull subacute pain retrospectively.

Finally, Chapter 4 reports the implementation of a clinical trial to assess the effects of open laparotomy, isobaric laparoscopy-assisted and insufflated laparoscopy-assisted ovariohysterectomy in rabbits. The trial confirmed the viability of the isobaric laparoscopic technique in clinical cases, with both subjective and objective assessments between isobaric and insufflated laparoscopy being indistinguishable or favourable towards the isobaric technique for surgeons that are relatively inexperienced in both methods. This finding may not be applicable to surgeons who are highly experienced in insufflated laparoscopy in the rabbit when attempting to implement unfamiliar isobaric techniques. It is likely that for the clinician naïve to laparoscopic surgery, that the isobaric method would carry a shallower learning curve, as it requires less specialised equipment compared with the insufflated method.

While no definitive benefit to morbidity could be established between isobaric and insufflated laparoscopic methods, there was a statistical and clinically significant benefit to isobaric laparoscopic-assisted techniques compared with laparotomy as defined by postoperative behavioural pain scores, and earlier time to voluntary eating, despite similar total surgery and anaesthesia times.

Laparoscopic surgery required a longer time under anaesthesia and generally a longer surgery time (though this failed to reach significance during the clinical trial, the trend was certainly towards longer laparoscopic surgery). As only one rabbit required conversion to open surgery out of 15, and that rabbit had uterine abnormalities, it was not seen as being particularly productive to discuss conversion rates of the techniques.

Of the six hypotheses postulated in Chapter 1, all were fully or partially confirmed as follows:

1. “As assessed using surgeon responses and subjective qualitative feedback, isobaric laparoscopy will be seen a viable method of performing laparoscopically-assisted ovariohysterectomy in the rabbit.”
 - This was supported during study 1 and further supported by successful implementation during the clinical trial (See Chapter 2 and Chapter 4)
2. “The use of abdominal wall-lift will provide equivalent working space to insufflated pneumoperitoneum in the rabbit abdomen to allow for completion of a laparoscopically-assisted ovariohysterectomy.”
 - This was supported during study 1 and further supported by successful implementation during the clinical trial (see Chapter 2 and Chapter 4)
3. “The establishment of an isobaric pneumoperitoneum will allow for a greater minimum and maximum thoracic volume and tidal volume, as measured by total intrathoracic volume at simulated inspiratory and expiratory phases during positive pressure ventilation.”
 - This was supported during the second part of study 1 (see Chapter 2)
4. As assessed by behavioural and grimace pain scores, perioperative morbidity of isobaric laparoscopically-assisted ovariohysterectomy will be significantly less than of open ovariohysterectomy in the rabbit.
 - This was partially supported. Only the behavioural scores were significantly different, and only between the isobaric laparoscopy and open laparotomy groups. (see Chapter 4)
5. Laparoscopic/Laparoscopy-assisted surgery time from first incision to closure will be significantly greater than open surgery time for ovariohysterectomy in the rabbit.
 - This was rejected, as the total surgery time was not significantly different, however the ovariohysterectomy time was significantly shorter for the open laparoscopy group, and the anaesthesia time was significantly longer in the insufflated laparoscopy group compared to the open laparotomy group (see Chapter 4)
6. There will be no statistical difference in the rate of conversion from laparoscopic to open surgery in the rabbit compared with historical data in other species for equivalent procedures.
 - This hypothesis was supported by the data during the clinical trial (see Chapter 4)

The summary findings of these studies support the clinical implementation of isobaric laparoscopy-assisted ovariohysterectomy as an alternative method of neutering female pet rabbits.

References

1. Varga M: Textbook of Rabbit Medicine (ed 2nd), Elsevier Ltd, 2014.
2. Barter LS: Rabbit analgesia. *Vet Clin North Am Exot Anim Pract* 14:93-104, 2011.
3. PDSA: PDSA PAW Report, in, Vol, 2015.
4. AVMA: U.S. Pet Ownership Statistics. (<https://www.avma.org/KB/Resources/Statistics/Pages/Market-research-statistics-US-pet-ownership.aspx>), 2012.
5. NZCAC: Companion Animals in New Zealand Report, in, Vol, 2016.
6. FEDIAF: Facts and Figures, in, Vol, 2014.
7. Alliance AH: Pet Ownership in Australia Summary, in, Vol Animal Health Alliance, 2013.
8. Queensland Biosecurity Act, in, Vol, 2014.
9. Kerr PJ: Myxomatosis in Australia and Europe: A model for emerging infectious diseases. *Antiviral Research* 93:387-415, 2012.
10. Baba N, von Haam E: Animal model: spontaneous adenocarcinoma in aged rabbits. *Am J Pathol* 68:653-656, 1972.
11. Bertram CA, Muller K, Klopfleisch R: Genital Tract Pathology in Female Pet Rabbits (*Oryctolagus cuniculus*): a Retrospective Study of 854 Necropsy Examinations and 152 Biopsy Samples. *J Comp Pathol* 164:17-26, 2018.
12. Walter B, Poth T, Bohmer E, et al: Uterine disorders in 59 rabbits. *Vet Rec* 166:230-233, 2010.
13. Richardson C, Flecknell PA: Routine neutering of rabbits and rodents. In *Practice* 28:70-79, 2006.
14. Bennett PC, Morenment K, Howell T: Pet-care practices in Victoria, 2013: a survey of bird, cat, dog and rabbit owners. *unpublished data*, 2013.
15. Divers SJ: Clinical Technique: Endoscopic Oophorectomy in the Rabbit (*Oryctolagus cuniculus*): The Future of Preventative Sterilizations. *Journal of Exotic Pet Medicine* 20:72-80, 2011.
16. Brown Y, Knowles S, Fiorello C, et al: Pathology in Practice. *JAVMA* 237:1257-1259, 2010.
17. Brodbelt DC, Blissit KJ, Hammond RA, et al: The risk of death: the Confidential Enquiry into Perioperative Small Animal Fatalities. *Veterinary Anaesthesia and Analgesia* 35:365-373, 2008.
18. Aeschbacher G: Rabbit Anaesthesia. *Compend Contin Educ* 17:1003-1010, 1995.
19. Schnellbacher RW, Divers SJ, Comolli JR, et al: Effects of intravenous administration of lidocaine and buprenorphine on gastrointestinal tract motility and signs of pain in New Zealand White rabbits after ovariohysterectomy. *Am J Vet Res* 78:1359-1371, 2017.
20. Oglesbee BL, Jenkins JR: Gastrointestinal Diseases, in Quesenberry K, Carpenter J (eds): *Ferrets, rabbits, and rodents: clinical medicine and surgery* (ed 3rd), Vol. St. Louis, Mo., Elsevier/Sanders, 2012, pp 193-204.
21. Huynh M, Vilmouth S, Gonzalez MS, et al: Retrospective cohort study of gastrointestinal stasis in pet rabbits. *Vet Rec* 175:225, 2014.
22. Pitt KA, Mayhew PD, Steffey MA, et al: Laparoscopic Adrenalectomy for Removal of Unilateral noninvasive Pheochromocytomas in 10 Dogs. *Veterinary Surgery* 45:O70-O76, 2016.
23. Fransson BA, Ragle CA: Lift laparoscopy in dogs and cats: 12 cases (2008-2009). *JAVMA* 239:1574-1579, 2011.
24. Gurusamy K, Samraj K, Gluud C, et al: Meta-analysis of randomized controlled trials on the safety and effectiveness of early versus delayed laparoscopic cholecystectomy for acute cholecystitis. *Br J Surg* 97:141-150, 2010.
25. Takacs JD, Singh A, Case JB, et al: Total laparoscopic gastropexy using 1 simple continuous barbed suture line in 63 dogs. *Veterinary Surgery* 46:233-241, 2016.

26. Yesildaglar N, Konninckx P: Adhesion formation in intubated rabbits increases with high insufflation pressure during endoscopic surgery. *Human Reproduction* 15:687-691, 2000.
27. Arung W, Drion P, Cheramy JP, et al: Intraperitoneal adhesions after open or laparoscopic abdominal procedure: an experimental study in the rat. *J Laparoendosc Adv Surg Tech A* 22:651-657, 2012.
28. Molinas CR, Koninckx PR: Hypoxaemia induced by CO₂ or helium pneumoperitoneum is a co-factor in adhesion formation in rabbits. *Human Reproduction* 15:1758-1763, 2000.
29. Shimada M, Kawaguchi M, Ishikawa N, et al: Saline-filled laparoscopic surgery: A basic study on partial hepatectomy in a rabbit model. *Minim Invasive Ther Allied Technol*:1-8, 2014.
30. Kabakchiev C, Valverde A, Singh A, et al: Cardiovascular and respiratory effects of carbon dioxide pneumoperitoneum in the domestic rabbit (*Oryctolagus cuniculus*). *Can J Vet Res* 84:108-114, 2020.
31. Fujii H, Aotake T, Kawakami Y, et al: Modified hemi-double-stapling technique combined with the temporal abdominal-wall-lift method for performing Billroth I anastomosis after laparoscopically assisted distal gastrectomy. *Surg Endosc* 22:2748-2752, 2008.
32. Galizia G, Prizio G, Lieto E, et al: Hemodynamic and pulmonary changes during open, carbon dioxide pneumoperitoneum, and abdominal wall-lifting cholecystectomy. *Surg Endosc* 15:477-483, 2001.
33. Liao CH, Kuo IM, Fu CY, et al: Gasless laparoscopic assisted surgery for abdominal trauma. *Injury* 45:850-854, 2014.
34. Luks FI, Peers KHE, Deprest JA, et al: Gasless Laparoscopy in infants: The Rabbit Model. *J Ped Surg* 30:1206-1208, 1995.
35. Smith RS, Fry WR, Tsoi EKM, et al: Gasless Laparoscopy and Conventional Instruments. *Arch Surg* 128:1102-1107, 1993.
36. Tintara H, Choobun T, Geater A: Gasless laparoscopic hysterectomy: a comparative study with total abdominal hysterectomy. *J Obstet Gynaecol Res* 29:38-44, 2003.
37. Kennedy KC, Fransson BA, Gay JM, et al: Comparison of Pneumoperitoneum Volumes in Lift Laparoscopy With Variable Lift Locations and Tensile Forces. *Vet Surg*, 2014.
38. Watkins C, Fransson BA, Ragle CA, et al: Comparison of thoracic and abdominal cavity volumes during abdominal CO₂ insufflation and abdominal wall lift. *Vet Surg* 42:607-612, 2013.
39. Corona R, Verguts J, Binda MM, et al: The impact of the learning curve on adhesion formation in a laparoscopic mouse model. *Fertil Steril* 96:193-197, 2011.
40. Kirlum H, Heinrich M, Tillo N, et al: Advanced paediatric laparoscopic surgery: repetitive training in a rabbit model provides superior skills for live operations. *Eur J Pediatr Surg* 15:149-152, 2005.
41. Marecos M, Torres R, Bailez M, et al: Pediatric Thoracoscopic Training in an Experimental Pleural Empyema Rabbit Model. *J Laparoendosc Adv Surg Tech A* 16:2006, 2006.
42. Molinas CR, Binda MM, Mailova K, et al: The rabbit nephrectomy model for training in laparoscopic surgery. *Human Reproduction* 19:185-190, 2004.
43. Sanchez-Salas: Autoaumento vesical por laparoscopia. Modelo experimental en conejos (*oryctolagus cuniculus*). *Actas Urologicas Espanolas* 32:722-726, 2008.
44. Uson-Casaus J, Perez-Merino EM, Rivera-Barreno R, et al: Evaluation of a Bochdalek diaphragmatic hernia rabbit model for pediatric thoracoscopic training. *J*

- Laparoendosc Adv Surg Tech A 24:280-285, 2014.
45. Valdivieso J, Contador M: The Rabbit: A Good Animal Model for Teaching and Training in Pediatric Laparoscopic Surgery. *Pediatric Endosurgery & Innovative Techniques* 7:303-307, 2003.
 46. Guzman DS-M, Graham JE, Keller K, et al: Colonic Obstruction Following Ovariohysterectomy in Rabbits: 3 Cases. *Journal of Exotic Pet Medicine* 24:112-119, 2015.
 47. Divers SJ: Personal communication, via email, 2015.
 48. Al-Badrany MS: Laparoscopic ovariectomy in rabbits. *Iraqi Journal of Veterinary Sciences* 23:51-55, 2009.
 49. Proenca LM: Two-Portal Access Laparoscopic Ovariectomy Using Ligasure Atlas in Exotic Companion Mammals. *Vet Clin North Am Exot Anim Pract* 18:587-596, 2015.
 50. Coleman KA, Monnet E, Johnston MS: Single Port Laparoscopic-Assisted Ovariohysterectomy in 3 Rabbits. *Journal of Exotic Pet Medicine* 27:21-24, 2018.
 51. Carbonell AM, Joels CS, Kercher KW, et al: A Comparison of Laparoscopic Bipolar Vessel Sealing Devices in the Hemostasis of Small-, Medium-, and Large-Sized Arteries. *J Laparoendosc Adv Surg Tech A* 13:377-380, 2003.
 52. Harold KL, Pollinger H, Matthews BD, et al: Comparison of ultrasonic energy, bipolar thermal energy, and vascular clips for the hemostasis of small-, medium-, and large-sized arteries. *Surg Endosc* 17:1228-1230, 2003.
 53. Marvel S, Monnet E: Use of a vessel sealant device for cystic duct ligation in the dog. *Vet Surg* 43:983-987, 2014.
 54. Mayhew PD, Brown DC: Comparison of three techniques for ovarian pedicle hemostasis during laparoscopic-assisted ovariohysterectomy. *Vet Surg* 36:541-547, 2007.
 55. Newcomb WL, Hope WW, Schmelzer TM, et al: Comparison of blood vessel sealing among new electrosurgical and ultrasonic devices. *Surg Endosc* 23:90-96, 2009.
 56. Barrera JS, Monnet E: Effectiveness of a bipolar vessel sealant device for sealing uterine horns and bodies from dogs. *Am J Vet Res* 73:302-305, 2012.
 57. Sindram D, Martin K, Meadows JP, et al: Collagen-elastin ratio predicts burst pressure of arterial seals created using a bipolar vessel sealing device in a porcine model. *Surg Endosc* 25:2604-2612, 2011.
 58. Barrera JS, Monnet E: Effectiveness of a bipolar vessel sealant device for sealing uterine horns and bodies from dogs. *Am J Vet Res* 73:302-305, 2012.
 59. Brown YK, Knowles S, Fiorello CV, et al: Pathology in practice. Morphologic diagnosis: Uterine adenocarcinoma with metastasis to the lungs, diaphragm, spleen, mesentery, and cecum. *J Am Vet Med Assoc* 237:1257-1259, 2010.
 60. Goldberg JM, Maurer WG: A Randomized Comparison of Gasless Laparoscopy and CO₂ Pneumoperitoneum. *Obstetrics & Gynecology* 90:416-420, 1997.
 61. Guido RS, Brooks K, McKenzie R, et al: A randomized, prospective comparison of pain after gasless laparoscopy and traditional laparoscopy. *J Am Assoc Gynecol Laparosc* 5:149-153, 1998.
 62. Malone L, Schuler C, Leggett RE, et al: Effect of estrogen and ovariectomy on response of the female rabbit urinary bladder to two forms of in vitro oxidative stress. *Int Urogynecol J* 25:791-798, 2014.
 63. Tapia-Araya AE, Martin-Portugués ID-G, Sánchez-Margallo FM: Veterinary laparoscopy and minimally invasive surgery. *Companion Animal* 20:382-392, 2015.
 64. Nylund AM, Drury A, Weir H, et al: Rates of intraoperative complications and conversion to laparotomy during laparoscopic ovariectomy performed by veterinary students: 161 cases (2010-2014). *J Am Vet Med Assoc* 251:95-99, 2017.

65. Nguyen NT, Lee SL, Goldman C, et al: Comparison of pulmonary function and postoperative pain after laparoscopic versus open gastric bypass: a randomized trial¹¹No competing interests declared. *Journal of the American College of Surgeons* 192:469-476, 2001.
66. Bailey J, Shapiro MJ: Abdominal compartment syndrome. *Critical Care* 4:23-29, 2000.
67. Kim J-S, Lee B-H, Jang I-S, et al: Tracheal Gas Insufflation-Aided Mechanical Ventilation during Carbon dioxide-Induced Pneumoperitoneum in Rabbits. *J Vet Med Scie* 65:907-912, 2003.
68. Kabakchiev CM, Zur Linden AR, Singh A, et al: Effects of intra-abdominal pressure on laparoscopic working space in domestic rabbits (*Oryctolagus cuniculus*). *Am J Vet Res* 81:77-83, 2020.
69. Hanley EJ, Aurora AR, Fuentes JM, et al: Abdominal Insufflation With CO₂ Causes Peritoneal Acidosis Independent of Systemic pH. *J Gastrointest surg* 9:1245-1252, 2005.
70. Ishida H, Murata N, Yamada H, et al: Influence of trocar placement and CO₂ pneumoperitoneum on port site metastasis following laparoscopic tumor surgery. *Surgical Endoscopy* 14:193-197, 2014.
71. Wang G, Wu R, Guo F, et al: Effects of carbon dioxide pneumoperitoneum on the inflammatory response and bacterial translocation in intraabdominal infection. *J Laparoendosc Adv Surg Tech A* 24:199-204, 2014.
72. Wong YT, Shah PC, Birkett DH, et al: Carbon dioxide pneumoperitoneum causes severe peritoneal acidosis, unaltered by heating, humidification, or bicarbonate in a porcine model. *Surgical Endoscopy* 18:1498-1503, 2004.
73. El-Hakim A, Chui K, Sherry B, et al: Peritoneal and systemic inflammatory mediators of laparoscopic bowel injury in a rabbit model. *J Urol* 172:1515-1519, 2004.
74. Chatzimavroudis G, Pavlidis TE, Koutelidakis I, et al: CO(2) pneumoperitoneum prolongs survival in an animal model of peritonitis compared to laparotomy. *J Surg Res* 152:69-75, 2009.
75. Uen Y-H, Liang A-I, Lee H-H: Randomized Comparison of conventional Carbon dioxide Insufflation and Abdominal Wall Lifting for Laparoscopic Cholecstectomy. *J Laparoendosc Adv Surg Tech A* 12:7-14, 2002.
76. Humphrey GME, Najmaldin A: Modification of the Hasson technique in paediatric laparoscopy. *British Journal of Surgery* 81, 1994.
77. Poole GH, Frizelle FA: Modifications to the Hasson Technique. *Aust NZJ Surg* 66, 1996.
78. Fahy BG, Barnas GM, Nagle SE, et al: Effects of Trendelenburg and reverse Trendelenburg postures on lung and chest wall mechanics. *J Clin Anesth* 8:236-244, 1996.
79. Fransson BA, Ragle CA: Lift laparoscopy in dogs and cats: 12 cases (2008-2009). *J Am Vet Med Assoc* 239:1574-1579, 2011.
80. Luks FI, Peers KH, Deprest JA, et al: Gasless laparoscopy in infants: the rabbit model. *J Pediatr Surg* 30:1206-1208, 1995.
81. Self I: A basic approach to small mammal anaesthesia. *Irish Veterinary Journal* 60:94-100, 2007.
82. Kennedy KC, Fransson BA, Gay JM, et al: Comparison of Pneumoperitoneum Volumes in Lift Laparoscopy With Variable Lift Locations and Tensile Forces. *Veterinary Surgery*:n/a-n/a, 2014.
83. Haefeli M, Elfering A: Pain assessment. *Eur Spine J* 15 Suppl 1:S17-24, 2006.
84. Hansen BD: Assessment of Pain in Dogs: *Veterinary Clinical Studies. ILAR Journal* 44:197-205, 2003.
85. Flecknell PA, Roughan JV: Assessing pain in animals -- Putting research into

- practice. *Animal Welfare* 13:71-75, 2004.
86. Holton L, Reid J, Scott EM, et al: Development of a behaviour-based scale to measure acute pain in dogs. *Vet Rec* 148:525-531, 2001.
 87. Firth AM, Haldane SL: Development of a scale to evaluate postoperative pain in dogs. *J Am Vet Med Assoc* 214:651-659, 1999.
 88. Lumley MA, Cohen JL, Borszcz GS, et al: Pain and emotion: a biopsychosocial review of recent research. *Journal of clinical psychology* 67:942-968, 2011.
 89. Cromwell HC, Atchley RM: Influence of emotional states on inhibitory gating: animals models to clinical neurophysiology. *Behavioural brain research* 276:67-75, 2015.
 90. Bayazit V: Evaluation of Cortisol and Stress in Captive Animals. *Australian Journal of Basic and Applied Sciences* 3:1022-1031, 2009.
 91. Flecknell PA, Liles J: Assessment of the analgesic action of opioid agonist-antagonists in the rabbit. *J Ass Vet Anaesth* 17:24-29, 1990.
 92. Keating SCJ, Thomas AA, Flecknell PA, et al: Evaluation of EMLA Cream for Preventing Pain during Tattooing of Rabbits: Changes in Physiological, Behavioural and Facial Expression Responses. *PLoS ONE* 7:1-11, 2012.
 93. Leach MC, Allweiler S, Richardson C, et al: Behavioural effects of ovariohysterectomy and oral administration of meloxicam in laboratory housed rabbits. *Res Vet Sci* 87:336-347, 2009.
 94. Leach MC, Keating SCJ: RbtGS Manual (draft), unpublished (provided via email, see Appendix B), 2015.
 95. Sprecher CM, Milz S, Suter T, et al: Retrospective analysis of corrosion and ion release from retrieved cast stainless steel tibia plateau leveling osteotomy plates in dogs with and without peri-implant osteosarcoma. *Am J Vet Res* 79:970-979, 2018.
 96. Chaniotakis I, Antoniou E, Kostomitsopoulos N, et al: Stress response to ovariohysterectomy in rabbits: role of anaesthesia and surgery. *J Obstet Gynaecol* 38:697-701, 2018.
 97. Leach MC: Personal communication, via email, 2015.
 98. Sánchez-Margallo FM, Tapia-Araya A, Díaz-Güemes I: Preliminary application of a single-port access technique for laparoscopic ovariohysterectomy in dogs. *Veterinary record open* 2:e000153-e000153, 2015.
 99. McCracken BD, Beths T, Herbert S, et al: Comparison of Isobaric and Insufflated Laparoscopy-Assisted Ovariohysterectomy in the Common Rabbit (*Oryctolagus Cuniculus*). *Journal of Exotic Pet Medicine* 28:193-204, 2019.

Appendices

Appendix A: Routine anaesthetic protocol

Pre-anaesthetic assessment

- Body condition (muscle bulk versus fat),
- Body weight
- Gut fill and gut sounds
- Dental condition
- Heart Rate
- Respiratory Rate and effort
- Rectal temperature
- Mucus membrane (gum) colour
- Hydration (subjective)
- PCV/TP

Anaesthetic protocol

- 5 minutes oxygen flow by in quiet space, either cuddled or allowed to shelter under towel depending on rabbit preference.
- Clip hair from marginal ear vein on the right side and apply EMLA local anaesthetic gel in preparation for later IV catheterisation³⁵
- Premedication intramuscularly in the paralumbar muscles after 5 minutes preoxygenation. Oxygen flow by continues while premedication takes effect (5-10 minutes).
- Monitor heart rate and respiratory rate every 2 minutes at this stage.
- Choice of drugs and doses dependent on individual patient health status, age and size. Rabbits with body fat and less muscle will get lower doses, giant breeds will get lower doses, very active or aggressive rabbits will get higher doses and dwarf breeds usually require higher doses.
 - Fentanyl 0.0074mg/kg IM/SC
 - Or
 - Hydromorphone 0.2mg/kg (0.15-0.25mg/kg) IM
 - Dexmedetomidine 0.02mg/kg (0.02- 0.03 mg/kg) IM
 - Ketamine 5-10mg/kg IM

- Induction once rabbit displays muscle relaxation and reduced jaw tone to allow visualisation of larynx.
 - Place IV catheter 22-24G in right marginal ear vein.
- Some rabbits are relaxed enough at this point to allow intubation without further sedation, for those that still have jaw tone or are chewing (one of):
 - Alfaxalone 1-2mg/kg IV bolus (if well sedated but still with significant jaw tone or if patient is compromised and has been prescribed lower dose rates of premedicants and extra caution upon induction)
- Spray larynx with local anaesthetic via scope.
 - Intubate with uncuffed 2.0mm - 3.5mm endotracheal tube.
- Maintenance
 - Isoflurane or sevoflurane per endotracheal tube in 100% oxygen
 - Alfaxan 1mg/kg IV bolus as needed to increase level of anaesthesia intermittently during procedure when jaw tone increases, heart rate and respiratory rate increase suddenly or there are muscle twitches.
 - Glycopyrrolate as needed to increase heart rate to normal pre anaesthetic range (as measured prior to anaesthesia) or to increase blood pressure if low and heart rate is low.
 - Ephedrine as needed to increase blood pressure if heart rate is not low and blood pressure is low.
- Reversal of dexmedetomidine with atipamezole (5mg/ml) IV at the same volume as the dose of dexmedetomidine given.

Anaesthetic monitoring

- Body temperature via oesophageal probe during procedure and rectal thermometer before and after procedure
- Pulse oximeter or ECG tabs for heart rate depended upon which gives the most reliable readings on individual patients
- Blood pressure via non-invasive via oscillometry cuff
- Capnography (end-tidal carbon dioxide and capnography trace)

Local anaesthesia and postoperative analgesia

- Lignocaine 2mg/kg (total) local nerve block along incision lines.

- Post operatively buprenorphine 0.03mg/kg IV or 0.05mg/kg subcutaneously q 6-8 hourly only if needed (if not eating voluntarily within 3 hours of surgery, OR grimace score excessive) (rescue analgesia)
- Meloxicam 1mg/kg post operatively subcutaneously and then 0.5mg/kg orally every 24 hours for 5 days.

Perioperative supportive care

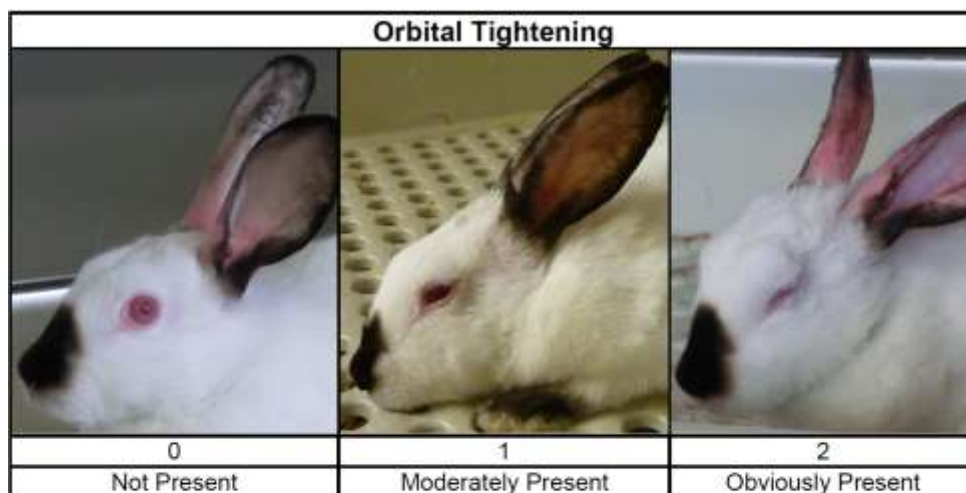
- Intravenous fluids (0.9% sodium chloride or isotonic lactated ringers solution) at 5ml/kg/hr routinely. May be increased if patient is haemoconcentrated or volume depleted.
- Supplementary heat during procedure (warmed fluid bags or bair hugger)
- Syringe feeding 20ml/kg OxBow Critical care formula upon recovery (when rabbit is able to sit up and is beginning to be ambulatory)

Appendix B: Rabbit Grimace-score

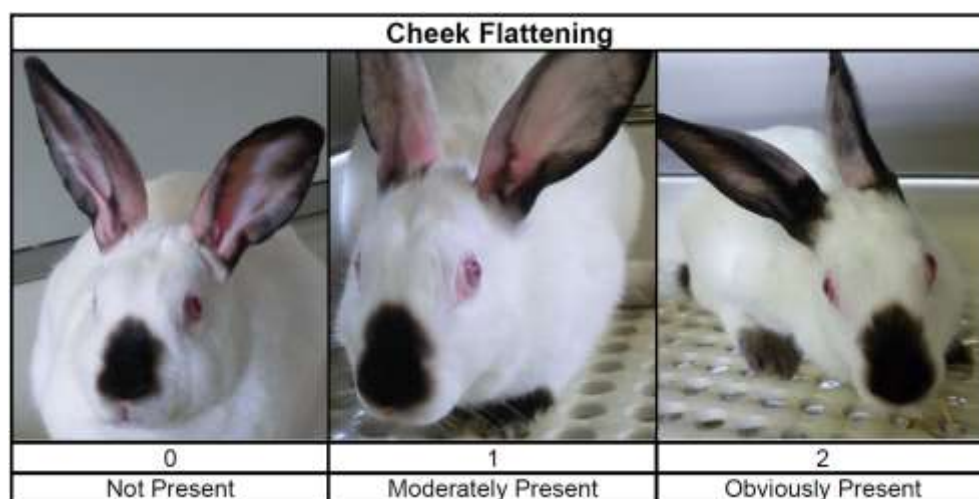
Reproduced from the Rabbit Grimace Score Manual, with permission from Dr Matt Leach, University of Newcastle.

The grimace pain-scale assesses for facial coding units (“FCUs”) of “orbital tightening”, “whisker position”, “cheek flattening”, “ear position” and “nose shape”. These FCUs are defined from still images taken at these set time-points and are defined as follows:

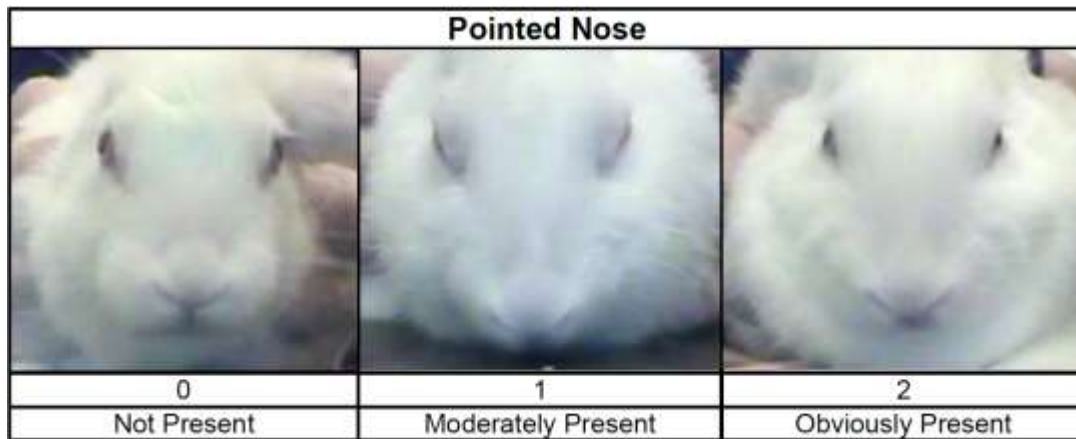
- **Orbital tightening:** The eyelid is partially or completely closed. The globes themselves may also be drawn in toward the head so that they protrude less. If the eye is partially closed, and/or the globe less pronounced, this FCU is scored as “1”. If the eye closure reduces the visibility of the eye by more than half, it is scored as “2”. Otherwise, this FCU is scored as “0”.



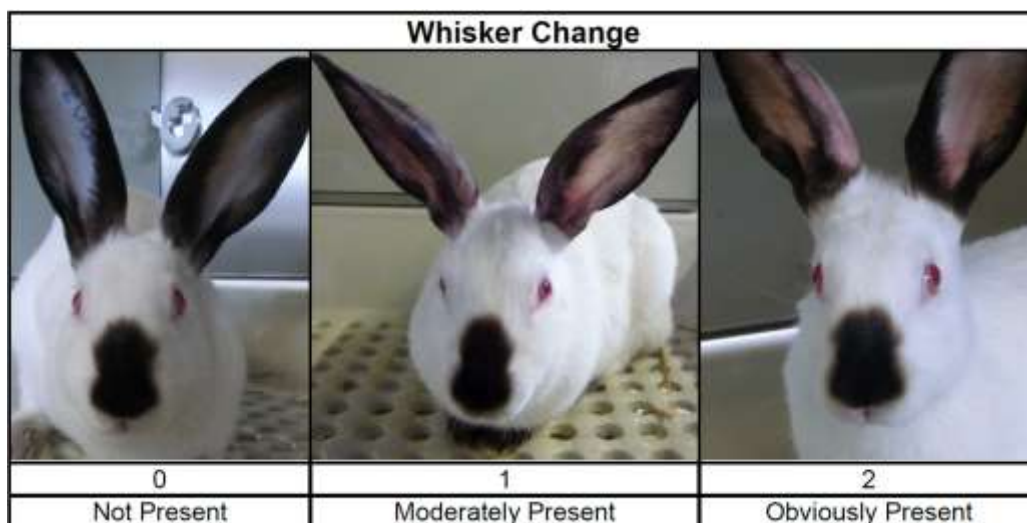
- **Cheek flattening:** The muzzle is contracted so that the whisker pads are pressed against the side of the face. The side contour of the face and nose is angular and the cheeks are not of a rounded appearance. If cheeks are slightly flattened, or not obviously rounded, this FCU is scored as “1”. If the cheeks are obviously flattened or concave, it is scored as “2”. Otherwise, this FCU is scored as “0”.



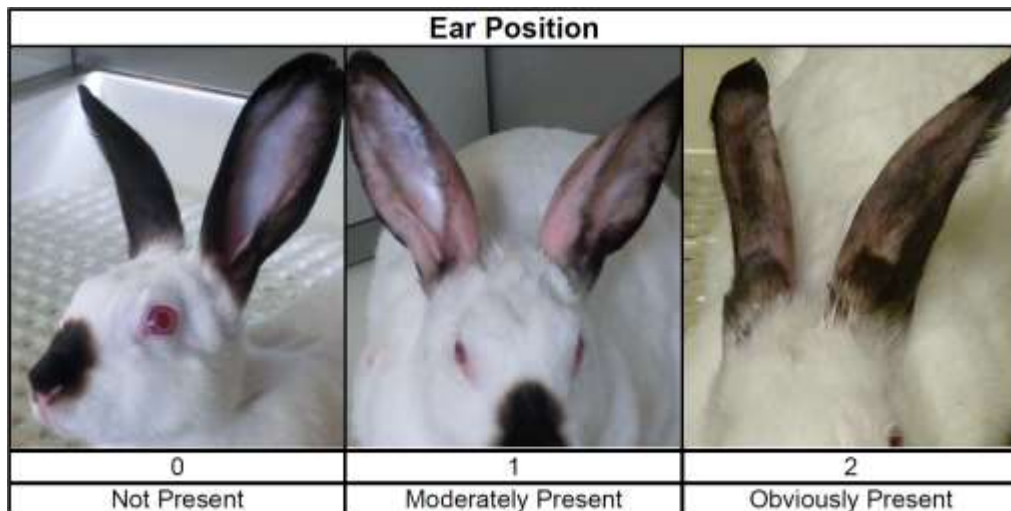
- Pointed nose: The nares (nostrils) which are usually present in a “U” shape are instead drawn vertically creating a “V”-shaped configuration. The tip of the nose may also be tucked under, which may exaggerate this change. If there is mild angulation in the nares forming a “Tall U-shape” or “pointed U-shape”, this FCU is scored as “1”. If the nares are sharply angulated in a “V-shape”, it is scored as “2”. Otherwise, this FCU is scored as “0”.



- Whisker position: The whiskers are straightened and extended horizontally or pulled back towards the cheeks instead of having a normal downward curve. If there is loss of the normal gentle neutral downward curving of the whiskers, this FCU is scored as “1”. If the whiskers are fully straightened and pulled-back, it is scored as “2”. Otherwise, this FCU is scored as “0”.



- Ear position: The ears are normally held roughly perpendicular to the angle of the top of the head and facing forward or to the side, away from the back and sides of the body with open/loosely curled shape. If the ears are held sloped towards the body, slightly curled and rotated backwards, this FCU is scored as “1”. If the ears are held tightly folded, rotated towards the body and against the back/shoulders, it is scored as “2”. Otherwise, this FCU is scored as “0”.



The final facial grimace score was recorded as the sum of the FCU values (total value out of 8). Rescue analgesia was administered if any subject scores a total pain score greater than 3/8 or if the subject is scored as “2” in any single FCU. If, for any reason, an individual FCU is unable to be assessed in a particular animal (e.g. the ears cannot be assessed due to catheters obscuring both ears), that FCU was excluded and the score was given out of six (the same minimum scores will trigger rescue analgesia). If two or more FCUs are unable to be assessed for multiple time-points, the patient was otherwise subjectively assessed for the need for rescue analgesia (e.g. heart rate, responsiveness) and data on facial grimace scores and rescue analgesia administration were excluded from statistical analysis.

The Behaviour assessment and grimace pain-score have been validated for postoperative morbidity assessment for the treatment of rabbits with ovariohysterectomy and ear tattoo.

Appendix C: Intervention Criteria

Clinical Trial AEC# 1513771 Intervention Criteria Sheet- Daily Monitoring

Steps:

1. Determine that an intervention criteria applies
2. Contact surgeon, investigator or anaesthesia officer (AO).
3. Take action in accordance with the Intervention Criteria below.

Intervention Criteria	
Observations	Action required
Grimace score total less than 3/8, each criterion less than 2/2, no other abnormalities present	None
Grimace score 4/8 or greater, or any criterion assigned 2/2 ->Rabbit is in "significant pain"	Notify anaesthesia, administer rescue analgesia dose of 0.3mg/kg IV if catheter in place, or 0.5mg/kg IM if no IV catheter in place. Repeat pain score in 10 minutes to reassess
Rabbit is in "significant pain" and there has been no response to rescue analgesia within 10 minutes	Notify anaesthesia officer, discuss if additional analgesia dose is warranted. • If so, administer additional dose, repeat pain score in 10 minutes to reassess • If not, notify surgeon/investigator, remove patient from study and administer alternate analgesia as required to achieve comfort
Rabbit is subjectively painful (e.g. tachycardic, distressed or otherwise assessed as painful) but does not qualify as "significant pain" based on grimace score	Notify anaesthesia, investigator/surgeon to assess/confirm If confirmed, remove patient from study and administer alternate analgesia as required to achieve comfort
Rabbit has not eaten voluntarily within 3 hours of extubation	Notify anaesthesia, administer rescue analgesia dose of 0.3mg/kg IV if catheter in place, or 0.5mg/kg IM if no IV catheter in place. Repeat pain score in 10 minutes to reassess

Rabbit is unwell for a reason other than mentioned above (e.g. not eating prior to surgery, scratching or rash, oculonasal discharge, etc...)	Notify investigator/surgeon Confirm assessment with GP rabbit veterinarian Remove patient from study and notify owner/carer. Transfer patient to GP for workup/treatment
Unexpected adverse events not otherwise listed	Notify investigator/surgeon to assess, Notify University's AWO

Grimace score should be assessed as per the provided grimace score instructions.

Clinical Trial AEC# 1513771 Intervention Criteria Sheet- Procedures

Steps:

4. Determine that an intervention criteria applies
5. Contact surgeon, investigator or anaesthesia officer (AO).
6. Take action in accordance with the Intervention Criteria below.

Intervention Criteria - Venepuncture	
Observations	Action required
Easy venepuncture from the lateral saphenous vein, relaxed animal, no more than 2ml total volume taken over the course of 1 week.	None
Patient is distressed with restraint (struggling, tachycardic, tachypnoeic, hyperthermic)	Adjust restraint method. If patient is still distressed, allow patient time to relax without restraint prior to attempting again. If patient is still distressed at repeated attempt, notify investigator/anaesthesia Delay venepuncture until after sedation/premedication if possible Consider use of EMLA gel if it hasn't already been attempted If none of the options are possible or effective, discontinue attempts at venepuncture.
Haematoma formation	Apply gentle pressure to the region to prevent further haematoma formation.
Lateral saphenous not available	Consider using marginal ear vein (if this will not compromise catheter placement). If this is unavailable, cephalic veins (preferably) or jugular (last preference) can be attempted, though restraint required can be distressing for some animals and preservation of the jugular vein is recommended in case larger volume blood draws are required.

	If restraint becomes stressful, refer to the intervention criteria above and continue abandoning attempts at venepuncture.
Blood in the syringe clots	Notify Investigator, confirm if additional blood required.
Repeated venepuncture attempts required	No more than three (3) skin punctures should be attempted for collection of blood for research purposes (aligned with Draft Animal Care & Use Standard: Blood collection in rats and mice [version 1], University of Melbourne, 2016)
Evidence of excessive blood loss, anaemia or hypovolaemia	Administer intravenous fluids (5ml/kg/hr, 0.9% sodium chloride or isotonic lactated Ringers solution) to maintain blood volume, monitor regularly for resolution of clinical signs.
Other unexpected adverse incidents	Notify Investigator/anaesthetist and the University's AWO.

Venepuncture regulations guided by Flecknell PA (2000) Anaesthesia in *Manual of Rabbit Medicine & Surgery*, Ed. Flecknell PA p 103-116, BSAVA Gloucester

Clinical Trial AEC# 1513771 Intervention Criteria Sheet- Procedures

Steps:

1. Determine that an intervention criteria applies
2. Contact surgeon, investigator or anaesthesia officer (AO).
3. Take action in accordance with the Intervention Criteria below.

Intervention Criteria - Surgery	
Observations	Action required
No complications, rapid recovery from anaesthesia	None
Patient is painful postoperatively	Refer to intervention criteria- "Daily Monitoring" document
Significant haemorrhage occurs intraoperatively	If using open technique: ligate bleeder if possible, if unable to rapidly access haemorrhaging vessel, extend incision to allow access to the bleeding vessel. Remove the patient from the data pool if: • The resulting incision is significantly longer than others in the open group OR • The surgery is significantly prolonged OR • A significant amount of blood is lost. Notify anaesthesia & the AWO if the patient shows clinical signs of anaemia or hypovolaemia. Administer intravenous fluids to maintain blood volume (may require bolus on top of standard 5ml/kg/hr surgical rates) If using laparoscopic technique: ligate or cauterise bleeder if possible to do quickly. If haemostasis is challenging or if visualisation is obscured, convert immediately to an open procedure, note the reason for conversion and remove patient from data pool for postoperative assessment. Continue postoperative assessment and notify as per the open technique above.
Inadvertent trauma to other abdominal organs	Assess the likely risk of complications to the patient. Notify the Investigator/anaesthesia.

	If no further measures are required to rectify the situation (e.g. trauma is mild and not likely to cause any complications), then record the adverse event. If further measures are required to rectify the situation (e.g. resection of parts of or whole organs), notify the Owner/Carer and the AWO. Then remove patient from study.
Wound breakdown /complications /dehiscence	Notify the Investigators and record the event If no further intervention is required (e.g. mild exposure of subcutaneous tissue through incision but no structural compromise of the incision) then no further actions are required. If further intervention is required (e.g. repeat surgery) then inform the AWO.
Other unexpected complications encountered	Record the adverse event, notify the investigators and the AWO

Appendix D: Publications from the thesis

Part of the data from the thesis was included in the following publication:

McCracken BD, Beths T, Herbert S, et al: COMPARISON OF ISOBARIC AND INSUFFLATED LAPAROSCOPY-ASSISTED OVARIOHYSTERECTOMY IN THE COMMON RABBIT (ORYCTOLAGUS CUNICULUS). Journal of Exotic Pet Medicine 28:193-204, 2019⁹⁹

COMPARISON OF ISOBARIC AND INSUFFLATED LAPAROSCOPY-ASSISTED OVARIOHYSTERECTOMY IN THE COMMON RABBIT (*ORYCTOLAGUS CUNICULUS*)



Blaine D. McCracken, MVS, Thierry Beths, PhD, Sasha Herbert, BVSc, and Stewart D. Ryan, BVSc (Hons), Dip. ACVS, ACVS Founding Fellow (Surgical Oncology)

Abstract

Surgical sterilization of female rabbits can be associated with a significant risk of postoperative morbidity. Laparoscopic ovariectomy has been reported previously to address this issue. The aim of the current study is to assess whether an isobaric (ISO) technique, utilizing an abdominal lifting technique, rather than insufflation (INS) technique, which involves pressurizing the abdomen, is feasible in this species. Four rabbit cadavers were used to subjectively compare the viability of the 2 techniques. Seven rabbit cadavers were used to assess the effects of ISO and insufflated pneumoperitoneum on the dimensions of the abdomen and the volume of the thorax at atmospheric and positive inspiratory pressures using CT imaging. There was a significantly higher ($P = 0.006$) difference between baseline and pneumoperitoneum thoracic volume in the ISO specimens versus the INS specimens. There was no significant difference in abdominal height in either technique and both afforded adequate working space. The implementation of ISO laparoscopy-assisted ovariohysterectomy was subjectively no more technically difficult than INS laparoscopy-assisted ovariohysterectomy in the rabbit. Based on this investigation, ISO laparoscopy is feasible and requires less specialized equipment compared with insufflated laparoscopy, making it more available in a general practice setting and for routine surgery. Use of ISO laparoscopy could potentially decrease ventilatory compromise compared with insufflated laparoscopy in rabbits. Copyright 2018 Elsevier Inc. All rights reserved.

Key words: rabbit; isobaric laparoscopy; laparoscopy; ovariohysterectomy; surgery; abdominal wall lift

The common rabbit (*Oryctolagus cuniculi*) is an increasingly common companion animal. Intact female rabbits have a high risk of developing uterine and mammary tumors,^{1,2} yet the majority of pet female rabbits are intact.³ Intact rabbits have a reproductive rate of up to 10 offspring per month, which can rapidly lead to overpopulation, abandonment or surrender of pet rabbits. Intact rabbits tend to fight if housed together and are more difficult to house-train.⁴ The rabbit uterus is bicornuate with a cervix on each horn, meeting together at a flaccid distensible vaginal body which retains urine during urination.⁵

Abbreviations: IPPV, intermittent positive pressure ventilation; PPV, positive pressure ventilation; OHE, ovariohysterectomy; ISO, isobaric laparoscopy; INS, insufflated laparoscopy; CT, computed tomography; PIP, positive inspiratory pressure

From the TRACTS, University of Melbourne, Melbourne, Australia

Address correspondence to: Blaine D. McCracken, MVS, TRACTS, University of Melbourne, 250 Princes Hwy, Werribee, Melbourne, Victoria 3030, Australia. E-mail: blainedmcc@gmail.com

Funding: The research was part of an MVSc degree, with funding from the University of Melbourne graduate research program.

Part of the results/data included in this report were presented to the ANZCVS Science Week Young Speakers Program, Gold Coast, Australia, July 6, 2017.

© 2018 Elsevier Inc. All rights reserved.

1557-5063/14/2101-\$30.00

<https://doi.org/10.1053/j.jepm.2018.08.008>

Perioperative mortality in rabbits undergoing anaesthesia for surgery is up to 5 times the rate seen in comparative domestic species.⁶ This elevated risk is multifactorial including high surface area to volume ratio (and hypothermia)^{7,8}; lack of veterinarian familiarity with species-specific physiology⁶; low proportionate lung volume; gastrointestinal stasis and factors associated being a prey species, including handling-associated stress and pain behaviour masking.^{7,9-11} Surgical adhesions have been studied in the rabbit as a model for humans, however, it is not well established whether these adhesions are associated with morbidity in rabbits in a similar manner to humans.¹²⁻¹⁴

Development of minimally invasive surgical methodologies in rabbits is driven by the goal of reducing perioperative morbidity through reduced need for pain management, reduction of stress and consequent risk of potentially life-threatening gastrointestinal stasis. The technique for laparoscopic ovariectomy has been reported in rabbits, but no studies have compared open surgery to minimally invasive techniques.^{15,16}

Isobaric (ISO), or abdominal wall lift, laparoscopy was developed in order to reduce the impact of laparoscopic surgical intervention in human patients at high risk of respiratory compromise by positive-pressure pneumoperitoneum, particularly in paediatric and trauma settings.¹⁷⁻²⁰ The ISO abdominal lift technique was subsequently modified for use in dogs.²¹⁻²³ One of the main findings in these studies was limited visualization of abdominal contents due to tenting of the abdominal wall restricting working space. The compliance of the abdominal wall in the rabbit is higher than humans and dogs, but there have been no studies published that quantify this difference.

Given the particular concern of proportionately low lung volume in the rabbit, 1 advantage of ISO laparoscopy could be the lack of positive pressure pneumoperitoneum, which limits diaphragmatic excursion.²⁴ Orotracheal intubation in rabbits is more difficult than in dogs and cats and establishing intermittent positive pressure ventilation to counteract these adverse effects can therefore be more difficult. The carbon dioxide used to insufflate the peritoneum has been reported to lead to additional morbidity due to local effects on the peritoneum and systemic affects associated with hypercapnia, which is likely to persist in a patient with depressed ventilation.²⁵⁻³⁰

The primary objective of this study is to determine the feasibility of a novel ISO laparoscopy-assisted technique for

ovariohysterectomy in the common rabbit. The secondary objectives are to compare the abdominal and thoracic volumes between normal, ISO and insufflated (INS) laparoscopic techniques under simulated nonventilated and positive pressure ventilation conditions.

Our hypothesis was that ISO laparoscopy will be technically feasible and will provide adequate visualisation of abdominal structures and have less effect on thoracic volume than INS laparoscopy for abdominal procedures in the rabbit.

MATERIALS AND METHODS

A 2-part cadaveric study was performed utilizing a total of 11 rabbit cadavers.

Animals

Rabbit cadavers were acquired after being euthanized humanely following the requirements of the ethics approval of an unrelated study. Ethics approval for the unrelated study was granted in compliance with AVMA Guidelines for the Euthanasia of Animals and the EU Directive (2010/63/EU) for animal experiments.

Study 1: Isobaric Laparoscopy Technique Feasibility Study

A subjective, descriptive feasibility study compared an ISO laparoscopy-assisted ovariohysterectomy technique (ISO) to a modified laparoscopy-assisted ovariohysterectomy (INS) technique. The cadaver specimens were frozen immediately upon being received and thawed gradually over a period of 48 hours prior to the procedure trials.

Four cadavers were utilized in this study; 2 using the ISO technique and 2 using the INS technique. The ISO and INS techniques were subjectively compared by surgeons. A small animal surgery resident performed all procedures as primary surgeon. A registered specialist in small animal surgery and a general practitioner experienced in rabbit ovariohysterectomy assisted with half of the procedures each. The surgeons commented on subjective difficulty of the procedure, whether conversion to open laparotomy would have been indicated in a live patient, and any particular difficulties they noted in the execution of that technique.

Qualitative assessment of the working space was made with the following ordinal rating system: 1—unable to visualize the uteri or ovaries, 2—able to visualize the uteri and ovaries, but unable to manipulate them effectively, 3—able to perform the technique on 1 side, but not the other without

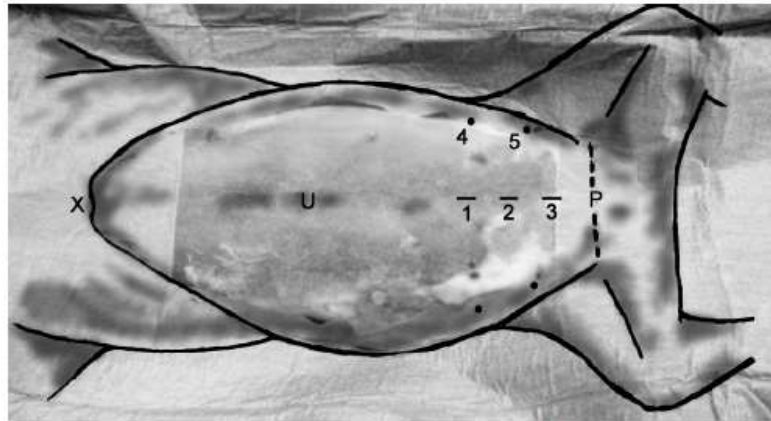


FIGURE 1. Diagram of landmarks for laparoscopy portals and lift points. The patient's head is oriented towards the left of the image and the position of the patient under the drape is outlined. The first portal incision (1) is located 50% of the distance between the umbilicus (U) and pubic rim (P). The second (2) and third (3) portals are located at one-third and two-thirds the distance between the first portal incision and the pubic rim. The 2 stay-suture lift points (4 and 5) are placed in line with incisions 1 and 2. Note there are also lift point positions on the contralateral side for when the patient is turned. The location of the xiphoid cartilage (X) is also noted for orientation.

conversion, 4—able to perform the entire procedure with some noted difficulties due to available working space, 5—able to perform the entire procedure without difficulty.

Laparoscopy Equipment

A tilting cradle (APEXX TT Endoscopic Positioner, apex Veterinary Equipment, Inc.) enabling rotation of the patient from left lateral to right lateral was used. The procedures were performed using a 5 mm diameter, 240 mm long, 30° fore-oblique rigid telescope (Karl Storz Endoscopy, Australia Pty. Ltd., 'KSEA') with Xenon (X8000) light source via fibre-optic light cable. Images were acquired via a 1088 3-chip HD endovideo camera (Stryker Australia Pty. Ltd., 'Stryker'). Different cannulas were utilized including 3 and 6 mm diameter threaded cannulas (KSEA) and 6 mm disposable plastic threaded cannulas (Covidien Versaport, Medtronic Animal Health Pty. Ltd., 'Medtronic'). Laparoscopic instruments of 2.7 and 5 mm including clickline scissors, monopolar cautery electrode and Babcock grasping forceps (KSEA) were used during the procedures. A 5 mm laparoscopic LigaSure (Medtronic) vessel-sealing device was also trialled during the study. Insufflation was achieved with a 40 L Hi-Flow Insufflator unit (Stryker) through the dedicated insufflation side-port on the main cannula.

Insufflated Laparoscopic-Assisted Surgery Technique

The 6 mm camera port was placed midway between umbilical scar and pubis utilizing a

modified Hasson technique,³¹ the abdomen was INS with carbon dioxide to 10 mm Hg and the remaining 2 (6 mm) laparoscopic cannulas were inserted on the midline approximately one-third and two-thirds the distance between the initial port and the pubis under direct visualization the endoscope (see Fig. 1). The Babcock forceps were utilized for grasping the ovaries, primarily through the caudal port, with the cautery/LigaSure instruments used through the cranial port. The abdomen was briefly inspected for abnormalities. The uteri were visualized as they diverge from the paired cervixes in the bladder region and run towards the dorsolateral gutter.

The ovary was located either in or suspended from the centre of the spiral-shaped terminal uterine horn. The mesometrium and mesovarium and vasculature of the rabbit are similar to that of other domestic species and were identified and divided with a 5 mm LigaSure vessel-sealing device. The cradle was tilted to the alternate recumbency and the procedure was repeated on the contralateral uterus. The uteri were grasped with forceps and exteriorized through the caudal portal. An encircling ligature was placed caudal to the cervixes extracorporeally and the vaginal stump was transected above the ligature and replaced into the abdomen. The stump was visualised with the endoscope and the portals were closed with a simple-interrupted suture of 3/0 polydioxanone (MonoPlus, B Braun Pty. Ltd.).

At the conclusion of the procedure, a laparotomy was performed to inspect the surgical site and assess for inadvertent tissue damage.

Isobaric Surgery Technique

The abdominal approach was identical to the INS technique, but rather than placing a laparoscopic cannula, the endoscope was fed through the incision directly. A blunt probe was placed through the same incision and elevated to cause the abdominal wall to tent away from the abdominal viscera. The tenting point was located in the inguinum, directly lateral to the initial incision. Under visualization of the endoscope, a USP-0 (Metric 3.5) stay suture (MonoPlus, B Braun Pty. Ltd.) was placed through the abdominal wall at this point. The same process was repeated caudally one-third of the distance from the previous suture to the level of the pubis (see Fig. 1).

Both stay sutures were secured in a haemostat and sterile elastic bandaging material was draped over a free-standing nonsterile long-armed intravenous drip pole and secured with a single half-hitch 1 end to each haemostat (see Fig. 2). The elasticity of the bandage was used to maintain tension on the abdomen, and the length of the bandage determined by the surgeon on tying it to the haemostat. The abdominal retraction could therefore be set while allowing the surgeon and assistant to operate the instruments and endoscope. At this point, ISO pneumoperitoneum had been established and the remaining instrument portals were made via stab-incision on the midline as per the INS technique. No cannulas were used in these portals; however, they were made a similar size to that used in the INS technique for comparison purposes. The remainder of the procedure was identical to that described under the INS technique aside from the need to

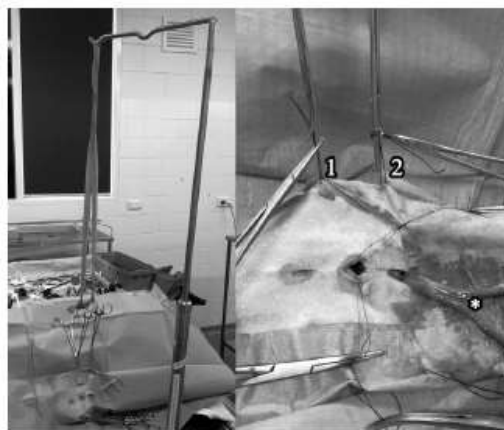


FIGURE 2. Image of isobaric lift construct, illustrating suspension of the stay sutures (1 and 2) from a drip stand with sterile bandaging material (left) and a close-up of the incisions postuterine (*) ligation.

place an additional 2 stay sutures in the contralateral inguinal region when the cradle was rolled into the alternate lateral recumbency.

Study 2: Volumetric Abdominal and Thoracic Imaging Study

Seven rabbit cadavers were used in this randomized cross-over study.

Each rabbit cadaver had computed tomography (CT) scans of the abdomen and thorax acquired in a standardized manner, under the following 5 conditions: baseline with no instrumentation, ISO technique, ISO with positive inspiratory pressure (PIP; ISO_{PIP}), INS and INS with PIP (INS_{PIP}).

Specimen Preparation

The specimens were prepared by placement of a laparoscopy cannula via a modified Hasson technique. A ventral midline approach was made to the trachea, which was transected between either the fourth to fifth or fifth to sixth tracheal rings. A cuffed endotracheal tube was placed into the transected trachea and, if necessary, a Rummel tourniquet was placed cranial to the inflated cuff to prevent inadvertent removal of the endotracheal tube. Five simulated breaths were made using intermittent positive pressure ventilation to an end-inspiratory pressure of 20 mm Hg to identify any leaking of the endotracheal tube cuff and to dilate the airways prior to the baseline images being acquired.

Specimens designated to the INS group were INS via the cannula to 10 mm Hg capnoperitoneum. Specimens designated to the ISO group had their cannulas removed and a single stay suture placed in the inguinal region at the level of the midway point between pelvic rim and umbilicus and were suspended by the stay suture from a radiolucent support jig custom assembled to fit within the CT machine aperture. Specimens were suspended by approximately 40% of their bodyweight, as measured by a flat scale placed under them and then replaced with an equal thickness mat.

Image Acquisition

Images were acquired using a 16-slice Siemens CT machine. Transverse and sagittal reconstructions were exported to the PACS (SYNAPSE, FUJIFILM Australia Pty. Ltd., 'Fujifilm') in 0.8 mm slice thickness and select representative 3-dimensional segmented reconstructions were created.

The specimens were placed in groups of 2 to 3 on the CT table and a baseline CT scan of the abdomen and thorax of all rabbit cadavers was taken under atmospheric airway pressure and

without pneumoperitoneum prior to allocation to treatment groups.

The rabbit closest to the CT gantry was assigned to undergo either ISO or INS procedure first as determined by coin flip. The second rabbit was assigned the opposite technique to the first rabbit to prevent numerical bias in the order of procedures. In cases where 3 specimens were imaged simultaneously, the third rabbit was allocated based on a separate coin flip. The images were acquired with the specimens maintained under their allocated condition.

The specimens were subsequently attached to an anaesthetic machine. A PIP of approximately 11 mm Hg was applied through the endotracheal tube and the trachea was clamped with a nontraumatic haemostat to prevent leakage and deflation of the lungs and the image acquisition was repeated. The same image acquisition and simulated inspiratory positive pressure procedure was then repeated under the ISO condition in cases where the insufflation condition was first performed, and vice versa.

Outcome Measures

Commercial medical imaging software (Synapse, Fujifilm) was used to collect measurements from the CT reconstructions in the soft tissue window. The sagittal thoracic reconstructions were assessed by tracing the thoracic space delineated by the internal surface of the bony structures of the thoracic cavity, the diaphragm and a straight line drawn from the fifth to sixth intervertebral space to the cranial tip of the manubrium. Each slice was assessed using the region-of-interest tool to calculate cross-sectional area. The slices were summed and multiplied by the slice thickness to yield an approximate volume in cubic millimetres.

The maximum sagittal height of the abdomen from the internal surface of the ventral abdominal wall to the ventral aspect of the relevant vertebral body was measured in millimetres and defined as the 'maximum abdominal height'. The same measurement was taken along this line from the internal surface of the ventral abdominal wall to the ventral-most visceral surface, and this was defined as 'maximum abdominal space'. The visceral height was also defined as the maximum abdominal height minus the maximum abdominal space and represented translation of the visceral level within the abdomen between techniques.

At the level of L5 to L6, a mid-sagittal line was drawn from the internal surface of the ventral abdominal wall to the ventral aspect of the L5 to L6 disc space. This line was subdivided into quarters

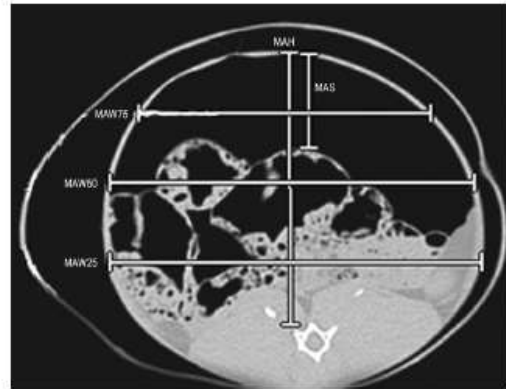


FIGURE 3. Example of measurement of maximum abdominal height (MAH), space (MAS) and width at 25% (MAW 25), 50% (MAW 50) and 75% (MAW 75) abdominal depth. MAW, maximum abdominal width.

and the width of the abdomen—defined as the distance between the points where a line perpendicular to the sagittal line intersects with the internal surface of the abdominal wall—was measured in millimetres at each of these points. These widths were assigned as '25%, 50% or 75% maximum abdominal width' (25%/50%/75% MAW) at the deep, middle and superficial quartile points, respectively (see Fig. 3).

Statistical Assessment

Part 1 was a subjective qualitative assessment and was not assessed statistically.

The sample size of the second study was estimated from preliminary scans on 3 rabbits, using the resulting calculated means and standard deviations with a power set at 80% and 5% Type I error rate using the calculated thoracic volumes as primary outcome measure.

Shapiro–Wilk testing was performed to assess consistency with normal distributions and subsequently, 2-tailed *t* tests (if normally distributed) or Wilcoxon signed rank test for paired samples (if not normally distributed) were applied to compare each individual variable. Significance was set at a *P* value of <0.05.

RESULTS

Study 1: Isobaric Laparoscopy Technique Feasibility Study

Both ISO cases were completed without deviation from the described methodology and were assigned subjective scores of 5/5.

One INS was subjectively rated as 3/5 and the other 4/5 for working space. Difficulties reported

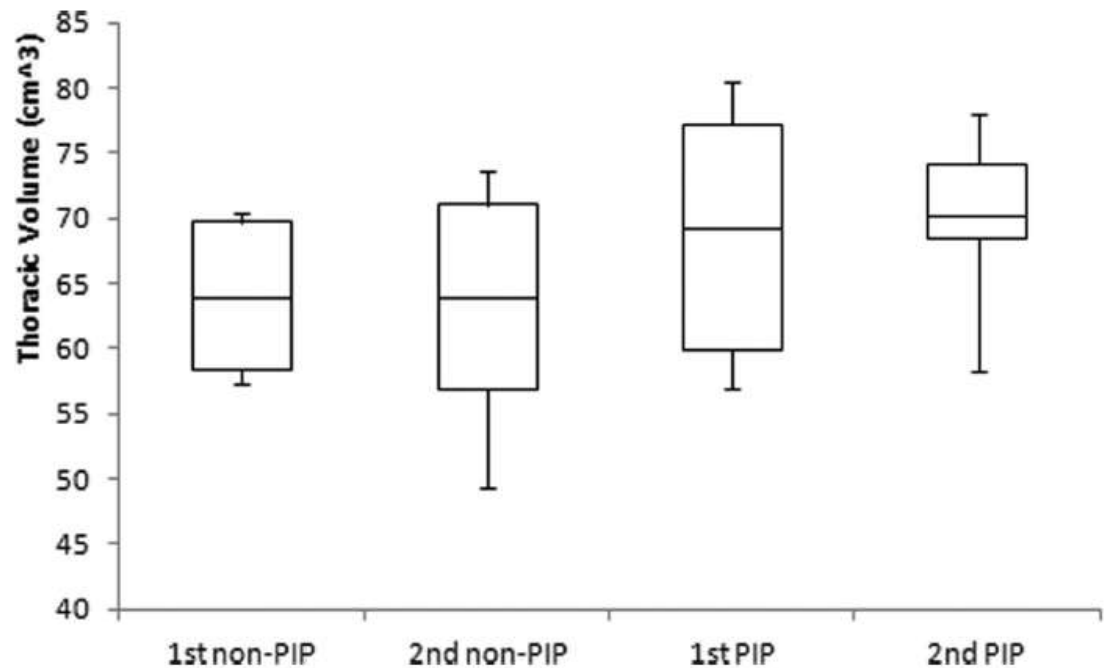


FIGURE 4. Box and Whisker plot: comparison ($n = 7$) of results grouped by order of procedures on each cadaver and based on whether positive inspiratory pressure (PIP) was applied, standardized by bodyweight: centre line indicates mean value, whiskers indicate range, box indicates first and third quartile values. No statistical differences observed.

included incomplete thawing of the retroperitoneal fat, friability of intestinal viscera and difficulty retracting the cecum, which had dilated postmortem.

In both cases treated with the INS technique, the surgeon reported difficulty in maintaining laparoscopic cannulas within the portal due to the pliability of the abdominal wall, inadequate depth of cannula thread, cannula weight and the length of the unthreaded portion of the cannula. Placement of a purse-string suture around the cannula was required to prevent air leakage. The excessive length of the cannulas utilized was noted as being problematic.

In some cases, retraction of the cecum was necessary. In the ISO cases, a curved Carmalt hemostat through the camera portal enabled retraction. In the INS cases, the pressure of the insufflation gas was generally reported to be sufficient to depress the cecum, though use of a fan-retractor or a blunt probe was required for full visualisation in 1 of the cases.

It was reported by the investigators that while use of a surgical assistant was 'useful' for the ISO technique, it 'was not essential'. On the other hand, it was 'very difficult' to perform the described procedure without an assistant when following the INS technique. This was partially attributed to the previously mentioned issues of cannula retention.

Study 2: Volumetric Abdominal and Thoracic Imaging Study

The cadavers had a mean bodyweight of 2.41 kg (range: 2.06 to 2.79 kg) with no difference between order (see Fig. 4) of ISO or INS conditions ($P = 0.18$). There were no differences between ISO_{PIP}, INS_{PIP}, ISO or INS groups in maximum abdominal height or space and all treatment groups had greater abdominal height than the baseline measurement ($P < 0.044$). There was decrease ($P < 0.01$) in the maximum visceral height in the INS groups compared with the ISO groups (see Fig. 5). There was no difference in visceral height between the baseline and any other groups.

The dorsal/deep abdominal width (25% MAW) at the level of L5 to L6 was not different between the ISO_{PIP}, INS_{PIP}, ISO or INS groups. In the middle and superficial abdomen, (50% MAW and 75% MAW), the width of the INS groups was greater than the ISO groups. The PIP groups were not different from the respective atmospheric pressure groups (see Fig. 6). In the volumetric assessment (see Table 1), thorax volume in the ISO_{PIP} group was larger than all other groups; thorax volume in the INS_{PIP} group was larger than ISO and INS groups but not different from baseline. There was no difference between INS, ISO and baseline thoracic volumes.

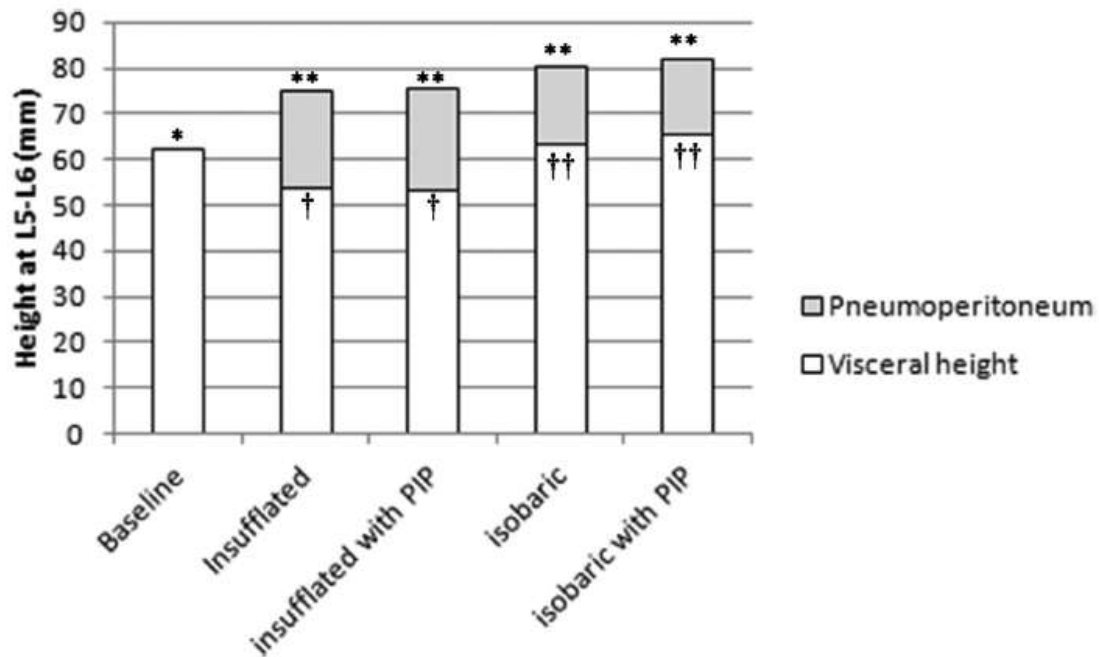


FIGURE 5. Comparison ($n = 7$) of the mean abdominal height (mm), abdominal space or pneumoperitoneum (mm) and visceral height (mm) between different groups. There was no significant difference in visceral height between baseline (*) and groups '†' or '††'.

*statistically different ($P < 0.044$) from groups '***'.

†statistically different ($P < 0.01$) from '††'.

The ISO groups had larger ($P = 0.006$) increase in thoracic volumes under PIP than INS groups (see Fig. 7) with a mean loss of 28% of potential PIP thoracic volume in the INS group.

DISCUSSION

The ISO laparoscopy-assisted ovariectomy technique developed was feasible and was subjectively assessed as easier to perform compared with the INS laparoscopy-assisted technique in rabbit cadavers. Some difficulties were encountered during this part of the study due to uneven thawing of the cadaver and friability of the specimens; however, these were accounted for in the subjective assessments and affected both techniques to a similar degree. The working spaces achieved in both techniques were subjectively equivalent.

The simulated thoracic expansion achievable over a specified and typical inspiratory and expiratory pressure range was significantly lower in cadavers where positive pressure insufflation of 10 mm Hg was utilized to achieve pneumoperitoneum compared with ISO pneumoperitoneum achieved by abdominal wall lift technique in the rabbit. It should be considered that the ventilatory pressures used in this study are

recommended for rabbits regardless of the abdominal pressure.^{4,5,7,9} While an increase in applied positive airway pressure to achieve normal tidal volume would not translate directly to increased risk of lung injury (compared with overdistension), there are currently no guidelines as to what pressure would safely compensate for this in the clinical patient.

The decrease in PIP thoracic volume in the INS specimens described in this study is potentially clinically significant due to the small lung capacity and tidal volume in rabbits, which places rabbits undergoing anaesthesia at a high risk of hypoventilation. In a study by Kim et al.³² in rabbits ventilated with 11 mm Hg PIP, PaCO₂ increased from a mean of 34.4 mm Hg to 82.1 mm Hg and blood pH decreased from 7.45 to 7.16 due to respiratory acidosis with the initiation of 8 mm Hg capnoperitoneum measured at 30, 60 and 90 minutes postinitiation of capnoperitoneum. The positive pressure ventilation values used in the current study were chosen to describe the physical volumetric changes that could reasonably be associated with the physiological changes as described in the Kim et al. study. This depression of ventilation was also described in the dog²³; however, both insufflation and ventilation

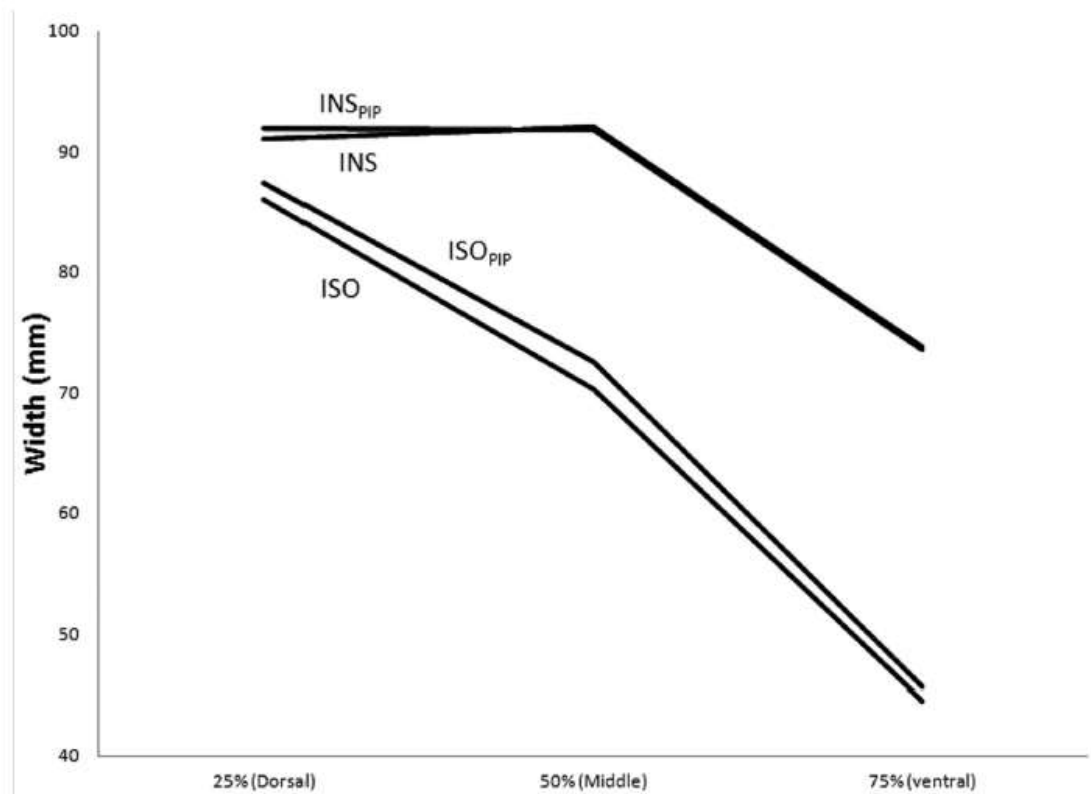


FIGURE 6. Chart demonstrating mean abdominal width (mm) at 25%, 50% and 75% abdominal height for insufflated (INS), insufflated with positive inspiratory pressure (INS_{PIP}), (isobaric ISO) and isobaric with positive inspiratory pressure (ISO_{PIP}) groups ($n = 7$).

pressures used in canines are significantly higher than that recommended for use in rabbits, so it was considered worthwhile completing the study in rabbits rather than attempting to extrapolate from other species.¹⁶

A substantial drawback to the ISO technique that has been reported²³ was the effect of abdominal 'tenting' on working space. Working space is provided by pneumoperitoneum and enables the surgeon/s to visualize viscera with the camera and operate their instruments. Tenting of the abdomen is caused by the upwards tension of the lifting device towards a point, rather than the generalized radially directed force applied to the abdominal wall in INS techniques. The latter force creates a 'domed' profile to the working space,²³ which is postulated to allow easier operation of instruments (see Fig. 8).

Mitigation of this tenting has previously been suggested²² by providing multiple lift points operating in slightly opposing directions to simulate a domed profile, or use of a wide, spiral-shaped, fan or spatula-shaped lifting device.²³ Blind placement of a lifting device as previously

reported was not favoured as an option by the authors due to the risk of trauma to the prominent and thin-walled cecum in the rabbit.

In contrast to the previous reports in other species, the abdominal wall tenting was not seen as a significant negative factor in the provision of working space in this study, and in fact, the surgeons described the working space as superior in the INS cases. The superiority of the working space in these cases may have been due to the difficulty the surgeons experienced in maintaining an airtight seal around the laparoscopic cannulas in the insufflation cases, or potentially the smaller surface area and increased pliability of the abdominal wall, allowing for more efficient lifting of the abdomen with 2 lift points compared with dogs and humans.

While a second lift point is recommended in the study design, this was more desirable than essential in order to easily access the caudal abdomen. A similar effect could also be achieved with downwards tilting of the cranial end of the table (trendelenburg position). It should be considered that this positioning would also increase pressure

TABLE 1. Mean difference (mm ³ /kg) in thoracic volume standardized by bodyweight between baseline, insufflated (INS), insufflated with positive inspiratory pressure (INS _{PIP}), isobaric (ISO) and isobaric with positive inspiratory pressure (ISO _{PIP}) groups (n = 7).						
	ISO	(P Value)	ISO _{PIP}	(P Value)	INS	(P Value)
Baseline	-1438.1	0.10 < P < 0.20 ^b	7863.94	0.00021 ^a	212.811	> 0.2 ^b
ISO	-	-	9302.09	<0.001 ^b	1650.96	> 0.2 ^b
ISO _{PIP}	-	-	-	-	-7651.1	<0.001 ^b
INS	-	-	-	-	-	<0.001 ^b
Comparisons of parametric datasets by 2-tailed t tests ^a have specific P values listed. Comparisons including nonparametric data by Wilcoxon Signed Ranks ^b indicate P value range. Duplicate values ('-') have been omitted for clarity.						
					INS _{PIP}	(P Value)
					2802.41	0.10 < P < 0.20 ^b
					4240.56	0.05 < P < 0.10 ^b
					-5061.5	0.02 > P > 0.05 ^b
					2589.6	<0.001 ^b

on the diaphragm and lungs by the weight of the abdominal organs. The use of 2 lift points also decreases the local strain on the abdominal wall at each point compared with if the same lifting force was applied to a single point, this could also be achieved with a spiral/fan-shaped lifting device as published previously.²³

The working space demonstrated in the second study supported the subjective findings of study 1. Tenting of the abdomen generally leads to a triangular cross-section of the abdomen in species with less flexible abdominal musculature; however, in the rabbit, the downwards displacement of the viscera due to gravity acts to blunt the abdominal profile into a comma-shaped cross-section, which relatively increased the width of the working space compared with what would be expected in species with less pliable abdominal walls (see Fig. 8). INS groups had significantly more width at middle- and ventral- abdomen levels, suggesting that the working space had increased, though the transverse reconstructions demonstrate that effective working space is more concentrated over the region of interest in the ISO technique, with much of the additional working space provided by the INS dome being cranial or contralateral to the region of interest and not of benefit to the execution of the surgical procedure in question.

The downwards displacement of the viscera in the INS group is likely to be of more clinical benefit than the increased abdominal width (see Fig. 5). The increased pliability of the abdominal wall in the rabbit allows the viscera to remain settled in the dorsal abdomen under their own weight rather than being pulled ventrally due to abdominal tenting (see Fig. 8). The increased pliability of the abdominal wall in this study is also present in cats and similar small species, and so the findings of this study could be extrapolated to those species. Although ISO laparoscopy has been described in cats previously,²¹ no direct comparison has been made between the propensity of the abdominal wall towards tenting.

An approximate lifting force of 40% body weight (split between 2 lift points) was used for standardization purposes for the CT study based on previous studies.²³ This force could be approximated in a clinical setting by placing a scale underneath the patient; however, subjectively, it was considered that lower lifting forces may be required to achieve similar effects due to the pliability of the abdominal wall and achieving a precise force is likely not relevant to the clinical picture. It is unknown what effect the forces used in this study would have on local perfusion in a live patient, however, at this

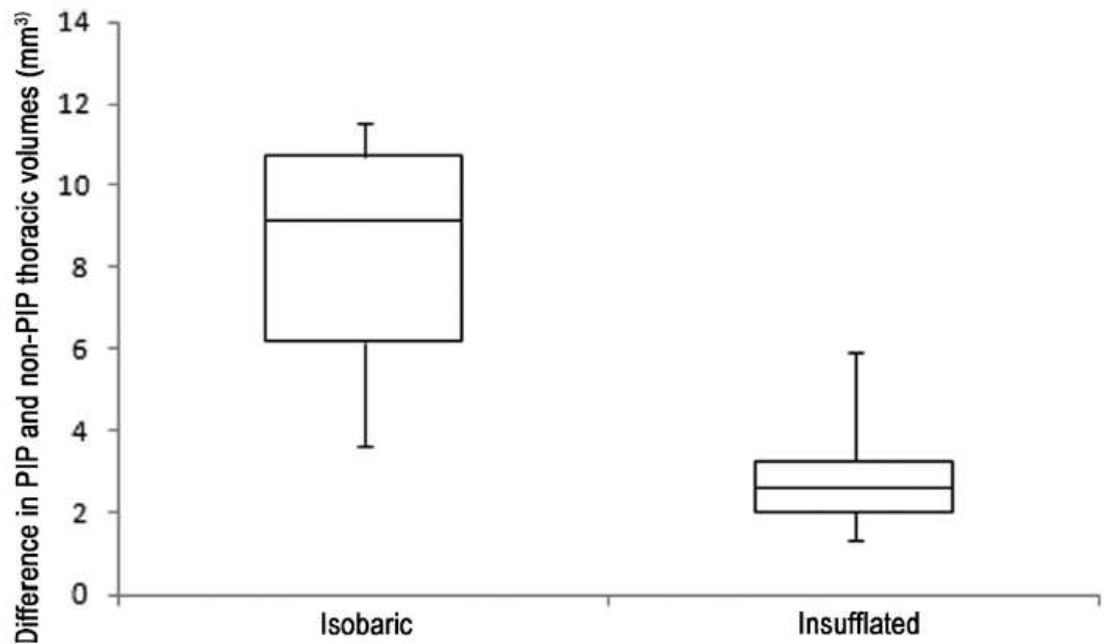


FIGURE 7. Box and Whisker plot: difference ($n = 7$) between PIP and non-PIP thoracic volumes, standardized by bodyweight: centre line indicates mean value, whiskers indicate range, and box indicates first and third quartile values. The difference between the isobaric and insufflated groups was statistically significant ($P = 0.006$).

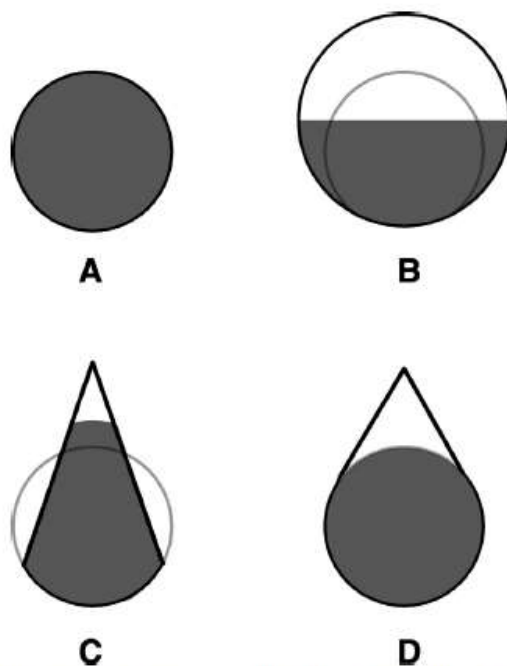


FIGURE 8. Diagram showing the changes from the baseline cross-section (A), under insufflation (B), and abdominal wall lift in a relatively rigid (C) and relatively pliable (D) abdominal wall. Visceral space is shown in grey and pneumoperitoneum space in white.

relatively high tension, no suture broke and there was no abdominal tearing.

The INS technique utilised in this study is a modification of previously reported techniques,^{15,16} which are entirely laparoscopic ovariectomy techniques. In this study, an ovariohysterectomy technique was reported in order to describe a method of completely resecting uterine tissues and enable comparison with the current standard technique.

To allow for ovariohysterectomy, extension of the caudal incision was required to exteriorise both cervixes prior to ligation due to the thickness of the reproductive tract in the rabbit. Sealing the reproductive tract cannot be done with electrocautery alone in the same manner as ovariectomy. While alternative methods of sealing the vestibular tract are possible, ligation was seen as the most analogous to that performed in the open laparotomy ovariohysterectomy. Alternatives for this approach include intracorporeal ligation and use of a vessel-sealing device. Using either of these techniques, retrieval of the uteri through the portals without the need to extend them would require morcellation, which complicates and increases the expense of the procedure.

While vessel-sealing devices have been reported for the sealing of reproductive tracts in dogs^{33,34} and pigs,³⁵ the strength of the resultant seal

strength is determined by the elastin/collagen composition,³⁴⁻³⁷ thus sealing strength cannot be extrapolated between species. Sealing strength is of particular relevance in the rabbit, due to normal urine distension of the vestibule. Ligation above the cervixes would result in retention of a small amount of uterine tissue, which could act as a source for adenocarcinoma.^{3-5,38-40}

A major difficulty in the implementation of the INS technique in rabbits is the thin and pliable abdominal muscles. There was difficulty in maintaining an airtight seal in the abdomen, and the cannulas were prone to stretching the muscle and sliding loose. The depth of the abdomen and working space also limits the intra-abdominal length of the cannula. As a result, it is recommended to use deep-threaded cannulas with threads that continue to the tip of the cannula (e.g., Ternamian cannulas, KSEA) to maximise engagement. It was also considered that the weight of the cannulas used may have been a factor in the difficulties observed. Use of a 3 mm cannula did decrease this effect; however, 2.7 mm LigaSure handpieces were not available for this project. Use of a purse-string suture was necessary in this study, though this may have been due to autolysis and is not expected to be necessary in a live patient. The ability to eschew cannulas and use conventional instruments in a laparoscopy-assisted manner is a major advantage of ISO laparoscopy.^{18,21,41,42}

The measurement of thoracic volume was achieved via an integrated region-of-interest function of the hospital imaging software (SYNAPSE, Fujifilm). Each slice was manually traced to calculate thoracic area. There was inherent inaccuracy in this technique, both in the difficulty of delineating the thoracic space, and the physical inaccuracy of the manual trace. To minimise this effect, the bounds of the thorax were strictly defined to the bony landmarks where sharp contrast was available. The soft tissue was able to be ignored as the specimens were compared with baseline values. It should be noted, that as the measurements are of total thoracic volume rather than lung volume, the change in diameter of the bronchial tree under pressure or compression of soft tissues could also contribute to the lung volumes; however, this was considered to be a minor effect and would affect both INS and ISO groups equally.

A limitation of this study is the use of cadavers rather than live patients. Cadavers were chosen for this study to develop the ISO technique and assess if it was feasible for use in live patients. While extrapolation to live patients is possible, further research utilizing the described ISO technique in

live patients is required to assess what impact the ISO technique may have on reduction in morbidity and mortality of these patients in the clinical setting. It is unclear whether the degree of changes seen with abdominal tenting may be different in a live patient, it is likely that compliance would increase in the postmortem patient; however, this degree of increase has not been described in cadaver studies in other species,¹⁷⁻²³ which leads the authors to surmise that the effect observed is genuine, though may not be as emphasised in live patients.

Furthermore, due to the study design, it was impossible to blind the assessor to the insufflation status of the specimen during CT assessment, while standardisation of methodology throughout the study aimed at minimising assessor bias, this cannot be eliminated as a limitation of the methodology.

In conclusion, while both ISO and INS laparoscopic or laparoscopy-assisted techniques are viable for the rabbit, the ISO techniques can be conveniently achieved without specialised lifting devices in this species. The mechanical inhibition of ventilation caused by positive pressure pneumoperitoneum necessitates ventilatory control in order to maintain thoracic volume. Further in vivo studies are recommended to assess the validity of these findings in clinical patients under different insufflation and ventilatory pressures.

REFERENCES

1. Baba N, von Haam E: Spontaneous adenocarcinoma in aged rabbits. *Am J Pathol* 68:653-656, 1972
2. Walter B, Poth T, Bohmer E, et al: Uterine disorders in 59 rabbits. *Vet Rec* 166:230-233, 2010
3. Howell TJ, Momement K, Bennett PC: Companion rabbit and companion bird management practices among a representative sample of guardians in Victoria, Australia. *J Appl Anim Welfare Sci* 3:18, 2015
4. Richardson C, Flecknell PA: Routine neutering of rabbits and rodents. *Pract* 28:70-79, 2006
5. Varga M: *Textbook of Rabbit Medicine*. ed 3 Elsevier Ltd; 2014
6. Brodbelt DC, Blissit KJ, Hammond RA, et al: The risk of death: the confidential enquiry into perioperative small animal fatalities. *Vet Anesth Analg* 35:365-373, 2008
7. Self I: A basic approach to small mammal anesthesia. *Ir Vet J* 60:94-100, 2007
8. Sikoski P, Young RW, Lockard M: Comparison of heating devices for maintaining body temperature in anesthetized laboratory rabbits (*Oryctolagus cuniculus*). *J Am Assoc Lab Anim Sci* 46:61-63, 2007
9. Barter LS: Rabbit analgesia. *Vet Clin North Am Exot Anim Pract* 14:93-104, 2011
10. Flecknell PA, Liles J: Assessment of the analgesic action of opioid agonist-antagonists in the rabbit. *J Ass Vet Anaesth* 17:24-29, 1990

11. Flecknell PA, Roughan JV: Assessing pain in animals—putting research into practice. *Anim Welfare* 13:71–75, 2004
12. de Sousa IDT, Candido dos Reis FJ, Garcia SB, et al: Adhesion formation after uterine horn resection in rabbit model: comparative study of laparoscopic procedure vs. laparotomy. *Gynaecol Endosc* 11:365–369, 2002
13. Gutt CN, Onui T, Schemmer P, et al: Fewer adhesions induced by laparoscopic surgery? *Surg Endosc* 18:898–906, 2004
14. Jorgensen JO, Lalak NJ, Hunt DR: Is laparoscopy associated with a lower rate of postoperative adhesions than laparotomy? A comparative study in the rabbit. *Aust NZ J Surg* 65:342–344, 1995
15. Al-Badrany MS: Laparoscopic ovariectomy in rabbits. *Iraqi J Vet Sci* 23:51–55, 2009
16. Divers SJ: Clinical technique: endoscopic oophorectomy in the rabbit (*Oryctolagus cuniculus*): the future of preventative sterilizations. *J Exotic Pet Med* 20:72–80, 2011
17. Liao CH, Kuo IM, Fu CY, et al: Gasless laparoscopic assisted surgery for abdominal trauma. *Injury* 45:850–854, 2014
18. Luks FI, Peers KHE, Deprest JA, et al: Gasless laparoscopy in infants: the rabbit model. *J Ped Surg* 30:1206–1208, 1995
19. Smith RS, Fry WR, Tsoi EKM, et al: Gasless laparoscopy and conventional instruments. *Arch Surg* 128:1102–1107, 1993
20. Tintara H, Choochun T, Geater A: Gasless laparoscopic hysterectomy: a comparative study with total abdominal hysterectomy. *J Obstet Gynaecol Res* 29:38–44, 2003
21. Fransson BA, Ragle CA: Lift laparoscopy in dogs and cats: 12 cases (2008–2009). *J Am Vet Med Assoc* 239:1574–1579, 2011
22. Kennedy KC, Fransson BA, Gay JM, et al: Comparison of pneumoperitoneum volumes in lift laparoscopy with variable lift locations and tensile forces. *Vet Surg* 44(S1):O83–O90, 2014. (Special issue: minimally invasive surgery)
23. Watkins C, Fransson BA, Ragle CA, et al: Comparison of thoracic and abdominal cavity volumes during abdominal CO₂ insufflation and abdominal wall lift. *Vet Surg* 42:607–612, 2013
24. Bailey J, Shapiro MJ: Abdominal compartment syndrome. *Crit Care* 4:23–29, 2000
25. Guler C, Sade M, Kirkali Z: Renal effects of carbon dioxide insufflation in rabbit pneumoretroperitoneum model. *J Endourol* 12:367–370, 1998
26. Guven S, Muci E, Unsal M, et al: The effects of carbon dioxide pneumoperitoneum on ovarian blood flow, oxidative stress markers and morphology during laparoscopy: a rabbit model. *Fertil Steril* 93:1327–1332, 2010
27. Shimada M, Kawaguchi M, Ishikawa N, et al: Saline-filled laparoscopic surgery: A basic study on partial hepatectomy in a rabbit model. *Minim Invasive Ther Allied Technol* 24(4):218–225, 2014
28. Sumpelmann R, Schuerholz T, Marx G, et al: Haemodynamic, acid-base and blood volume changes during prolonged low pressure pneumoperitoneum in rabbits. *Br J Anaesth* 96:563–568, 2006
29. Wang G, Wu R, Guo F, et al: Effects of carbon dioxide pneumoperitoneum on the inflammatory response and bacterial translocation in intraabdominal infection. *J Laparoendosc Adv Surg Tech A* 24:199–204, 2014
30. Lindgren L, Koivusalo A-M, Kellokumpu I: Conventional pneumoperitoneum compared with abdominal wall lift for laparoscopic cholecystectomy. *Br J Anaesth* 75:567–572, 1995
31. Hasson HM: Open Laparoscopy as a method of access in laparoscopic surgery. *Gynaecol Endosc* 8:353–362, 1999
32. Kim J-S, Lee B-H, Jang I-S, et al: Tracheal gas insufflation-aided mechanical ventilation during carbon dioxide-induced pneumoperitoneum in rabbits. *J Vet Med Sci* 65:907–912, 2003
33. Barrera JS, Monnet E: Effectiveness of a bipolar vessel sealant device for sealing uterine horns and bodies from dogs. *Am J Vet Res* 73:302–305, 2012
34. Mayhew PD, Brown DC: Comparison of three techniques for ovarian pedicle hemostasis during laparoscopic-assisted ovariectomy. *Vet Surg* 36:541–547, 2007
35. Sindram D, Martin K, Meadows JP, et al: Collagen-elastin ratio predicts burst pressure of arterial seals created using a bipolar vessel sealing device in a porcine model. *Surg Endosc* 25:2604–2612, 2011
36. Harold KL, Pollinger H, Matthews BD, et al: Comparison of ultrasonic energy, bipolar thermal energy, and vascular clips for the hemostasis of small-, medium-, and large-sized arteries. *Surg Endosc* 17:1228–1230, 2003
37. Marvel S, Monnet E: Use of a vessel sealant device for cystic duct ligation in the dog. *Vet Surg* 43:983–987, 2014
38. Brown Y, Knowles S, Fiorello C, et al: Pathology in practice. *J Am Vet Med Assoc* 237:1257–1259, 2010
39. Malone L, Schuler C, Leggett RE, et al: Effect of estrogen and ovariectomy on response of the female rabbit urinary bladder to two forms of in vitro oxidative stress. *Int Urogynecol J* 25:791–798, 2014
40. Sommerville LM: Treatment of a uterine adenocarcinoma in a domestic rabbit by ovariectomy. *Vet Rec* 142:550–551, 1998
41. Goldberg JM, Maurer WG: A randomized comparison of gasless laparoscopy and CO₂ pneumoperitoneum. *Obstet Gynecol* 90:416–420, 1997
42. Guido RS, Brooks K, McKenzie R, et al: A randomized, prospective comparison of pain after gasless laparoscopy and traditional laparoscopy. *J Am Assoc Gynecol Laparosc* 5:149–153, 1998