UNIVERSIDADE DE LISBOA

FACULDADE DE MEDICINA VETERINÁRIA

LAPAROSCOPIC TECHNIQUES FOR REPRODUCTIVE SURGERY IN DOGS

INÊS MARIA TENREIRO TAVARES

Orientador (es): Professor Doutor Ramón Rivera Barreno
Professor Doutor José Paulo Pacheco de Sales Luís

Tese especialmente elaborada para obtenção do grau de Doutor em Ciências Veterinárias na especialidade de Clínica

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Resumo

TÉCNICAS LAPAROSCÓPICAS PARA CIRURGIA REPRODUTIVA EM CÃES

As cirurgias laparoscópicas são já a rotina na medicina humana. Nos animais de companhia estão gradualmente a ser mais aplicadas e os tutores já as requisitam. A esterilização é a cirurgia mais realizada nos animais de companhia. Desta forma surgiu o interesse de avaliar a esterilização de cães machos e fêmeas e a dor associada.

No primeiro trabalho, comparámos a viabilidade e os efeitos sobre a dor e inflamação provocados por uma nova técnica de castração laparoscópica de cães machos com as da técnica clássica. Tempos cirúrgicos, escala de dor, cortisol sanguíneo e salivar e proteína C-reactiva foram comparados entre os dois grupos. O grupo laparoscópico teve níveis de dor, cortisol e valores de proteína C-reactiva significativamente mais baixos do que o grupo de orquiectomia clássica. Os resultados sugerem que esta castração laparoscópica é uma alternativa segura e benéfica à orquiectomia clássica em cães.

Estudos em medicina humana concluíram que a acetazolamida reduz a dor associada à insuflação de dióxido de carbono durante a cirurgia laparoscópica. Até agora nenhum estudo avaliou a acção da acetazolamida para esse fim nos animais de companhia. No segundo trabalho comparámos os valores de cortisol sérico, cortisol salivar e da Escala de Dor da Universidade de Melbourne em três grupos de cadelas sujeitas a: ovariectomia clássica, ovariectomia laparoscópica e ovariectomia laparoscópica com administração pré-operatória de acetazolamida. Encontrámos evidências que a administração pré-operatória de acetazolamida pode ser benéfica no controle da dor pós-operatória em cadelas após cirurgia laparoscópica.

O terceiro trabalho relata a resolução com sucesso de um achado intra-cirúrgico de teratoma no ovário esquerdo durante a ovariectomia laparoscópica. Este é o primeiro caso relatado de um teratoma removido por ovariectomia laparoscópica.

Palavras-passe: Ovariectomia laparoscópica; Castração laparoscópica; Cão; Dor; Acetazolamida.

Abstract

LAPAROSCOPIC TECHNIQUES FOR REPRODUCTIVE SURGERY IN DOGS

Laparoscopic surgeries are already routine in human medicine. In companion animals, they are gradually being applied more and tutors are already requesting them. Sterilization is the most common surgery performed on companion animals. In this way, the interest in evaluating the sterilization of male and female dogs and the associated pain arose.

In the first study, we compared the viability and the effects on pain and inflammation caused by a new technique for laparoscopic castration of male dogs with the classic technique. Surgical times, pain scale, blood and salivary cortisol and C-reactive protein were compared between the two groups. The laparoscopic group had significantly lower pain, cortisol, and C-reactive protein values than the classical orchiectomy group. The results suggest that this laparoscopic castration is a safe and beneficial alternative to traditional orchiectomy in dogs.

Studies in human medicine have concluded that acetazolamide reduces pain associated with carbon dioxide insufflation during laparoscopic surgery. So far, no study has evaluated the action of acetazolamide for this purpose in companion animals. In the second study we compared the values of serum cortisol, salivary cortisol and the University of Melbourne Pain Scale in three groups of bitches subjected to: open ovariectomy, laparoscopic ovariectomy and laparoscopic ovariectomy with preoperative administration of acetazolamide. We found evidence that preoperative administration of acetazolamide may be beneficial in controlling postoperative pain in bitches after laparoscopic surgery.

The third paper reports the successful resolution of an intra-surgical finding of teratoma in the left ovary during laparoscopic ovariectomy. This is the first reported case of a teratoma removed by laparoscopic ovariectomy.

Keywords: Laparoscopic ovariectomy; Laparoscopic castration; Dog; Pain; Acetazolamide.

Resumo Alargado

TÉCNICAS LAPAROSCÓPICAS PARA CIRURGIA REPRODUTIVA EM CÃES

A esterilização eletiva de cães é um dos procedimentos mais realizados na prática veterinária, e que pode contribuir para aumentar a expectativa de vida dos cães. É usado para contraceção, para controlar a superpopulação de cães, prevenir doenças e reduzir comportamentos sexuais e agressivos indesejados.

Até ao momento, vários procedimentos de esterilização foram descritos, incluindo orquiectomia por via aberta ou fechada, ablação escrotal, vasectomia, oclusão vasal com cloreto de cálcio, vasectomia laparoscópica e esterilização e vasocistostomia bilateral. Em cadelas, está descrita a ovariohisterectomia e a ovariectomia.

Nos últimos anos, a cirurgia minimamente invasiva desenvolveu-se rapidamente tanto na medicina humana quanto na veterinária, e muitas novas técnicas e instrumentos foram criados. As cirurgias laparoscópicas são agora mais comuns na medicina veterinária devido aos seus benefícios, como redução da dor pós-operatória, redução da incidência de infeção do campo cirúrgico, menor tempo de internamento hospitalar, feridas muito menores que não requerem pensos pós-operatórios e menor morbilidade. À medida que os donos de animais de companhia se tornam mais conscientes destas técnicas, eles esperam que uma cirurgia minimamente invasiva seja uma opção para os seus animais de companhia.

A castração laparoscópica é considerada uma escolha adequada se combinada com outra cirurgia com abordagem laparoscópica, como cistopexia, deferentopexia, colopexia e gastropexia. Além disso, pode ser aplicada para criptorquidectomia em cães e como opção para programas de esterilização em massa em áreas urbanas e industriais superpovoadas.

Tendo em conta os benefícios da cirurgia laparoscópica quisemos comparar a castração por técnica aberta e laparoscópica em canídeos machos. Este é o primeiro estudo controlado a descrever esta técnica cirúrgica e a avaliar a dor pos-cirurgica em cães castrados por laparoscopia. Um total de 20 animais foram randomizados e colocados em grupo castração aberta ou grupo castração laparoscópica. O mesmo protocolo anestésico foi aplicado aos dois grupos. Pre-medicação com acepromazina e tramadol endovenoso. A anestesia foi induzida

com propofol e mantida com isoflurano em 100% oxigénio num circuito sem reciclagem de ar.

A orquiectomia clássica foi realizada com o método standart pré-escrotal com encerramento com fio de sutura monofilamentar de gliconato. O pneumoperitoneu foi estabelecido pela introdução de CO_2 (dióxido de carbono) pela agulha de Veress colocada caudalmente ao apêndice xifoide. Colocaram-se dois trocares de 5,5mm lateralmente às cadeias mamarias 3cm abaixo da linha da cicatriz umbilical. A técnica laparoscópica foi inovadora porque apenas se usaram dois trocares roscados de 5,5mm e uma pinça bipolar de alta-frequencia com tesoura integrada (5 mm, RobiPlus; Karl Storz; 78532 Tuttlingen, Germany) para fazer a oclusão, coagulação, secção do ductus deferens e dos vasos testiculares. As feridas foram encerradas fio monofilamentar de gliconato. Após a cirurgia e nos dois dias seguintes administrou-se meloxicam subcutâneo. Todos os animais tiveram alta dois dias após a cirurgia. E foram reavaliados uma semana depois.

Não se detectaram diferenças significativas nos seguintes valores: na média de idades, na média dos pesos, na média do tempo cirúrgico dos dois grupos. Não houve complicações intra nem pos cirúrgicas nos dois grupos. O grupo de laparoscopia teve as menores classificações de dor pós-cirurgica da Escala de Dor da Universidade de Melbourne em todos os momentos avaliados. As classificações da Escala de Dor da Universidade de Melbourne foram significativamente mais baixas para o grupo laparoscópico às 12h e às 24h pós-cirurgico. As concentrações de cortisol sérico e cortisol salivar foram significativamente mais baixas para o grupo laparoscópico 1h após a cirurgia. E encontrou-se uma correlação forte (r=0,58) entre os valores de cortisol sérico e salivar em todos os tempos avaliados. A concentração de PCR, ligada à inflamação, foi significativamente mais baixa para o grupo laparoscópico uma semana após a cirurgia em comparação com o grupo da castração aberta. Os resultados sugerem que esta técnica cirúrgica é segura, permite recuperação rápida e sem complicações para os animais, e que é exequível em tempo semelhante à técnica clássica aberta.

A dor pós-operatória laparoscópica está associada à neuropraxia do nervo frénico, que é secundária à distensão abdominal com o gás dióxido de carbono e à temperatura intra-abdominal, humidade e quantidade de gás residual. O número de cânulas utilizadas, o tipo de procedimento cirúrgico e a experiência do cirurgião também influenciam a intensidade da dor pós-operatória. Em procedimentos laparoscópicos, o dióxido de carbono (CO_2) usado para o pneumoperitoneu difunde-se nos tecidos. É absorvido para a circulação sistémica através do peritoneu, o que resulta em diminuição do pH peritoneal e plasmático pela libertação de iões de hidrogénio, que podem ser responsáveis por algum grau de dor. Múltiplas técnicas têm sido investigadas para reduzir ou prevenir as alterações do pH intraperitoneal, como uso de aquecimento, humidificação, bicarbonato, lavagem intraperitoneal, laparoscopia sem gás e uso de hélio como substituto do CO_2, todas com resultados mistos. A acetazolamida tem potencialmente um papel na inibição das formas citoplasmática e ligada à membrana da anidrase carbónica, o que retarda a produção de iões H+ pela catálise enzimática. Ao inibir a anidrase carbónica, possivelmente o ácido carbónico é removido por difusão ou fluxo sanguíneo em velocidade capaz de diminuir a estimulação dolorosa. Nos cães as alterações no fluído peritoneal provocadas pelo CO_2 são semelhantes às registados em humanos. Assim, supomos que tal como nos humanos, a acetazolamida poderá ajudar a reduzir a dor pos-cirurgica nos cães sujeitos a cirurgia laparoscópica onde houve insuflação abdominal com CO_2.

No segundo trabalho 30 cadelas de raça não definida foram incluídas e aleatoriamente designadas para um dos três grupos: OVE (ovariectomia mediana com celiotomia), OVEL (ovariectomia laparoscópica) e OVELA (ovariectomia laparoscópica com administração pré-operatória de acetazolamida). A acetazolamida foi administrada por via oral (na dose de 25 mg/kg) 2 horas antes da indução no grupo OVELA. O mesmo protocolo anestésico foi usado para os três grupos. Pre-medicação com acepromazina, tramadol e fentanilo IV. Indução com propofol e manutenção anestésica com isoflurano. Foi usado circuito respiratorio semi-fechado. Durante o procedimento fez-se CRI de fentanilo e ventilação mecânica. No grupo de ovariectomia realizou-se a técnica clássica. Nos grupos de ovariectomia laparoscópica, a técnica consistiu nos seguintes passos sequenciais: estabelecimento pneumoperitoneu com CO_2 com ajuda de Agulha de Veress caudal ao xifoide; o primeiro trocar foi colocado 2 cm caudal à cicatriz umbilical; os seguintes trocares foram colocados 3 cm cranial e caudal ao primeiro trocar. Colocou-se animal em decúbito lateral direito, expondo o ovário esquerdo e com pinça bipolar fez-se cauterização, transseção do ligamento próprio do ovário ao nível do corno uterino, depois o mesovário e o ligamento suspensor. Utilizando uma agulha que atravessou a parede abdominal, fixou-se o ovário percutaneamente. O animal foi colocado em decúbito lateral esquerdo expondo o ovário direito. Usando a mesma técnica fez-se resseção do ovário. O ovário direito foi apreendido por pinça. Colocou-se novamente animal em decúbito lateral direito para retirada de ambos ovários pela incisão do trocar. As incisões foram encerradas com fio absorvível de gliconato. Após a cirurgia foi administrado meloxicam subcutâneo e nod dias seguintes. A alta ocorreu dois dias pos-cirurgia. Os animais voltaram para reavaliação após uma semana. Não se verificaram diferenças significativas nos pesos e idades dos animais dos três grupos. O tempo de cirurgia nos grupos OVEL e OVELA foram significativamente superiores que no grupo OVE. Não se registaram complicações que justificassem a conversão para cirurgia aberta. Não foram observados efeitos adversos potencialmente provocados pela acetazolamida. A dor pós-operatória foi avaliada por meio do cortisol sérico, do cortisol salivar e da Escala de Dor da Universidade de Melbourne (UMPS). Diferenças estatísticas foram observadas nas pontuações UMPS quando o grupo OVELA foi comparado ao grupo OVEL em 1 hora após a cirurgia, 12 horas e 24 horas. Os animais submetidos à cirurgia aberta (grupo OVE) tiveram pontuações de dor significativamente maiores em todos os momentos após a cirurgia quando comparados aos grupos OVEL e OVELA. Uma alta correlação positiva foi encontrada entre as concentrações de cortisol sérico e salivar. A concentração média de cortisol na saliva não foi significativamente menor para o grupo OVELA em comparação com os outros grupos. Este estudo encontrou evidências de que a administração pré-operatória de acetazolamida pode ser benéfica no controle da dor pós-operatória em cães após cirurgias laparoscópicas.

No terceiro trabalho apresenta-se a resolução cirúrgica de excisão de um teratoma periovárico esquerdo. Numa cadela saudável detectou-se uma estrutura cística no ovário esquerdo, com uma consistência dura e um útero não-grávido grosseiramente normal. Mantendo-se o plano cirúrgico de ovariectomia laparoscópica, o pedículo ovárico esquerdo, o ligamento próprio e o ligamento suspensor foram selados e seccionados com pinça bipolar de alta frequência com lâmina integrada, e removidos. O procedimento cirúrgico foi bem-sucedido. O exame histopatológico mostrou a presença de um teratoma adjacente ao ovário. Um ano após a cirurgia, a cadela apresentava-se clinicamente normal e a ecografia abdominal não indicou a presença de alterações anatómicas nos órgãos abdominais nem a presença de líquido livre no abdómen. De acordo com o conhecimento dos autores, este é o primeiro caso relatado de um teratoma ovárico removido por ovariectomia laparoscópica numa cadela.

Mais estudos com amostras mais numerosas são necessários para demonstrar que a acetazolamida deveria ser considerada como adjuvante num protocolo analgésico multimodal em cirurgia laparoscópica em animais.

Índice

List of Publications

This tesis was based on the following publications:

Tavares, I.T.; Barreno, R.R.; Sales-Luís, J.P.; Vaudano, C.G.; Jaber, J.R. Laparoscopic Castration Using Bipolar Forceps vs. Orchiectomy in Dogs: A Comparison of Two Techniques. *Animals* (Basel). 2021 Oct 24; 11 (11), 3041.

Tavares I.T., Rivero R, Sales-Luís JP, Vaudano CG, Correia SD, Corbera JA, Jaber JR. Premedication with acetazolamide: Is its use for postoperative pain and stress control after laparoscopic ovariectomy in dogs ruled out? *Vet Med Sci.* 2023 May; 9(3):1114-1123.

Tavares I.T., Barreno RR, Sales-Luís JP, Vaudano CG. Ovarian teratoma removed by laparoscopic ovariectomy in a dog. *J Vet Sci.* 2018 Nov 30; 19(6):862-864

From this Project also resulted the following conference posters:

Tavares, I.T.; Barreno, R.R.; Sales-Luís, J.P.; Vaudano; "Estudo comparativo entre orquiectomia e castração laparoscópica em cães."; 9º Encontro de Formação da Ordem dos Médicos Veterinários, 6 e 7 de Abril de 2019

Tavares, I.T.; Barreno, R.R.; Sales-Luís, J.P.; Vaudano; Ovariectomia laparoscópica com excisão de teratoma ovárico", XIV Congresso Hospital Veterinário de Montenegro, 24-25 Fevereiro 2018

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List of abbreviations and symbols

AENG2	new generation articulating enseal G2
ASA	American Society of Anesthesiologists
AWL	Abdominal wall lift
CBC	complete blood count
CMPS	Glasgow Composite Measuring Pain Scale
COR	cortisol
COX	cycloxigenase
CRI	Constant Rate Infusion
CRP	Serum C-reactive protein
DIVAS	Dynamic and interactive assessement of patients
DSAMAR	Deformable self-assembled magnetic anastomosis ring
ERAS	Enhanced Recovery After Surgery guidelines
IAP	intrabdominal pressure
IPLAs	intraperitoneal local anesthetics
LMSD	Ligasure Maryland Sealer/Divider
LS	Laparoscopic sterilization
MAG	magnetically anchored gasper
MMAC	miniature magnetically anchored camera
MNT	Mechanical Nociceptive Testing
NIR	Near-infrared
NMDA	N-Methyl-D-Aspartate
NOTES	Natural Orifice Transluminal Endoscopy Surgery
NRS	Numerical rating scales
NSAIDs	non-steroid antinflammatory drugs
OFA	opioid free anesthesia
OR	orchiectomy
OSA	opioid sparing anesthesia
OVE	ovariectomy
OVEL	laparoscopic ovariectomy
OVELA	laparoscopic ovariectomy with acetazolamide

OVH	ovariohysterectomy
PCT	paracetamol
PNBs	Peripheral nerve blocks
QBA	Qualitative behavioral assessement
QLB	quadratus lumborum block
SC	subcutaneos
SDS	Simple descriptive scales
SF-CMPS	Short-Form Glasgow Composite Measuring Pain Scale
SILS	Single-Incision Laparoscopic Surgery
TAP	transverse abdominis plane block
TPR	temperature, pulse, respiration
UMPS	University of Melbourne Pain Scale
VAS	Visual analog scale
WBC	white blood cells

Chapter 1- Introduction

The elective sterilization of dogs is one of the most commonly performed reproductive tract surgeries in veterinary practice (Greenfield, Johnson, & Schaeffer, 2004). Laparoscopic techniques are already being applied in the sterilization of dogs due to their peri-surgical benefits (Austin, Lanz, & Hamilton, 2003; Davidson, Moll, & Payton, 2004; Devitt, Cox, & Hailey, 2005; Gower & Mayhew, 2008; Lansdowne, Mehler, & Bouré, 2012; Mahalingam et al., 2009). As pet owners become more aware of these techniques, they expect minimally invasive surgery to be an option for their animals (Howe, 2006). Since it is a recent technique, international companion animal veterinary associations' guidelines on peri-surgical pain management do not have specific protocols for laparoscopic surgery.

Because there is a need to continue the development of laparoscopic surgery for the reproductive tract of dogs, we conducted this research project with male and female dogs, with three studies on reproductive laparoscopic surgery techniques.

We established the following objectives:

In the first study:

- Use a new surgical technique that combines laparoscopic vasectomy and deferenctomy in healthy male dogs, with two trocars and a high frequency bipolar energy forceps.
- Compare the surgical time between the classic castration and the new castration technique
- Compare the inflammation associated with classic castrations and the new technique
- Use the University of Melbourne Pain Scale to evaluate pos-surgical pain and find significant differences between groups

In the second study:

- Use acetazolamide as a diuretic and assess its effect on post-surgical pain in laparoscopic ovariectomy with OC_2 pneumoperitoneum
- Compare the surgical time between the classic and the laparoscopic ovariectomy
- Compare the post-surgical pain associated with the classic and the laparoscopic ovariectomy
- Assess the correlation between the serum and the salivary cortisol
- Use the University of Melbourne Pain Scale to evaluate pos-surgical pain and find significant differences between groups

The results of these studies allowed three scientific articles.

First article

In male dogs, laparoscopic castration is described for cryptorchid animals, and we wanted to evaluate the safety, pain, and feasibility of using a new laparoscopic castration technique for dogs without this malformation.

Second article

Innovations in laparoscopic techniques originated in human medicine and were later adapted to veterinary medicine. There are studies demonstrating the usefulness of acetazolamide in reducing post-surgical pain in reproductive tract laparoscopy in women. Therefore, we wanted to evaluate if acetazolamide could have a similar effect in female dogs.

Third article

In one of the laparoscopic ovariectomy surgeries from the second study of this project, we successfully excised a teratoma (an intra-surgical finding). This female dog was excluded from the study, but the fully laparoscopic resolution resulted in the third publication.

In designing this experimental project, we considered several concerns:

- To replicate as closely as possible the procedures of daily hospital practice to reproduce the real behaviors and physiological changes resulting from surgical interventions in this environment. Therefore, we used healthy animals that were already going to undergo elective sterilization surgery. We maintained the animals' hospitalization and visits from their guardians as with any other procedure performed in the hospital.
- Although acetazolamide is a medication used in dogs, it was approved for experimental use with an application different from that described. Thus, its application in Portugal would be subject to a prescription under the cascade.

This project was designed and approved in 2011. The development and execution of the experimental work and the publication of the three articles from this thesis took place over 13 years. The state of the art and surgical practices in 2011 contributed to the experimental design used, in which, as of today, we recognize the passage of time. However, the novelty of the contribution of this project's results remains despite the time elapsed because the published work has been recently cited in the 2022's book Small Animal Laparoscopy and Thoracoscopy from the American College of Veterinary Surgeons and in the 2024

Guidelines for the Control of Reproduction in Dogs and Cats from the Word Small Animal Veterinary Association.

Finally, a few words about the structure of the thesis. All the works presented in this thesis were published in peer-reviewed journals, with the doctoral candidate listed as the first author in all of them. The thesis structure contains the following chapters: summaries; extended summaries; this Introduction; a literature review on the thesis topic; the results presenting the three published works; the discussion on the results; and finally, the conclusion and future perspectives. The order of presentation of the works in the thesis follows the logical development of the themes, not the chronological order of their publication.

Chapter 2 – Review on laparoscopic techniques for reproductive surgery in dogs

2.1 Laparoscopy

2.1.1 History of laparoscopy in small animals

The performance of minimally invasive therapy had its origin in endoscopy, which in turn began from the observation of cavities using speculums or similar instruments (Brun & Beck, 1999). The idea of looking inside a body is old, but surgeons had to wait until 1806 (first cystoscopy performed by Bozini) before they could perform it (Dupre, 2008). Among the difficulties observed in that period were the impossibility of transmitting enough light to the place to be examined and the restriction of the field of vision. These difficulties were almost eliminated with the creation of the Nitze cystoscope, since this device had optical lenses and illumination at its distal end (Brun & Beck, 1999). It was not until the beginning of the 20th century (1901) that George Kelling, in Dresden (Dupre, 2008) ventured to observe the abdomen using a needle to insufflate filtered air into the peritoneal cavity of a live dog (Brun & Beck, 1999). Since then, several authors have contributed to the development of laparoscopy (see Table 1):

Table 1– Discoveries in the 20th century that contributed to the development of laparoscopy (Brun & Beck, 1999)**.**

Author	Era	Contribution to laparoscopy
H.C. Jacobaeus	1911	Performance of laparoscopic exams in 45 patients
W. E. Stone	1924	Use of a nasopharyngoscope to visualize the peritoneal cavity of dogs
Nadeau e Kampmeier	1925	Performance of laparoscopy in three patients and detailed description of the technique used
H. Kalk	1927	Development of a lens system that enabled 135º viewing

H.Kalk	1929	Use of two trocars and performance of 100 laparoscopic exams
C.Ferves	1933	Use of laparoscopy in performing biopsies and treating abdominal adhesions
J.C. Ruddock	1937	500 laparoscopies performed, including 39 biopsies
J. Veress	1938	Development of a needle for creating pneumoperitoneum
Fourestier, Gladu, Valmiére	1952	Use of quartz for light transmission through the laparoscope
K. Semm	1960s	Development of an automatic inflator and laparoscopic instruments
Hopkins	1960s	Development of the rod-lens system

The word laparoscopy derives from the Greek "lapará" and "skopein" which mean, respectively, abdomen and examine (Usón Gargallo, Sanchez Margallo, & Soria Gálvez, 2003). In 1986, a camera with a computer chip was developed to be coupled to the laparoscope, starting the era of video-assisted surgery (Brun & Beck, 1999).

However, some gynecologists, under the influence of Bruhat (Clermont-Ferrand) switched from diagnosis to treatment and performed many gynecological procedures. In 1987, Mouret and Dubois, in Lyon, performed the first laparoscopic cholecystectomy. This event gave a great impetus to the initiation of minimally invasive surgery. In humans, this type of surgery has become the "gold standard" for many procedures (Dupre, 2008). The dog, as an experimental model, has shown great importance in the development of laparoscopy, both in human medicine and in veterinary medicine. In veterinary medicine, the first works involving the performance of laparoscopic techniques in dogs as patients date back to the 1970s (Brun & Beck, 1999). Dr David E. Wildt was the first to report on female and male sterilization using occlusion of the uterine horn and the vas deferens respectively (Dukelow, 1980). At that time, the pneumoperitoneum gas of choice was the nitrous oxide, carbon dioxide and air were also recommended (Johnson & Twedt, 1977).

Advancements in laparoscopic surgery during the 21st century have been substantial, driven by a combination of technological innovation and procedural refinement. The introduction of high-definition cameras in urological surgeries (Niu et al., 2023) and three-dimensional imaging systems (Azuma & Monnet, 2024) has greatly improve visual clarity and depth perception during laparoscopic procedures in a canine cadaveric study. Near-infrared (NIR) cameras allow for real-time, high-definition visualization of vessels, anatomic structure and perfusion. New uses of NIR technologies during laparoscopy are continuing to grow for vascular, lymphatic, and oncologic-related techniques (Thomson, 2024). The development of robotic systems like the Da Vinci robot has enabled surgeons to perform more precise and complex procedures in beagles with enhanced dexterity and control (Jiang, Xiang, He, & Tian, 2024). Advances in instrument design have led to the development of smaller, more versatile laparoscopic tools, minimizing the invasiveness of procedures. Laparo-endoscopic single-site cholecystectomy through a 10-mm incision using the miniature magnetically anchored camera (MMAC) and magnetically anchored gasper (MAG) was attempted in six dogs. The MMAC provided clear imaging, and the MAG provided sufficient exposure to perform the cholecystectomy (Haoyang Zhu et al., 2019). Techniques which involve accessing the abdominal cavity through natural orifices, have been developed to reduce the need for external incisions, improving recovery times and cosmetic outcomes. Deformable self-assembled magnetic anastomosis ring (DSAMAR) is a safe and feasible option for the treatment of colon stenosis. Its specific deformation and self-assembly capability maximize the applicability of the minimally invasive in seven dogs (M.-M. Zhang et al., 2023). The innovation in energy devices, such as ultrasonic and advanced bipolar instruments, has improved tissue dissection, coagulation, and sealing, enhancing the safety and efficiency of laparoscopic surgeries. New generation Articulating Enseal G2 (AENG2) provides a good alternative to Ligasure Maryland Sealer/Divider (LMSD) in laparoscopic ovariectomy, with only minor differences in measured variables. Subjectively, the articulating feature of AENG2 did not improve surgical performance in laparoscopic ovariectomy and the use of LMSD appeared more straight-forward for this specific procedure (Driessen, Marrero, Grinwis, & van Nimwegen, 2023). The development of Single-Incision Laparoscopic Surgery (SILS) techniques allows for laparoscopic surgery through a single incision, typically in the umbilicus, reducing the number of incisions and potentially improving cosmetic outcomes. A meta-analysis was performed on the eight controlled studies, showing that SILS ovariectomy and gastrointestinal procedures had a comparable surgical time to multiport techniques (Lacitignola, Guadalupi, & Massari, 2021). Artificial Intelligence technologies are being utilized to enhance image recognition, helping surgeons identify critical landmarks and potential complications in real-time. AI has the potential to revolutionize surgery in the coming years. Still, it is essential to clarify what the meaningful current applications are and what can be reasonably expected (Spinelli et al., 2023).

2.2 Indications for laparoscopic surgery

There are several laparoscopic surgical techniques for diagnosis, elective and treatment procedures. For the gastrointestinal tract: laparoscopic-assisted feeding tube placement (Matsukubo et al., 2024), gastrotomy (Nasher et al., 2024), enterotomy (Katzen et al., 2023), and intertinal resection and anastomosis (Vivas López et al., 2024); laparoscopic and laparoscopic-assisted gastropexy (Kamran et al., 2024); treatment of sliding hiatal hernia (Singh et al., 2024) and gastroesophageal reflux disease (G. Li et al., 2023); laparoscopic splenectomy (McGaffey et al., 2022), laparoscopic liver biopsy (Larose, Brisson, Foster, & Monteith, 2024), resection, ablation and cholecystocentesis ; laparoscopic cholecystectomy (Azuma & Monnet, 2024). For endocrine surgery: Laparoscopic adrenalectomy (van Bokhorst et al., 2023); laparoscopic surgery of the pancreas (Poggi et al., 2024). Urological surgery: laparoscopic renal biopsy (Xu et al., 2024); laparoscopic ureteronephrectomy (Pathak & Hemal, 2024); transurethral cystoscopy and laparoscopic-assisted cystotomy for urolith removal and mass resection (Almutairi, 2024), total prostatectomy (Geraghty, Keane, & Davis, 2024) (Monnet, 2007), For the reproductive trat: laparoscopic ovariohysterectomy (Thurston, Sertich, McDonnell, & Parente, 2024), ovariectomy (Tavares et al., 2023) and hysterectomy; laparoscopic castration and sterilization techniques of the male dog, including removal of undescendent testicles (David, de Rooster, & Van Goethem, 2024); cancer staging (Koek et al., 2024); diaphragmatic and inguinal herniorrhaphy and natural orifice translumenal endoscopic surgery (NOTES) (Fransson & Mayhew, 2022).

2.3 The advantages and disadvantages of laparoscopic surgery

Research in dogs, pigs, and humans indicates that laparoscopic procedures are preferable to open surgery due to reduced morbidity from less incisional trauma, fewer healing complications, adhesion formation, less perioperative and postoperative pain, and lower physiological stress response. Laparoscopic surgery is associated with increased visibility of abdominal structures (Popat, Smith-Mathus, Lucioni, & Lee, 2024) (van Nimwegen & Kirpensteijn, 2007). It has a low complication rate, very short recovery time and better patient convalescence when compared to open surgery (Emmen et al., 2024) (Hewitt, Brisson, Sinclair, Foster, & Swayne, 2004). This type of surgery has specific advantages for biopsy collection procedures. The biopsy is collected under camera observation and the biopsy site can be observed immediately after collection. All this with less postoperative discomfort (Moore, 2007).

The direct visualization and diagnosis of the pathological processes with the laparoscope presents advantages (Sun, Guo, Kang, Tao, & Wang, 2023) in relation to what is possible to do with radiography and ultrasound (S.K. Maiti et al., 2007). Laparoscopy is associated with the possibility of performing the procedure during the diagnosis and the reduction of complications and inconveniences associated with celiotomy (Antoun et al., 2023). The laparoscopic approach is, however, limited by factors such as the high cost of equipment, specific surgical training and longer operative procedure time (Costa Neto, Teixeira, Baraúna, Gordilho Filho, & Baraúna, 2006). Disadvantages such as increased surgical time, high cost of equipment and visceral trauma by trocar, are overcome by less post-surgical pain and faster return to normal activity for the patient (Minami, Okamoto, Eguchi, & Kato, 1997) (Antoun et al., 2023).

These procedures are available in specialty practices and teaching hospitals due to the necessary specialty training, the expertice to safely perform the surgeries and the costs associated with the equipment. However, due to increasing client requests the basic sterilization and gastropexy laparoscopic techniques are being performed by general veterinarians (Jones, Case, Evans, & Monnet, 2017) (Massari & Kelly, 2024).

When the surgeon is considered the difficulties of performing minimally invasive surgery are using different and expensive equipment, the “unnatural ” position or non ergonomic positions in which the surgeons have to stay during surgery, magnified tremor, the haptic feedback (a limited tactile sensitivity) for the use of long instruments, the fulcrum effect (the limited movement of the instruments in the port), as the vision shifts from binocular to monocular leads to the loss of depth perception, the loss of “bird’s eyeview” as the reduction of the field of view leads and the surgical laparoscopic instruments outside of the field of view are a liability and the reduction of movement of the instruments is reduced (Usón, Sánchez, Sánches, Pérez, & Hashizume, 2010) (Dejescu, Bel, Melega, Muresan, & Oana, 2023)

2.4 Fundamental techniques in laparoscopic surgery

Laparoscopy is a type of endoscopy that is performed with a rigid endoscope or laparoscope. For laparoscopic procedures an endoscopy tower is required. The tower is a mobile car that carries several components: monitor, camera, light source with light cable, mechanical insufflator and an image/video recording device (Mayhew, 2009). It may also contain a power source (monopolar and/or bipolar electrocoagulation system) and an aspirator (Usón et al., 2010).

2.4.1 Anesthesia

A multimodal protocol is recommended, which may include as premedication an opioid for analgesia and sedation, plus a tranquilizer or sedative when the animal is excited or fractious; an anticholinergic in case of bradicardia; induction with propofol, ketamine or alfaxalone (Fransson & Mayhew, 2022). Laparoscopy is usually performed under general anesthesia (Lazzarini et al., 2024). General anesthesia allows patients to be easily ventilated, and promotes analgesia, good muscle relaxation, and an immobile surgical field. It also allows immediate conversion to laparotomy if a more serious complication occurs or if something unexpected is discovered (J. D. Smith, Allen, & Quandt, 1999). After intubation, anaesthesia is maintained with an inhaled anesthetic like isoflurane or sevoflurane. Local anesthetic infiltration can be used on trocar sites. Postoperative protocol can include opioid and nonsteroidal anti-inflamatory drug (Fransson & Mayhew, 2022).

2.4.2 Animal preparation

As with open surgery, patients must be prepared for surgery. The abdomen must be shaved and prepared for a sterile procedure. The entire surface (not just the trocar insertion sites) must be prepared for surgery so that, should conversion to open surgery be necessary, the field is prepared (Fransson & Mayhew, 2022). Ideally, the bladder and stomach should be empty of contents. If the bladder is full, it must be emptied so that there is no risk of accidental puncture. A full stomach makes it difficult to visualize the liver (Richter, 2001) .

2.4.3 Pneumoperitoneum

To visualize the abdominal structures and safely place the trocars, a pneumoperitoneum must be created to lift and separate the wall of the abdominal cavity from the viscera. This is achieved by insufflating gas into the abdominal cavity (Richter, 2001) (Chatterjee, Kumar, & Chattoraj, 2024).

The instruments and basic equipment needed for pneumoperitoneum establishment are an electronic insufflator and an insufflation needle or laparoscopic trocar (Merlin, Cinti, & Charlesworth, 2022). The best position to create the pneumoperitoneum is dorsal recumbency and two techniques can be used: open or closed. In the closed technique, an insufflation needle or Veress needle is used, which has a mechanism to protect the perforating needle. When pressure is exerted with the end of the needle, the protective rod retracts allowing the passage of the needle through the wall. Once the wall has been crossed, the protective device is activated, preventing damage to the internal organs (Leschnik, Bockstahler, Katic, Schramel, & Dupré, 2018).

The needle can be introduced below the xiphoid process (in the case of dogs, it will have to be lateral to the midline so as not to touch the falciform ligament), in the umbilical region or in the left hypochondrium. It is important to approximately calculate the thickness of the abdominal wall in order to hold the needle at as many centimeters from its end as the wall is thought to measure, thus avoiding organ damage. It may help to pull the wall upwards to increase the distance between the wall and the viscera (Tapia-Araya, Martin-Portugués, Bermejo, & Sánchez-Margallo, 2015).

There are several methods of checking that the needle is in the correct position inside the abdominal cavity: instill saline solution with a syringe, observing that it flows without resistance and that later, if we aspirate, we do not recover the serum; place a syringe without a plunger filled with saline solution at the end of the needle and check how the serum is diffused by gravity into the abdominal cavity; insufflate air with a syringe and if the needle is well placed, the air enters without resistance. However, when there is negative pressure in the abdomen, there may be a certain resistance in air aspiration, so it is necessary to connect the CO_2 insufflator and check that the pressure is low and that there is an inlet flow. If the needle is not well placed, it must be withdrawn and reinserted (Taliento et al., 2023).

The open technique for obtaining the pneumoperitoneum consists of making a small 3 cm skin incision and dissecting the abdominal wall in layers, then inserting a trocar through a 1 cm incision through the peritoneum under direct vision. Once the correct position of the needle or the trocar has been verified, the cable is connected to the end of these and the insufflator is turned on (Usón et al., 2010) at a flow of 1 L/min or up to 2 L/min in larger patients (Richter, 2001). In anesthetized dogs, it is recommended that the intra-abdominal pressure oscillate between 8 and 12 mmHg to avoid complications caused by hemodynamic changes (Merlin et al., 2022).

Carbon dioxide is the most commonly used gas for several reasons: it is rapidly absorbed and excreted; it does not combust unlike oxygen, it is more soluble in blood than other gases such as nitrogen monoxide and is relatively harmless to peritoneal tissues. CO_2 is readily absorbed by the splenic vasculature, and the entry of small amounts of gas into the circulation usually does not give rise to complications (J. D. Smith et al., 1999) (Ghamari et al., 2024). The disadvantage of carbon dioxide is the irritation it causes to the peritoneal wall, which requires a slightly higher anesthetic level (Richter, 2001) (Bataineh, Qudaisat, Banihani, Obeidat, & Hamasha, 2021).

Most of the physiological changes caused by laparoscopic procedures result from the insufflation of gas into the abdomen which is used for better visualization of structures (J. D. Smith et al., 1999) (Noor et al., 2021).

A “trampoline” effect when touching the abdomen indicates that the pneumoperitoneum has been reached (Richter, 2001).

2.4.4 Placement of trocars

Patient position and trocar placement sites depend on the procedure and the organ or organs being examined (Richter, 2001). The next step consists of introducing the first trocar (by closed technique), whose location will vary depending on the procedure to be performed. A small skin incision the size of the trocar thread is made. With a conventional surgery hemostat, the subcutaneous tissue is dissected until reaching the muscular layer of the abdominal wall. The trocar is introduced perpendicularly to the wall. By exerting pressure on the end of the trocar, the protection system retracts, allowing the cutting part to pass through the wall. Once the muscle and peritoneum have been crossed, the protection system triggers, preventing damage to internal organs with the cutting edge. The cutting spike part is extracted and the trocar cannula is threaded into the abdominal wall (Usón et al., 2010).

According to Potter and Hendrickson (1999) and also according to McCarthy and Monnet (2005), the trocars must be placed in such a way that the instruments passing through them do not interfere with each other, so that the examined area can be reached and that the manipulations and the instruments remain within the field of view of the laparoscope (Bonfada, 2005). One of the principles of laparoscopy is triangulation (Willard M. D., 2008), that is, the trocars are placed in a triangle, with the laparoscope tending to be placed on the medial trocar and the instruments on the lateral trocars (Chatterjee et al., 2024).

The site to insert the laparoscope trocar should not be exactly over the site to be examined and operated on, but close enough to allow access and visualization (Bonfada, 2005).

In addition to trocars previously mentioned, there are more access techniques to be used in laparoscopic surgeries.

Single-Port laparoscopy procedure can be performed by a single surgeon and represents a less invasive alternative to multi-port laparoscopic procedure (David et al., 2024). It has been described in veterinary medicine since 2009 applied in different surgeries such as ovariectomy, ovariohysterectomy, criptorchidectomy, ovarian remnant syndrome, gastropexy, combining gastropexy with ovariectomy, splenectomy, laparoscopic assisted gastrointestinal surgery (Lacitignola et al., 2021).

Natural Orifice Transluminal Endoscopic Surgery (NOTES) (M. Zhang et al., 2024), is a technique that reduces external incisions, and the access is through mouth or anus, but is mostly used in veterinary in canine models for human sugery techniques.

2.4.5 Laparoscope introduction

The laparoscope is inserted into the trocar connected to the light cable and the camera. The abdominal cavity is examined, and the Veress needle insertion site is noted if used. The gas cable is disconnected from the Veress needle and connected to the newly placed trocar, and the needle is withdrawn under direct visualization. The skin incision is widened at this location if necessary and a second trocar is inserted under direct visualization. More trocars can be introduced in different number and position according to the surgical procedure (Richter, 2001). At the beginning of the procedure, it is normal for the laparoscope to fog up due to the difference in temperature in the operating room and inside the patient's body. This condensation only disappears when the laparoscope is heated or cleaned with a warm saline solution or is carefully cleaned on a tissue such as the omentum (Van Lue & Van Lue, 2009) (Evans & Taylor, 2024).

In gynecological surgery, the laparoscope is introduced close to the midline, several centimeters cranial to the umbilicus and approximately equal distance from the xiphoid process. In this position the ovaries, uterine horns, uterine body, bladder and prostate are easily visualized (Richter, 2001).

2.4.6 Animal's recumbency

Depending on the procedure, the animal will be recumbent. With the animal in the same position, we can have a better visualization of the abdominal structures if we place the operating table with different degrees of inclination.

The “Trendelenburg” position (patient in dorsal decubitus, on an inclined plane, in order to keep the head lower in relation to the body) is necessary for caudal and pelvic procedures and aims to remove the abdominal viscera from the pelvic canal, allowing a better visualization of cryptorchid testes or ovaries (E. Hofmeister, Peroni, & Fisher, 2008) (Heishima et al., 2023).

2.4.7 Hemostasis

One of the great challenges of laparoscopic surgery is the ability to achieve hemostasis. A small hemorrhage already has a detrimental effect on the visualization of the surgical field, in such a way that it can be said that controlling hemorrhage in laparoscopy is even more important than in open surgery, (Mayhew, 2009) since the cleaning of the field with sponges or suction cannot be immediate (Mayhew & Brown, 2007).

In addition to blood obscuring the surgical field, it has the ability to absorb a lot of light. Even if the light intensity is increased, the field remains dark, which limits the surgeon's ability to achieve hemostasis (Shettko & Hendrickson, 2008b).

Hemostatic agents such as gelatin sponges (Gelfoam®, Pfizer Inc., New York, USA) or oxidized regenerated cellulose (Surgicel®, Johnson & Johnson Inc., Paramus, New Jersey, USA) can be passed through the trocars and placed in sites of small hemorrhages (Culp, Mayhew, & Brown, 2009).

Electrocautery (monopolar and bipolar) and ultrasonic devices, as well as steel sutures, hemoclips, laser and extracorporeal sutures have already been used successfully in the laparoscopic sterilization of dogs, cats, birds and humans. LigaSure® (Valleylab, Covidien, USA) has been extensively evaluated in laparoscopic studies in humans as well as in horses for the removal of normal and neoplastic ovaries. LigaSure® is an effective means of achieving hemostasis in both humans and animals and is indicated for ligating arteries and veins up to 7 mm in diameter (Culp et al., 2009) (Driessen et al., 2023).

Electrosurgery, ideally bipolar or a more advanced instrument such as the LigaSure®, is the most practical technique for coagulating the ovarian vessels (Moore, 2007).

In bipolar electrocoagulation, the bipolar clamps are coupled and the electric current passes from one end (active electrode) to the other (neutral electrode). Only the tissue trapped between the forceps is coagulated. The advantages of bipolar electrocoagulation are that less current is required for the same hemostatic effect and coagulation can be performed in a wet field. The incidence of perisurgical hemorrhage decreases with the use of bipolar electrocoagulation and, when there is hemorrhage, coagulation is facilitated by the use of this technique (Van Goethem, Rosenveldt, & Kirpensteijn, 2003).

The ultrasonic scalpel consists of an electric generator, a manual device, a system of two blades (one active and the other inactive) and a pedal. The electrical energy produced by the generator is converted into ultrasound through the piezoelectric ceramic transducer in the handheld device. This small piezoelectric crystal vibrates at a frequency of approximately 55000 Hz and causes longitudinal movements against the inactive blade (Holub et al., 2002) (Düsterdieck, Pleasant, Lanz, Saunders, & Howard, 2003). The mechanical energy resulting from oscillating the blades is transferred to tissue proteins that undergo denaturation and form a clot that seals vessels less than 5 mm in diameter (McCarus, 1996). The pressure variations caused by this device allow cellular vaporization and tissue dissection (Düsterdieck et al., 2003) (Dusterdieck K. F., 2003) (McCarus, 1996).

It has already been proven that the use of these devices reduces surgical time, which is an advantage in procedures that are time-consuming (Culp et al., 2009). It is known that maintaining an intracavitary CO_2 pressure of 10 to 12 mmHg during the inspection stage of the abdominal cavity can mask the observation of hemorrhages. Some authors state that small hemorrhagic vessels may not be observed at a pressure of 15 mmHg, however they may be the cause of significant hemorrhage after cavity deflation. In humans, at the end of the laparoscopic procedure, it is indicated to decrease the intracavitary pressure to 5 mmHg, to evaluate the occurrence of hemorrhages. Based on these statements, it is advisable to apply this decrease in pressure during ovariohysterectomies in dogs (Hellyer et al., 2007) (Brun, FILHO, Beck, MARIANO, & Mello, 2000).

At the end of the surgery, the entire interior of the abdominal cavity should be observed to detect any accidental trauma or hemorrhage. These situations are resolved, all the instruments are removed from the cannulas and the abdomen is emptied by opening the valves in all the cannulas. Slight pressure is applied to the wall to "force" the gas out, the cannulas are removed and the incisions are closed with simple stitches in two or three layers (abdominal wall, subcutaneous tissue and skin) (Richter, 2001).

2.4.8 Complications of laparoscopic surgery

The laparoscopic approach presents immediate complications related to visceral, vascular and hemorrhagic injuries caused by the “blind” introduction of the Veress needle or the first trocar (David et al., 2024).

The occurrence of complications makes it difficult to visualize the abdominal cavity or even makes the surgical procedure unfeasible.

Bleeding can be minimal and self-limiting when small vessels are injured, while bleeding from larger vessels requires immediate action such as cauterization, application of clips, ligatures, use of vasopressor agents or even conversion to laparotomy (Malm et al., 2004).

Deaths resulting from bleeding during laparoscopic surgery have been reported in both humans and dogs (Brun et al., 2000). Visceral lesions (bladder, intestines, stomach, spleen, liver and ureters) are considered rare. They may occur during the blind introduction of the Veress needle or the trocar, during dissection maneuvers or due to the incorrect use of surgical instruments (Malm et al., 2004).

Insufflation with CO_2 to create pneumoperitoneum can have the following complications: hypercapnia, cardiac arrhythmias, depression of cardiac output, pulmonary impairment, air embolism, gastric reflux and increased intracranial pressure. The effects of pneumoperitoneum, the increase in intraperitoneal pressure and the decrease in residual functional capacity, as well as the complications caused by CO_2 (hypercapnia, respiratory acidosis and hypoxia) can be minimized with ventilation assisted by positive pressure (Quandt, 1999).

2.4.8.1 Hypercapnia

Absorption of carbon dioxide into the blood may contribute to the occurrence of hypercapnia (Rawat, Modi, & Sharma, 2024) . Hypercapnia increases the risk of arterial hypertension, tachycardia, cardiac arrhythmias, vasodilation, and myocardial depression. Pneumoperitoneum and hypercapnia can increase sympathetic tone, leading to an increase in circulating catecholamines and consequently to vasoconstriction, elevated central venous pressure and increased cardiac chronotopia, inotropy and sympathetically mediated arrhythmias. Plasma concentrations of cortisol, prolactin and glucose also increase in response to the stress caused by abdominal distension (Quandt, 1999).

2.4.8.2 Cardiovascular changes

In dogs and humans, peritoneal space insufflation depresses stroke volume, cardiac output, and inferior vena cava flow by up to 60% (Quandt, 1999). The degree of hemodynamic changes is directly dependent on the patient's intravascular volume. Hypotension will be more severe in hypovolaemic animals and therefore it is important to maintain proper hydration. Administration of 35 crystalloids at a dose of 10 to 20 ml/kg will increase intravascular volume and help minimize cardiac changes. Hemodynamic changes at laparoscopy are well tolerated in healthy patients, but patients with a compromised cardiac condition are very sensitive to any hemodynamic changes (Scharf, 2024).

2.4.8.3 Lung changes

The increase in intra-abdominal pressure and the consequent decrease in intrathoracic volume have a deleterious effect on gas exchange in the lung during spontaneous breathing. If intra-abdominal pressure is greater than 20 mmHg, it increases airway pressure, intra-thoracic pressure and the mechanical impedance of lungs and chest wall. In turn, increased chest wall impedance may have inhibitory effects on cardiac output (Kim et al., 2023) .

2.4.8.4 Air embolism

It is one of the most serious and fatal complications of laparoscopic surgery. Severe hemodynamic and respiratory compromise may arise if large amounts of CO_2 enter the central venous circulation from vessel lacerations caused by accidental trauma, if splenic venous flow is decreased by excessive intra-abdominal pressure (20 to 40 mmHg), or if there is vasoconstriction peripheral.

Clinical signs of air embolism are a sudden and profound decrease in blood pressure, cardiac arrhythmias, heart murmurs (eg, mill type), cyanosis, pulmonary edema, and an increase in the final tidal volume of CO_2 as the gas embolizes. This is followed by an abrupt drop in final tidal CO_2 volume as right heart failure due to pulmonary hypertension and hypoxemia occurs (Quandt, 1999).

2.4.8.4.1 Emergency treatment of air embolism:

- deflate the pneumoperitoneum immediately
- place the animal in left lateral recumbency with the head below the level of the right atrium
- gain access to the central venous circulation for aspiration of gas in the heart
- prevent further gas entry
- start assisted ventilation if not being done
- start or continue rapid intravenous infusion of fluids
- cardiac massage
- if after 2 or 3 minutes all these maneuvers fail, perform a non-sterile thoracotomy and the air must be aspirated directly from the right atrium (Quandt, 1999).

2.4.8.5 Cardiovascular collapse

In addition to being caused by an air embolism, it can occur due to hemorrhage, myocardial infarction, pneumothorax, pneumomediastinum, excess intra-abdominal pressure and deep vagal reflex (Quandt, 1999).

2.4.8.6 Increased intracranial pressure

This complication is explained by the mechanical effect. Decreased or restricted outflow from the venous plexus increases pressure in the spinal canal, increases cerebrospinal fluid pressure, and consequently increases intracranial pressure. The increase in arterial PCO_2 increases cerebral blood flow which leads to increased intracranial pressure (Quandt, 1999).

2.4.8.7 Passive gastric reflux

Pnemoperitoneum may be sufficient to increase the risk of passive reflux of gastric contents. Patients with a history of diabetes due to gastroparesis or with hiatal hernia, obesity or any type of obstruction are at greater risk of aspiration of gastric contents. It is therefore important to ensure that the endotracheal tube is correctly cuffed. A gastric tube can also be used to aspirate the contents (Quandt, 1999).

2.4.9 Effects of CO_2

Laparoscopy requires adequate visualization, which is typically maintained via insufflation of the abdominal cavity with carbon dioxide (CO_2), which is a common gas for insufflation because it is inexpensive, inert, highly soluble, and easily excreted by the lungs. The adverse effects of capnoperitoneum on the cardiorespiratory system have been documented in multiple species. These effects are produced via two primary mechanisms. First, direct absorption of CO_2 into the circulation leads to systemic acidosis and subsequent vasodilation and myocardial depression. Second, increased intra-abdominal pressure causes compression of high capacitance vessels, such as the vena cava, resulting in decreased preload and stroke volume, although cardiac output is usually maintained due to a compensatory increase in heart rate (Parlier et al., 2024).

CO_2 can cause diaphragmatic and peritoneal irritation, which can lead to the presence of pain on abdominal palpation in the postoperative period (Brun et al., 2000).

Subcutaneous migration of CO_2 or subcutaneous emphysema depends on three factors: the method used to access the abdominal cavity, the intraabdominal distending pressure and the time of surgery. An open method with a skin incision smaller than that of the abdominal wall, a pressure greater than 15 mmHg and a very long surgery increase the likelihood of this complication. If subcutaneous emphysema sets in during the surgical procedure and gives rise to a pneumomediastinum or pneumothorax, the surgery must be stopped immediately (Devitt et al., 2005).

Changes in the trocar incisions such as edema, suture dehiscence, hematoma, serous, bloody or purulent secretions are complications already associated with this type of surgery (Brun et al., 2000).

2.4.10 Learning the laparoscopic technique

Learning laparoscopic surgery is challenging because it requires the spatial adjustment of changing from a three-dimensional visual field to a two-dimensional one. Depth perception and spatial orientation can be difficult for an inexperienced surgeon. The tactile sensation of open surgery is altered and a new “tissue sensation” is learned through the instrumentation. The skills needed to learn laparoscopic surgery are ability to work in a two-dimensional visual field, with a coordinated technique of dissection with both hands, ability to suture and knot laparoscopically and work with technologically advanced instruments. Spatial orientation for correct placement of the laparoscope and instruments is a learned skill (Shettko & Hendrickson, 2008a) (S. Several studies have shown that the rate of complications is higher when the surgeon is acquiring knowledge. It is therefore necessary to train these new surgical procedures to prevent the occurrence of surgical complications when this new technique is put into practice (Maia et al., 2021).

Training for laparoscopic surgery includes didactic instruction, simulator training, ex vivo organ training (Usón et al., 2010) and live patient training. The basic teaching of principles and theory can be done in classes, on the internet or with CD ROM. Reviewing surgical cases recorded on video is a good method to learn how instruments are used and how techniques are put into practice (Shettko & Hendrickson, 2008a).

2.5 Reproductive system laparoscopic surgery

2.5.1 Female

The field of gynecological surgery was one of the pioneers regarding laparoscopic therapeutic techniques. Techniques carried out laparoscopically follow essentially the same steps as the open approach but benefiting from the advantages of minimally invasive surgery (Usón Gargallo et al., 2003). Several procedures have already been described in reproductive system surgery: ovariectomy, ovariohysterectomy in healthy bitches, pregnant dogs, or carriers of ovarian and uterine diseases (Fransson & Mayhew, 2022).

On the other hand, one cannot forget the diagnostic applications of laparoscopy in the genital system, since anatomical and functional studies of said device can be carried out, diagnosis of ectopic pregnancy, collection of fluids and secretions, aspiration of cystic liquids and guided insemination by laparoscopy (Usón Gargallo et al., 2003).

Ovarian activity (presence, number, and stage of follicles), uterine size and signs of disease can be assessed. Laparoscopy is a simple method of transuterine artificial insemination in cases where the transcervical route is not feasible (Richter, 2001).

2.5.1.1 Laparoscopic Sterilization Surgical Procedures

The first laparoscopic sterilization in female dogs and cats was described in 1985 by Wildt and Lawer. Through two studies, they performed tubal occlusion with two different methods and locations. In the first study, electrocoagulation or plastic clips were applied to the mid-region of one horn and the uterotubal junction in the contralateral horn. In the second study, they only applied electrocoagulation at the uterotubal junction next to the ovarian pouch in both horns (Wildt & Lawler, 1985).

Tubal occlusion is a process that prevents egg fertilization by temporarily or permanently breaking the permeability of the uterine horns. There are several methods: monopolar electrocoagulation - causes great tissue damage, necrosis if there is loss of blood supply and is incompatible with the recanalization of the uterine tubes -; bipolar electrocoagulation – here the tissue destruction is localized and the entire tube is sectioned -; placement of metallic or plastic clips – the clips occlude the lumen of the tube, preventing fertilization and can be removed, therefore constituting the best method if we want to reverse the process (Usón Gargallo et al., 2003).

Tubal occlusion is a method that may be irreversible and considered as an alternative to ovariohysterectomy or ovariectomy (Wildt & Lawler, 1985). This technique is not recommended because, in addition to having animals that develop pyometra, only conception is avoided by maintaining sexual behavior (Van Goethem et al., 2003).

Ovariectomy (OVE) or ovariohysterectomy (OVH) is usually performed through three incisions in the midline, with three trocars, one cranial to the umbilicus for the introduction of clamps, one trocar caudal to the umbilicus for the laparoscope, and a third trocar between the umbilicus and the pubis for instruments.
The simplest technique is an ovariectomy, in which each ovary is removed from the abdominal cavity by a trocar, although some surgeons prefer an ovariohysterectomy. Studies indicate the latter as a more difficult procedure and that often the dissection of the uterine body is performed extracorporeally (Moore, 2007).

2.5.1.1.1 Laparoscopic Ovariohysterectomy

Indications similar to indications for open ovariohysterectomy.

2.5.1.1.1.1 Technique

As in conventional surgery, there are numerous techniques that can be used. They can be divided into two groups: assisted or semi-closed and closed. The assisted technique combines the advantages of minimally invasive surgery with the possibility of more complex procedures being performed extracorporeally. Procedures such as gastropexy are now routinely performed by assisted laparoscopy. A description of the technique is: placement of two trocars, using a laparoscope with an instrument channel, bipolar electrocoagulation to coagulate, dissect and cut the structures, using an operating table that allows rotation of the animal, placing it in right and left lateral decubitus (Devitt et al., 2005).
According to Austin *et al*, the technique can have the following sequence: insert three trocars into the abdominal cavity - one umbilical and two paramedial - with the animal in dorsal decubitus. With a Babcock forceps on the left trocar, separate the intestines and clamp and lift pulling the suspensory ligament of the right ovary with forceps placed on the right trocar. Electrocoagulate or suture this ligament, the broad ligament of the uterus and the ovarian pedicle and then transect the whole to the uterus. Section the uterus and uterine artery from right to left. Cut the uterus and seal it one centimeter from the cervix, coagulating and then cutting the right uterine artery. Raise the left uterine horn to visualize it and the ovary. Coagulate the broad ligament of the uterus, the ovarian pedicle and the suspensory ligament and transect in the reverse order to that performed on the right side. Enlarge the left paramedian incision to remove the organs and

observe any signs of hemorrhage, if not observed, remove the trocars and suture the incisions (Fossum, 2007).

Suspension of the ovaries for visualization of the ovarian pedicle can be performed with a transabdominal suture that crosses the abdominal wall, the mesovarium and the abdominal wall again. This suture allows: to eliminate a trocar that would be placed to enter the clamp that would perform this suspension and eliminates the need for an assistant to support this clamp (Rosin, Kuriansky, Rosenthal, Brasesco, & Ayalon, 2001). Various equipment can be used: ultrasonic scalpel, vascular clips and bipolar electrocoagulation clamps that coagulate and/or cut. The electric scalpel is expensive and difficult to use while electrocoagulation devices are safe, less expensive and reduce the material to be used because they cut and coagulate simultaneously (Devitt et al., 2005). The application of clips and the use of sutures increases surgical time, the material to be used and the surgeon's movements (Mayhew & Brown, 2007).

2.5.1.1.1.2 Benefits

It has been reported that the animal has little post-surgical pain when using this technique (Davidson et al., 2004). Minami et al reported the treatment of pyometra with this technique and suggested that laparoscopic OVH may be superior to the conventional one because the incision is much smaller, the elevations of interleukin-6 and C-reactive protein are considerably less, which demonstrates that tissue trauma was lower, the animals have less post-surgical pain and return to normal activity more quickly (Minami et al., 1997). In highly demographic, urban and industrialized areas, a birth control program is needed to reduce the length of hospital stay, the cost of surgery and the appearance of post-surgical complications. According to Maiti, OVH with clips and electrocoagulation are successfully applied techniques for mass reproductive sterilization (Swapan Kumar Maiti & Dutta, 2008).

2.5.1.1.1.3 Complications

Subcutaneous emphysema, rupture of the uterine horn, loss of clip in the abdominal cavity, breakage of instruments inside the abdominal cavity, hemorrhage, perforation of the viscera (the spleen and bladder are most frequently mentioned), conversion to open surgery and death (Brun et al., 2000).

There is a risk of collateral thermal injury from the use of electrocoagulation when structures are transected too close to the abdominal wall (Devitt et al., 2005).

2.5.1.2 Laparoscopic ovariectomy

One of the first references to laparoscopic ovariectomy dates to 1996 (Malm et al., 2004).

2.5.1.2.1 Indications

Where there is no specific indication to remove the uterus.

2.5.1.2.2 Technique

Animal preparation is similar to that required for laparoscopic OVH with regard to recumbency, trocar placement, instruments for dissection, cutting, and hemostasis. The surgery consists of identifying the ovaries, coagulating and cutting the suspensory ligament, proper ligament and ovarian pedicle, extracting the ovaries from the abdominal cavity and closing the incisions. The possibility of tilting the operating table to expose the ovaries is extremely useful, whether you want to lower your head 15 degrees, which is called the Trendelenburg position, or sideways up to 45 degrees. After identification of the ovary, it is captured and then the ovarian pedicle is dissected. With practice and good patient placement this procedure can be completed in 30 minutes or less (Moore, 2007). A technique has already been described in which only a trocar with an instrumentation channel and a transabdominal suture was used (Dupré et al., 2009).

2.5.1.2.3 Benefits

It is a fast, minimally invasive technique and allows excellent visualization of the genitourinary tract. Compared to laparoscopic OVH, it is faster, easier and no enlargement of incisions is required to remove the uterus (Van Goethem et al., 2003).

It has already been shown to be a safe and effective technique in dogs, horses, cows, llamas, rhesus monkeys and humans (van Nimwegen & Kirpensteijn, 2007).

It is the technique of choice for sterilization in the Netherlands. It has multiple advantages over the conventional one: less invasive, better visibility of abdominal structures, smaller incisions, less post-surgical pain, less adhesion formation and shorter recovery times (Van Nimwegen, Van Swol, & Kirpensteijn, 2005).

Bipolar electrocoagulation has many advantages when applied to ovariectomy: it shortens surgery time, shortens the time to remove the ovaries, decreases the incidence of perisurgical bleeding, and facilitates clotting if bleeding occurs (Goethem, Rosenveldt, & Kirpensteijn, 2003).
A study showed that this technique in dogs is associated with beneficial effects on oxidative stress status compared with open procedures during surgery (Lee & Kim, 2014).

2.5.1.2.4 Complications

Several complications resulting from laparoscopic ovariectomy can be listed, namely: subcutaneous emphysema, loss of clip in the abdominal cavity, breakage of instruments inside the cavity, bleeding, perforation of the viscera (most commonly referred to are the spleen and bladder), conversion to open surgery and death (Brun et al., 2000).
There is a risk of collateral thermal injury from the use of electrocoagulation when structures are transected too close to the abdominal wall (Devitt et al., 2005).
Surgical difficulty increases in obese patients and in the presence of highly vascularized ovarian tumors (Usón Gargallo et al., 2003).
There is a risk of losing the ovaries within the abdominal cavity after being transected (Dupré et al., 2009).
When applying vascular clips to the ovarian pedicle, it has to be divided into smaller segments so that they can fit the width of the clips. In obese animals this material is more difficult to apply, with a greater probability of hemorrhage. A suture is applied to the pedicle when clips or staples cannot be applied or when there is bleeding. However, the pedicle must resist the tension caused by the knots. Endoscopic loop sutures increase the surgical budget and the risk of complications such as: trauma to friable adipose tissue, destruction of the ovarian pedicle and accidental ligation of the ureter (Van Goethem et al., 2003). The use of the laser produces smoke that decreases visibility within the abdomen and does not prevent hemostasis of the mesovarium (Van Nimwegen et al., 2005).

2.5.2 Male

Techniques carried out laparoscopically follow essentially the same steps as the open approach but benefiting from the advantages of minimally invasive surgery (Usón Gargallo et al., 2003). Several laparoscopic procedures have already been described in reproductive system surgery: orchiectomy in cryptorchid dogs, occlusion of the vas deferens in male dogs and total prostatectomy (Brun & Beck, 1999).

2.5.2.1 Laparoscopic Sterilization Surgical Procedures

To date, there are few references regarding laparoscopic sterilization (LS) of male dogs with descended testis. The first study on this used the occlusion of the ductus deferens, resulting in rapid azoospermia but maintained libido and mating behavior. The development of this technique had the goal of sterilizing the male animal without changing its physical characteristics or endogenous hormone secretion (Mayhew & Brown, 2007). Other studies described a technique in which a segment of the vas deferens was cauterized and resected with monopolar grasping forceps (Jana & Samanta, 2007) or a laparoscopic-assisted colopexy and sterilization, where both vessels and the deferens were double clipped and then coagulated, and the tissues in the area between the clips cut (Mathon et al., 2011). Other report of a laparoscopic sterilization technique was performed by Mahalingam *et al.*, where besides cauterizing and resection of 2 to 3 cm of vas deferens, the spermatic artery–vein plexus was double clipped (Mahalingam et al., 2014).

2.5.2.2 Laparoscopic vasectomy

2.5.2.2.1 Indications

Laparoscopic vasectomy has the same indications as the open technique with the advantage of being minimally invasive.

2.5.2.2.2 Technique

Animal preparation is similar to the required for laparoscopic in female dogs with regard to recumbency, trocar placement, instruments for dissection, cutting, and hemostasis. The surgery consists of identify the vas ductus deferens, its cauterization and removal (Wildt, Seager, & Bridges, 1981).

2.5.2.2.3 Benefits

Due to the time required for healing of surgical wounds could be particularly advantageous for the sterilization of stray or abandoned animals (Wildt et al., 1981).

2.5.2.2.4 Complications

The laparoscopic approach presents immediate complications related to visceral, vascular and hemorrhagic injuries caused by the “blind” introduction of the Veress needle or the first trocar (Malm et al., 2004).

2.5.2.3 Laparoscopic castration

Animal preparation is similar to that required for laparoscopic in female dogs with regard to recumbency, trocar placement, instruments for dissection, cutting, and hemostasis.

2.5.2.3.1 Indications

Laparoscopic castration has the same indications as the open technique with the advantage of being minimally invasive.

2.5.2.3.2 Technique

The surgery consists of identifying the vas ductus deferens and spermatic artery vein plexus. Coagulating the vas ductus deferens multiple times and cut. Oclusion of the ductus deferens were considered complete when the structure was coiled and blanched. After resection and removal of vas ductus deferens, the spermatic arteryvein plexus were identified. Clipping or cutting of spermatic artery-vein plexus should also be performed.

2.5.2.3.3 Benefits

Due to the time required for healing of surgical wounds could be particularly advantageous for the sterilization of stray or abandoned animals (Wildt et al., 1981).

2.5.2.3.4 Complications

The laparoscopic approach presents immediate complications related to visceral, vascular and hemorrhagic injuries caused by the “blind” introduction of the Veress needle or the first trocar (Malm et al., 2004).

2.6 Pain

2.6.1 Introduction

The most widely accepted definition, proposed by the International Association for the Study of Pain, describes pain as an unpleasant sensory or emotional encounter linked to actual or potential tissue damage, expressed in terms of that damage (Loeser & Treede, 2008).

There are several types of pain such as neuropathic, maladaptive, dysfunctional, nociceptive, and inflammatory. It can be of short duration (postoperative pain that subsides with recovery) or long term; superficial or deep, and somatic or visceral in origin. Contrary to humans, animals cannot be conforted by the knowledge that pain will subside or improve (Sharkey, 2013).

Pain in the fourth vital sign and should be incorporated into the TPR (temperature, pulse, respiration) assessment of everys patient physical examination (B. P. Monteiro et al., 2023).

As part of the customary medical protocol, veterinarians are required to incorporate measures to alleviate pain and distress in their patients (Holton, Pawson, Nolan, Reid, & Scott, 2001). The initial stage involves the identification of this condition in order to establish an accurate diagnosis and recommend appropriate analgesic therapy. The assessment of animal pain can be complicated by their survival instinct, cats and dogs may hide their pain as a protective mechanism, such as wagging its tail although being in pain (Hellyer et al., 2007).

Consequently, specialized tools and protocols have been developed to assess acute pain (Bleijenberg, van Oostrom, Akkerdaas, Doornenbal, & Hellebrekers, 2011; Boselli, Logier, Bouvet, & Allaouchiche, 2016; Mansour et al., 2017), resulting in enhanced understanding of nociceptive pathways and mechanisms that can aid veterinarians in addressing pain proactively (preemptive analgesia).

Regarding the stimulation experienced during surgery, the primary aim is to ensure sufficient pain relief throughout the procedure in order to mitigate potential risks that may impede the patient's recovery (Kehlet, Jensen, & Woolf, 2006; KuKanich, Bidgood, & Knesl, 2012; Rodgers et al., 2000). This is essential not only due to ethical considerations concerning animal well-being but also to guarantee the effectiveness of surgical interventions. Inadequate pain management leads to unnecessary suffering, predisposes patients to medical complications, prolongs hospital stays, delays the recovery process, and escalates surgical costs. Hence, it is imperative to prevent pain by implementing optimal pharmacological and/or physical interventions that target the specific source of pain (Duncan, 2008; Watkins & Maier, 2000).

2.6.2 The neurobiology of acute pain

Due to the intricacies of pain assessment and the various aspects and variations in animal behavior when experiencing pain (Holton et al., 2001) (Morton, Reid, Scott, Holton, & Nolan, 2005), it is now understood that the neuronal processes involved in encoding and processing potentially harmful stimuli to tissues (nociception) encompass a multidimensional experience that includes sensory, cognitive, and emotional components (Price, 2000) (Grimm et al., 2015).

2.6.2.1 Neurophysiology processes

The underlying pathophysiological mechanisms of nociception (i.e., the neurophysiological processes of pain) are similar across mammals, which are susceptible creatures capable of experiencing pain. Furthermore, observations indicate that both animals and humans undergo comparable neuronal processes involved in the recognition, transmission, and modulation of pain (Hellyer et al., 2007) (Karol Mathews et al., 2014) (Rutherford, 2002) (Weary, Niel, Flower, & Fraser, 2006). Similar to other sensory and alarm systems, nociception comprises mechanisms that allow the perception of pain to be received at some peripheral level (such as skin, viscera, or skeletal muscle) and subsequently transmitted to the central nervous system (CNS), where it is processed and consciously integrated (at spinal and supraspinal levels) (Romera, Perena, Perena, & Rodrigo, 2000) (Zegarra Piérola, 2007). Throughout this process, peripheral responses to tissue damage involve the local release of substances like histamine, prostaglandins, hydrogen ions, and potassium (Zegarra Piérola, 2007).

The neurophysiological process of pain is divided into five consecutive processes once the painful or nociceptive stimulus enters in contact with the organism(W. W. 3rd Muir & Woolf, 2001). These stages are: (A) Transduction: the nociceptive stimulus is transformed into an electrical signal in the receptors (nociceptors) (Lamont, Tranquilli, & Grimm, 2000), causing peripheral changes that are recognized as indicators of pain, including redness, swelling and translucence of the skin. (B) Transmission: is the conduction of the electrical signal generated in the nociceptors through the axons of the first-order neurons, making synapsis with the second-order neurons in the dorsal horn of the spine (Gaynor & Muir, 2009). This information is transmitted through two primary afferent nociceptive neurons, the C fibres or C polymodal nociceptors (which transmit chemical, thermal, and mechanical nociceptive information), and the alfa-delta fibres (which respond to high-intensity mechanical stimuli and thus are known as high-threshold mechanoreceptors). (C) Modulation: the process through which the excitatory and inhibitory mechanisms alter the transmission of the nerve impulse (Lamont et al., 2000). (D) Projection: the nociceptive information is transported to the brain through the nervoustracts that originate in the dorsal horn, especially spinothalamic and spinoreticular (supraspinal structures) (Gaynor & Muir, 2009). (E) Perception: consists in processing and integrating the information that occurs in multiple, specific areas of the brain, such as the thalamus and cerebral cortex, where sensory characteristics are defined, including the onset, location, and type of nociceptive stimulus (Gaynor & Muir, 2009).

When tissue injury or inflammation occurs, there are release of inflammatory mediators and consequently peripheral nociceptors become sensitized and hyperexcitable. Then activation of silent nociceptors and the expansion of receptive field results in peripheral sensitization. The constant noxious input from the periphery to the spinal cord results in central sensitization. Central sensitization can occur as a result of surgery. Surgical procedures are associated with tissue damage and adaptative/acute pain (B. P. Monteiro et al., 2023) .

Surgery can change the neuroplasticity of spinal cord, leading to central sensitization (Latremoliere & Woolf, 2009)(W. W. 3rd Muir & Woolf, 2001). The mechanism behind the central sensitization is a long-term potentiation (LPT) that consists of a long lasting increase of sypnatic strength at the C-fibers synapses, as well as stimulation of glial cells and dorsal root ganglion cell body cross-talk in the spinal dorsal horn (S. Li et al., 2017) (Ruscheweyh, Wilder-Smith, Drdla, Liu, & Sandkühler, 2011)(Drdla & Sandkühler, 2008)(Ren & Dubner, 2010). In this process of LPT we know that N-methyl-D-aspartate (NMDA) receptors, the sodium channels and opioid receptors play a critical role. the sodium channels play a role on transmission of nociceptive inputs through C-fibers from the site of surgery Opioid receptors play a role in modulation and NMDA receptors are mainly responsible for the plasticity of the CNS (Babos, Grady, Wisnoff, & McGhee, 2013)(Gold & Gebhart, 2010)(Klein et al., 2007).

2.6.2.2 Physiological level

Several physiological changes (autonomic responses) occur in animals after a noxious stimulus. These changes include mydriasis (pupil dilation), tachycardia (increased heart rate), tachypnea (rapid breathing), and arterial hypertension (elevated blood pressure with increases of 20% or more). It's important to note that these measurements are taken from basal levels rather than reference values (Saritas et al., 2015)

Nevertheless, these indicators are influenced by various factors, such as anxiety and tension (Cambridge, Tobias, Newberry, & Sarkar, 2000) (Franzini de Souza, Dias, de Souza, & de Medeiros, 2018), making it unreliable to rely solely on a single physiological parameter to accurately assess discomfort (Gaynor & Muir, 2009). Nonetheless, some researchers suggest using them as a foundation for identifying this phenomenon (Hellyer et al., 2007) (Robertson et al., 2018). However, in the case of animals enduring persistent discomfort, these fluctuations are generally less apparent (Grimm et al., 2015).

Post operative pain which is acute and pathological, produces the following side effects: reduction in food intake, negative protein balance due to increased protein catabolism, suppression of the immune system, slower wound healing, lower threshold for painful stimuli (Gwendolyn & Carrol, 1996) (Flecknell & Waterman-Pearson, 2000)

Other described indicators include changes in concentrations of hormones and measurement of chemical mediators. Examples of these indicators encompass changes in plasma hormone levels such as cortisol, β-endorphins, and catecholamines, which have been regarded as indirect markers of pain (JESÚS GUTIÉRREZ BAUTISTA, 2017).

Cortisol is produced in the adrenal glands after the release of adrenocorticotrophic hormone from the pituitary gland. It increases in the blood transiently in response to stressors such as pain, excitement, fear, anxiety, chronic stress, anesthesia and surgery (Devitt et al., 2005)(Freeman et al., 2010) (Hancock et al., 2005). Cortisol is a known marker of surgical stress, it increases during surgery due to tissue damage, which prevails in abdominal surgery then in surface surgery (Evangelista et al., 2014; Fazio, Medica, Cravana, Pupillo, & Ferlazzo, 2015; Fox, Mellor, Firth, Hodge, & Lawoko, 1994; Gutiérrez-Bautista et al., 2018; Nenadovic et al., 2017). In dogs and cats, lactate levels (a biomolecule generated through cellular metabolism) have been examined, as they have traditionally been utilized as parameters to assess the extent of tissue damage following a traumatic event or a severe metabolic condition that compromises blood perfusion to the tissues. These levels are used as an indicator for prognoses. In certain cases, elevated lactate levels have been linked to pain, as they can be influenced by catecholamine production (Pang & Boysen, 2007) (Allen & Holm, 2008)(Bakker, Nijsten, & Jansen, 2013). Alpha amilase is also considered an important tool for the evaluation of pain-related stress in dogs (Kang, Park, Oh, & Seo, 2022).

Pain can increase the blood's glucose due to unbalance of the hypothalamic-pituitary-adrenal axis which impacts the adrenal glands; so this parameter is used as an auxiliary tool for accessing pain. Pain tolerance is also reduced by high blood glucose (Morley, Mooradian, Levine, & Morley, 1984). In addition the high blood glucose can rise the pain intensity (Cradock & Hawthorn, 2002) .

Serum C-reactive (CRP) protein is a sensitive and specific marker for the systemic inflammatory activity (Kjelgaard-Hansen & Jacobsen, 2011). Studies show it is a quantitative marker of the inflammatory stimulus of soft tissue surgery (Kjelgaard-Hansen et al., 2013). CRP is an acute-phase protein produced in the liver in reaction to inflammation, infection, tissue damage or neoplasia (Tobias & Johnston, 2017). CRP production is induced by inflammatory citokines such as IL-1 and IL-6 (Nishikawa et al., 2008). IL-6 may can be usefull as objective indicators of inflammation in animais and for that have been chosen to acess the role of cytokines in pain control (Tobias & Johnston, 2017).

2.6.2.3 Behavioural level

In animals, it is possible to observe a motor response to pain, leading to a range of complex behaviors that are specific to each species (Leon-Olea, 2002). Due to this, behavioral changes have been acknowledged as indicators of pain in animals (J Reid, Nolan, & Scott, 2018). This distressing emotional and sensory experience elicits alterations in behavior, providing indications of the presence, location, and severity of pain (de Grauw & van Loon, 2016). Wiseman-Orr et al. (Wiseman-Orr, Scott, Reid, & Nolan, 2006) identify several behavioral changes that should be considered in animals, including aggression, vocalizations, self-mutilation, social interaction, sleep disturbances, restlessness, and reluctance to move (lethargy) (Bonagura, Kirk, & Samperio, 1995). It is important to note that these behavioral manifestations of pain or discomfort are unique to each species and cannot be applied universally to other species.

Dogs often exhibit exaggerated responses to noxious stimuli, with aggression being a characteristic response to acute pain. However, it is more common to observe signs of depression, submission, anxious expressions, anorexia, licking of the affected area, and reluctance to move. In cases of high-intensity pain, behaviors such as vocalizations, increased tear production, constant touching of the affected area, restlessness, and protective behaviors may be observed (Franzini de Souza et al., 2018) (Landa, 2012). Dogs with acute abdominal pain may also display specific body postures, commonly known as the "prayer" position, where they raise their hindquarters while keeping their heads and front limbs on the floor (Gaynor & Muir, 2009)(Essner et al., 2017).

2.6.2.4 Emotional level

Emotions consist of four interconnected aspects. The first aspect involves the cognitive process that gives rise to feelings. The second aspect is biological and serves an evolutionary function. The third aspect encompasses physiological elements, primarily involving the autonomic nervous system and hormones. Finally, the fourth aspect is the social component, which serves a purposeful function and is expressed through behavior (Merola, Prato-Previde, Lazzaroni, & Marshall-Pescini, 2014) (Albuquerque et al., 2016). Therefore, an individual's emotional state is the outcome of the interplay between physiological activity and cognitive evaluation of the situation (Merola et al., 2014). Consequently, a mental state leading to pain reflects a low level of well-being resulting from the inability to achieve adequate adaptation (Galvan & Vonk, 2016) (Hemsworth, Mellor, Cronin, & Tilbrook, 2015).

Moreover, research has revealed that animals may exhibit changes in facial expression (Gleerup, Forkman, Lindegaard, & Andersen, 2015) in response to noxious stimuli, which can be accompanied by disruptions in behavior and physiological parameters. However, studies conducted thus far with dogs and cats have yielded inconclusive results in terms of demonstrating a direct association with pain, as these responses can be influenced by stress or undesired social interactions (Franzini de Souza et al., 2018) (Galvan & Vonk, 2016).

2.7 Evaluating pain

According to Lord Kelvin, the ability to treat pain in humans and animals depends in being well measured (Kelvin, 1893).

Quantifying pain presents a challenge as the physiological perception of this sensation involves multiple dimensions. Consequently, the complexity of identifying pain in animals arises from the variations among species, behaviors, and environments (Viñuela-Fernández, Jones, Welsh, & Fleetwood-Walker, 2007) (Ashley, Waterman-Pearson, & Whay, 2005) (Grimm et al., 2005).

Along with the absence of normal animal behaviour, examining thoroughly the location of pain and its characteristics we can diagnose the type of pain. Nevertheless, the change of the animals normal environment, such as being in the hospital, can influence the dogs normal behaviour (Sharkey, 2013).

There is the need to use different pain assessement tools to evaluate different types of pain and its different dimensions. Currently there are objective and subjective methods to assess pain. The subjective are validated and reliable pain scales, and the objective are the measurement of hormones and other molecules in blood and saliva, electrical (Kaka, Hui Cheng, et al., 2015), thermal (Hoffmann, Kästner, Kietzmann, & Kramer, 2012) and mechanical nociceptive testing (with Semmes-Weinstein von Frey filaments and with an algometer) (Ross et al., 2022).

As found in several postoperative pain evaluating studies, the use of only subjective pain scales is insufficient to detect significant differences between surgical procedures and draw valid conclusions. Therefore the evaluation of postoperative pain to be effective should use the measurement of biomarkers such as cortisol (COR), c-reactive protein (CRP) and others (Haraguchi et al., 2017).

2.7.1 Mechanical Nociceptive testing (MNT)

The von Frey filaments are used to determine tactile, mechanic parameters in order to objectively quantify nociceptive processes since the filament deformation can be transformed in in force per unit (g) parameter, giving this pain evaluation an objective caracter (Valadão, Mazzei, & Oleskovicz, 2002). The method consists of applying mechanical pressure using the smallest filament (0,008g – 0,00008Pa) a couple centimeters from the incision at three locations: the cranial, middle and caudal aspect of it. Filaments are also applied over the caudodorsal flank just cranial to the ilial wing to assess the dogs natural response to the MNT. If enough pressure is applied to bend the filament without the animal responding, a sequential heavier filament is used to repeat the process, up to 300g (2,94Pa) until a positive response is provoked, such as vocalization, head turning, or

attempt to bite. Finally this evaluation is performed in triplicate in each location and scores are averaged (Ross et al., 2022).
The limitation of this evaluation is that the pain assessed is superficial, around the incision, as such it is less sensible to the evaluate the pain associated with the muscular wall and the abdominal organs (Pohl, Carregaro, Lopes, Garlet, & Marques, 2011).
The algometry is a similar methodology instead of filament it uses a probe over testing points in the animal body in a gradual incrising force to produce noxious stimulus. The force applied to the probe is transmitted to a load cell and a voltage output is produced. The signal is transformed, and the output is displayed in newtons or kilograms (Kaka, Chen, et al., 2015). It has been used to evaluate the efficacy of analgesics in postoperative pain (L. Slingsby & Waterman-Pearson, 2000). This method can stimulate both the Aδ and C fibers, which are responsible for encoding clinical pain (Le Bars, Gozariu, & Cadden, 2001).

2.7.2 Scales for evaluating pain

The literature encompasses various scoring methods that incorporate both physiological and behavioral variables (Grimm et al., 2005). In veterinary medicine, the pain scoring methods employed for domestic animals are adapted versions of scales initially developed for humans (Morton et al., 2005). The use of these scales has demonstrated several advantages in clinical veterinary practice. The implementation and validation of an instrument specifically designed to evaluate pain significantly enhance pain management by utilizing the scores obtained during assessment. These scores serve as indicators of the level of pain and the necessary analgesic treatment (Jacky Reid et al., 2007).

2.7.2.1 Subjective or unidimensional scales

When pain is considered solely as a subjective experience, valid measurement attempts to access the individual's subjective perception. However, the subjective nature of pain poses a significant limitation in this approach since the observer must rely on their impressions and experiences to assign a degree of pain. To address this limitation, it is suggested that the same evaluator performs all procedures of pain assessment for consistency (J Reid et al., 2018).

2.7.2.1.1 Preventive scoring systems

This system involves the evaluator anticipating the level of pain the animal will experience following a specific procedure. The evaluator assigns a score based on their belief of the pain level, using categories such as none, slight, moderate, or severe. The main disadvantage of this scale is that it does not assess the individual pain level of each patient, but it offers the advantage of simplicity and the ability to quickly plan a preventive analgesic strategy (Gaynor & Muir, 2009).

2.7.2.1.2 Simple descriptive scales (SDS)

Simple descriptive scales involve predefined categories or degrees of pain assigned with corresponding numerical values for statistical analysis. For example, 0 may represent no pain, 1 a medium level, 2 a moderate level, and 3 a severe level. Some scales may use 4 or 5 categories. It is important to note that as the number increases, the difficulty in accurately assigning the pain semiology to each category also increases. One drawback of such scales is their limited sensitivity in detecting subtle changes in pain intensity (Grimm et al., 2015).

2.7.2.1.3 Numerical rating scales (NRS)

The numerical rating scale involves a horizontal line divided into ten segments numbered from '0' to '10'. The value '0' represents the absence of pain, while '10' indicates the worst possible pain. This scale does not allow for decimal scores between the integers, making it a "discontinuous" scale with potential statistical errors. However, its advantage lies in considering various parameters, including physiological aspects, locomotor activity, and vocalizations (Grimm et al., 2015).

2.7.2.1.4 Visual analog scale (VAS)

Visual analog scale uses a 100-mm line with two endpoints: 0 mm representing the absence of pain and 100 mm representing maximum pain. The evaluator marks the point on the line that corresponds to the perceived level of pain experienced by the animal. The score is determined by measuring the distance from the initial point (0) to the marked spot. The linearity of this scale has been subject to debate, as it raises uncertainty regarding the proportional relationship between different intensity values. For instance, a mark of 60 mm may not necessarily indicate precisely twice the amount of pain compared to a mark made at 30 mm (JESÚS GUTIÉRREZ BAUTISTA, 2017). Holton et al. applied these three methods (NRS, SDS, VAS) to assess pain and found significant variability of 29-36% in the results depending on the observer (Holton et al., 2001). In response to this issue, Lascelles et al. introduced an improved version of the scale that has been used in various studies (Lascelles, Cripps, Mirchandani, & Waterman, 1995) (L. S. Slingsby, Jones, & Waterman-Pearson, 2001) (Morgaz et al., 2013). Their approach includes a dynamic and interactive assessment of patients (DIVAS), which involves observing the animal from a certain distance (DIVAS I), approaching and interacting with the animal (DIVAS II), and finally, auscultating the injured area (DIVAS III). When this pain assessment scale is applied, and the score for the animal is ≥40 mm, it indicates the need for rescue analgesia.

2.7.3 Objective or multidimensional scales

While subjective scales assess only one aspect, such as behavior or physiological parameters, multidimensional scales take into account both aspects. Validated pain scales that demonstrate reliability and responsiveness are essential to the recognition and management of pain in dogs (Sharkey, 2013).

2.7.3.1 Glasgow Composite Measuring Pain Scale (CMPS)

The Glasgow Composite Measuring Pain Scale is currently validated for assessing acute pain in dogs. It is a scoring system primarily based on behavior (Holton et al., 2001). The CMPS employs a structured questionnaire filled out by an observer following a standardized protocol. The questionnaire covers evaluations of spontaneous behavior, interactions with the animal, and clinical observations. The scale comprises seven assessment items, including behavior and reactions towards people, posture, mobility, activity, response to auscultation, treatment of the painful area, and vocalizations. Specific questions within each item are selected by the observer based on the description that best reflects the observed behavior in the animal. While other composite scales have been developed, the CMPS is specifically designed for veterinary science, employing psychometric principles to establish a procedure for selecting relevant items. For practical use in the dynamic environment of daily clinical practice, the questionnaire must be concise, simple, and require minimal time. Reid et al. published a simplified version of the scale known as the Short Form of the Glasgow Composite Measuring Pain Scale (SF-CMPS), which prioritizes practicality and specifies the score threshold for determining the need for analgesic treatment. The total score on this scale ranges from 0-24 if the animal is mobile, or 0-20 if mobility cannot be assessed. If the animal's score is ≥6 on the former or ≥5 on the latter, rescue analgesia is necessary. (Jacky Reid et al., 2007).

2.7.3.2 University of Melbourne Pain Scale (UMPS)

The University of Melbourne Pain Scale was originally developed as a multidimensional scale for assessing pain in dogs, taking into account their behavior and physiological constants as the basis of evaluation (Firth & Haldane, 1999). This scale incorporates six variables: physiological constants (heart rate, respiratory rate, rectal temperature), response to auscultation, activity level, emotional state, posture, and vocalizations (Brainard & Snyder, 2015). After observing the animal, the evaluator assigns a value to each variable based on the scale. This tool provides higher precision than unidimensional scales, with increased specificity and sensitivity due to the consideration of multiple factors. However, a disadvantage is that knowledge of typical animal behavior before undergoing a surgical-anesthetic procedure is required, making it less applicable for sedated animals (Saritas et al., 2015). Based on the scores obtained from this assessment, pain can be categorized as slight (1-5 points), moderate (6-13), severe (14-21), or unbearable (21-27). Rescue analgesia is necessary when the animal's score is ≥10.

2.7.3.3 Colorado State University Feline and Canine Acute Pain Scale

The Colorado State University Feline and Canine Acute Pain Scale is a specific scale designed for evaluating pain in both dogs and cats. It incorporates appropriate descriptions selected in the form of checkboxes and considers the following components: psychological/behavioral aspects and response to auscultation. A third component assesses body rigidity, which is evaluated using a subjective scale known as the Simple Descriptive Scale (SDS). The scale includes visual diagrams to assist evaluators in identifying the level of pain based on animal posture. Furthermore, diagrams illustrating various body positions are provided to help evaluators mark the areas where the animal exhibits signs of pain, tension, or increased temperature (Seymour, Duke-Novakovski, & Mendenhall, 2008).

2.7.3.4 Free Choice Profiling (FCP)

This is a methodoly of integrative animal welfare measurement, firstly used in food science (Dijksterhuis & Punter, 1990; GONZÁLEZ-VIÑAS, MOYA, & CABEZUDO, 2003; GUERRERO, GOU, & Arnau, 1997; Piggott, Paterson, Fleming, & Sheen, 1991) and later in qualitative behavioral assessment (QBA) in animals (Wemelsfelder, Hunter, Mendl, & Lawrence, 2000). It evaluates the emotional statues of the animal by focusing on how the animal does what he does, instead of what the animal does. The users of this methodology are allowed to generate their own words to describe the emotional expressions of the observed animal, estimulating the individual interpretation instead of using a fixed list of terms. This evaluation is made by watching the animal inside a cage, then the animal exiting the cage and being put on a leash, and at last the animal being gently palpated from the non wounded area, starting in the limbs over to the wound. In a study, an excellent correlation was found between the QBA assessment and the GCPS-SF, suggesting that the first could be applied to pain assessment (Zanusso, Contiero, Normando, Gottardo, & De Benedictis, 2024).

2.7.3.5 Automated measurement of movement

Video recording of animals has been used to evaluate their movements after surgery, detecting where each subject spends its time, its spatial relationship to objects in the environment, frequency/duration of movement, distance moved, velocity, speed moving to/from other animals or objects. Two 24 hour recording sessions are made, the first of the healthy dog and then another after surgery in two different days. The behavioral observation software uses an automatic video tracking system to evaluate the movements of animals inside their cage. The comparison between the two sessions can help determine which analgesic protocol produces less deviation from the control visit behaviour (Bernie D Hansen, 2003). The difficult applicability of this protocol in the clinical practice might be the reason it is not currently used. Using video of animais facial expressions, body pose and posture as been suggested to allow computer pain estimation in animals. It poses a challenge as different dog breeds have different appearances, and different behavioral coping mechanisms. (Hongyi Zhu, Salgırlı, Can, Atılgan, & Salah, 2023)

2.7.3.6 Accelerometers

Miniature portable accelerometers have the ability to give information about the active movement of the animal enabling the production of reliable mobility information. Nonetheless more data is necessary about their performance across breeds and ages of dogs, to determine the correlation between activity and pain, and what degree of change in activiy translates into a significant aleeviation of pain (Bernard D Hansen, Lascelles, Keene, Adams, & Thomson, 2007) (Wernham et al., 2011).

The evidence suggests that behaviour classification is more successful when the motion sensor is attached to the harness at the back of the dog rather from the sensor attached to the collar (Kumpulainen et al., 2021).

2.7.3.7 PainTrace

PainTrace is a noninvasive device that measures acute and chronic pain using skin-based electrical activity as an indicator. It is a small device about the size of a deck of cards that is attached by a cable to two small metal sensors created to measure a novel biosignal, skin-based electrical activity, much like an ECG that is indicative of pain within the body. The sensors are applied to the clipped in skin in the axillary region with an adhesive, acquire a systemic signal comprising the whole body, regardless of the suspected pain localization. the device communicates wirelessly with an iPad equipped with PainTrace software, allowing for real time electrical readings that determine a patient's "pain score," which is a cumulative measurement of pain throughout the patient's body. On the iPad, users will see a graph with a line tracing of the pain score of the patient and how it changes (if applicable) as the patient moves or when certain parts of the body are touched or manipulated. This helps veterinary professionals to localize the pain and determine its severity (Dullen L.; Mohanty, M., 2018). It has several clinical applications such as detection of acute pain during nail clipping, dental cleaning, pre and post-operative pain assessment in dogs and equines (Cooley, 2023).

2.8 Pain in laparoscopic surgery

In humans, three types of pain have been described after laparoscopic surgery: somatic pain derived from the incision of the skin, visceral pain from the trauma in the visceral organs and from the irritation of the CO_2 pneumoperitoneum, and the third kind from shoulder pain, caused by the stretching of the phrenic nerves (Case, Marvel, Boscan, & Monnet, 2011) also from the pneumoperitoneum (Bisgaard, Klarskov, Rosenberg, & Kehlet, 2001)(Tsai et al., 2011).

This type of neurologic discomfort is not reported in the veterinary literature, however abdominal wall lift (AWL) laparoscopy has been suggested as an alternative modality to minimize this source of pain (Fransson, Grubb, Perez, Flores, & Gay, 2015) (Fransson & Ragle, 2011). In a study by Fransson et al. the degree of pain experienced in both $STCO_2$ and AWL was similar, suggesting that stretching of the peritoneal tissues is responsible for laparoscopic postoperative discomfort (Fransson et al., 2015).

The number of cannulas used, the type of surgical procedure, and the surgeon's experience also influence the severity of postoperative pain (Leggett, Churchman-Winn, & Miller, 2000).

The use of scissor and bipolar coagulation provokes broad intraperitoneal thermal damage. Evidence from models of pain from inflammation, show that the liberation of nitric oxid and excitatory amino-acid and the release of prostaglandin E2 play an important role in the development of peripheral tactile and thermal hypersensivity (Seybold, Jia, & Abrahams, 2003)(Ma & Eisenach, 2002).

The degree of perioperative pain can be influenced by the surgical technique and location. Gentle tissue handling and techniques that minimize trauma like small incisions: minimally invasive surgery such as laparoscopy should be employed whenever possible (Monteiro BP, et al.,2023).

Ovariohysterectomy postoperative pain in considered as of moderate category (Carpenter, Wilson, & Evans, 2004) (Bleedorn, Dykema, & Hardie, 2013).

2.8.1 Management of pain

Analgesia depends on decreasing the signs of ascending nociception, since inhibiting nociceptive transmission prevents the perception of a harmful stimulus; however, inadequate analgesia leads to the activation of brainstem structures which coactivate areas that induce cortical excitement [88,89]. It is important to consider that the capacity of response will depend not only on the level of analgesia and hypnosis, but also on the type and magnitude of the stimulus (March & Muir, 2005).

The latest guidelines and recommentations for perioperative pain management in laparoscopic gynecological surgery in humans are the following: First, the combination of three or more interventions or regional techniques combining local anesthetics with adjuvant drugs reduce postoperative pain and opioid consumption within 24 hours, with better efficacy and longer duration. Second, for combination therapies, regional techniques including peripheral nerve blocks (PNBs) and intraperitoneal local anesthetics (IPLAs), in combination with nonopioid systemic analgesics, or combining local anesthetics with adjuvant drugs are recommended for better efficacy. The basic analgesics commonly used in such combination therapies include paracetamol (PCT) and/or NSAIDs. Third, PNBs are the most effective monotherapy intervention in reducing postoperative pain within 24 hours, with the ultrasound-guided quadratus lumborum block (QLB) being the most effective techniques. Dexmedetomidine was found to be the most frequently used adjuvant in regional analgesic techinques. Fourth, dexmedetomidine, ketamine, and NSAIDs were the most effective nonopioid single-drug interventions in reducing postoperative pain and opioid consumption withn 24 hours (Ding et al., 2023).

Multi-modal analgesia consists of the use of pharmacological and non-pharmacological therapies. Because they target different pain mechanisms to produce a sinergistic effect (Meunier et al., 2019), lower doses of each drug can be administered, minimizing the occurrence of adverse effects. Non-pharmacological therapies should be added to the pain management protocol whenever practicable (B. P. Monteiro et al., 2023). This method removes the over reliance on pharmacotherapy (Bradbrook & Clark, 2018).

In a healthy animal for an elective laparoscopic procedure the standard anesthetic drug plan consists of an opioid used for premedication to provide analgesia and some sedation. Additionally, a sedative or tranquilizer is used if the animal is excited or fractious. To conteract the opioid bradycardia an anticholinergic is administered (Fransson & Mayhew, 2022). Premedication is administered in small animal practice to calm the animal before induction of anesthesia, allowing procedures such as intravenous catheterization. Is reduces fear and anxiety, contributes to an balanced anesthetic technique by providing extra analgesia, counteracting the side effects of medications and allowing a smooth and quiet recovery (Seymour et al., 2008)(Rankin, 2017).

For anesthesia induction propofol or other are used and facilitate intubation. After intubation, the anesthesia is maintained with volatile anesthetic such as isoflurane or sevoflurane. A NSAID and a local anesthetic infiltration at portal sites plus postoperative opioids provide additional analgesia (Fransson & Mayhew, 2022).

2.8.1.1 Opioid analgesics

Opioids are used as part of multimodal and preventive analgesia, for its sparing effects of inhalant anaesthetics in perioperative situations and for postoperative analgesia when administered via epidural (like morphine). Its administration intravenous and intramuscular is preferred (B. P. Monteiro et al., 2023).

Opioids bind to opioid receptors (mu, kappa, delta, nociception and their subtypes) in the central and peripheral nervous systems inhibiting release of excitatory neurotransmistters from afferent fibres in the spinal cord, thereby inhibiting synaptic transmission of nociceptive stimuli. Postsynaptically, enhanced potassium efflux causes neuronal hyperpolarization of spinal cord projection neurons and inhibits ascending nociceptive pathways. Opioids do not interfere with motor function or proprioception (Simon & Steagall, 2017).

Opioids are usually divided in four groups: full agonists (morphine, metadone, fentanyl and its derivatives, pethidine (meperidine, etc)), agonist-antagonists (butorphanol and nalbuphine), partial agonists (buprenorphine) and antagonists (naloxone, nalmefene and naltrexone)) (B. P. Monteiro et al., 2023).

They produce several adverse effects (usually associated with high doses) such as nausea, vomiting, dysphoria, panting, bradycardia, histamine release, urinary incontinence/retention, respiratory depression. Less often they can produce inappetence, restlessness, constipation, hypothermia or hyperthermia. Naloxone or butorphanol titration can rapidly reverse these adverse effects (B. P. Monteiro et al., 2023).

2.8.1.1.1 Tramadol

Is a central acting opioid analgesic with dual mechanism of action and has minor respiratory, cardiovascular and gastrointestinal effets than other typical opioids (Eduardo Raposo Monteiro, Junior, Assis, Campagnol, & Quitzan, 2009). It reduces the doses of volatile and injectable agents (Seddighi, Egger, Rohrbach, Cox, & Doherty, 2009) (Wordliczek, Banach, Garlicki, Jakowicka-Wordliczek, & Dobrogowski, 2002). Its analgesic effects derive from the inhibition of noradrenaline reuptake and the increased release and inhibition of serotonin reuptake; it activates the two systems involved in pain inhibition: the opioid and the descendant monoaminergic system. It has low affinity for the μ, δ, κ opioid receptors and weaker affinity for the kappa subtype (Kukanich & Papich, 2004) (McMillan et al., 2008).

The most important metabolite of tramadol, O-desmethyl tramadol (M1) is related with the mu opioid agonist effects. This metabolite is produced at much faster rates in cats when compared with dogs (Perez Jimenez, Mealey, Grubb, Greene, & Court, 2016). Dogs are unable to produce significant concentrations of *O*-desmethyl tramadol were not observed in dogs in postoperative pain (Donati et al., 2021).

Constant rate infusion administration of analgesics keeps the plasma concentrations stable preventing transient subtherapeutic concentrations (Lucas, Firth, Anderson, Vine, & Edwards, 2001)(Guedes, 2012). The mu opioid agonist fentanyl is frequently used in veterinary patients as a CRI for its rapid onset and minimal hemodynamic effects (Arndt, Mikat, & Parasher, 1984) (Grimm et al., 2005).

2.8.1.1.2 Fentanyl

It acts in the opioid receptor with high affinity and intrinsic action (Villiger, Ray, & Taylor, 1983) (Boas & Villiger, 1985). It has a rapid onset, short duration, and an analgesic potency 75-100 times the morphine's (Yaksh, Noueihed, & Durant, 1986). Compared to morphine it has fewer side effects such as respiratory depression, excessive sedation and dysphoria (Freye, Schnitzler, & Schenk, 1991). Due to its pharmakocinetic characteristics it has to be administered repeteadly or in CRI to allow prolonged analgesia (Sano et al., 2006).

2.8.1.2 Acepromazine

Is a long acting phenotiazine derivative, with antagonistic action at dopaminergic receptors within the brain, as such the degree of sedation following its administration in canine patients is mild to moderate (Montané et al., 2003) (Eduardo R Monteiro, Figueroa, Choma, Campagnol, & Bettini, 2008) (Eduardo Raposo Monteiro et al., 2009)(E. H. Hofmeister, Chandler, & Read, 2010). It has several properties like antihistaminic, anticonvulsant, antiarrhytmic, hypotensive and hypothermic (Fallis, 2023).

It is a widely used tranquiliser with no analgesic properties. Therefore, is usually administered in combination with opioid analgesic to provide sedation and analgesia, which enables handling of dogs for preparation for surgery, placement od venous catheters and diagnostic procedures (Eduardo Raposo Monteiro et al., 2009). Is allows the use of lower doses of the injectable and inhalant anaesthetic requirements (B. P. Monteiro et al., 2023).

2.8.1.3 Maintaining anesthesia

Propofol is a intravenous agent, non barbiturate, with short action used for induction and maintenance of anesthesia (Glowaski & Wetmore, 1999). Its advantages over other CNS depressants are rapid complete recovery, rapid action, a non-cummulative effect, good muscle relaxation, excellent hypnosis (Adetunji, Ajadi, Adewoye, & Oyemakinde, 2002). It can be used after administration of premedication of acepromazine plus an opioid analgesic to achieve a balanced analgesia (Suarez, Dzikiti, Stegmann, & Hartman, 2012) (Herbert, Bowlt, Ford-Fennah, Covey-Crump, & Murrell, 2013).

2.8.1.4 Nonsteroidal anti-inflammatory drugs

Given the anti-inflammatory, analgesic and antipyretic effects of nonsteroidal anti-inflammatory analgesics they are commonly used for control of postsurgical pain in dogs and cats (KuKanich et al., 2012). There are several NSAIDs available all with the mechanism of inhibiting cycloxigenase (COX). The COX enzyme is in most tissue types and three forms have been identified: COX-1, COX-2 and COX-3. The latter has been identified in the canine cerebral cortex and in minimal amounts peripherally (Chandrasekharan et al., 2002).

NSAIDs may also prevent central sensitisation to painful stimuli caused by persistent stimulation dorsal horn neurons in spinal cord. Their postsurgical use is moderate given their possible side effects in renal function. Furthermore its use depends on the medical assessment of animals that are in a renal state dependent on prostaglandin (Kay-Mugford, Benn, LaMarre, & Conlon, 2000). Hypotension and increased sympathetic tone are surgical and anaesthesia side effects that can induce renal blood flow changes dependent on prostaglandin E2 and prostacyclin I2 (Crandell, Mathews, & Dyson, 2004). The renal protective prostaglandins are primarily derived from the actions of COX-1, hence the preferential use of NSAIDs that target COX-2 in postoperative pain management because they may cause less changes in renal blood flow and haemodynamics. Nonetheless, evidence shows that COX-2 also has a constitutive role in the kidneys (Harris, 2000).

Carprofen and meloxicam have analgesic effect up to 72 hours post-surgery, even in ovariohysterectomy in dogs Meloxicam may control pain after laparotomy up to 20 hours with greater effectiveness than butorphanol . (Leece, Brearley, & Harding, 2005).

The treatment of postoperative pain with paracetamol, meloxicam or carprofen was equally effective between these three drugs when pain is scored using Dynamic Interactive Visual Analog Scale (DIVAS) and UMPS (Hernández-Avalos et al., 2020).

2.8.1.5 Alfa2-adrenoceptor agonists

They produce sedation and hypnosis, analgesia and muscle relaxation. The agonists (medetomidine, dezmedetomidine, xylazine, romifidine) can be reversed by the administration of an antagonist (atipamezole or yohimbine). They bind to different alpha2-adrenoceptor subtype receptors in the dorsal horn of the spinal cord (spinal analgesia) and cerebral cortex and locus coeruleus (sedation and supraspinal analgesia).

2.8.1.6 Intraperitoneal anesthetics

Intraperitoneal administration of local anesthetic or analgesic in the surgical site and viscera before applying the final abdominal suture has been used to control abdominal postoperative pain in human and veterinary medicine (Carpenter et al., 2004) (Campagnol, Teixeira-Neto, Monteiro, Restitutti, & Minto, 2012) (Kalchofner Guerrero, Campagna, Bruhl-Day, Hegamin-Younger, & Guerrero, 2016)(Lambertini, Kluge, Lanza-Perea, Bruhl-Day, & Kalchofner Guerrero, 2018)(Chilkoti et al., 2019).

This administration is risk-free, inexpensive, and non-invasive procedurethat is easily performed. It also allows the administration of higher doses compared with the intravenous method, possibly increasing the duration of the effects (Karsli, Kayacan, Zorlu, Arici, & Erman, 2003) (Wilson, Barnes, & Hauptman, 2004).

The intraperitoneal administration of a combination of topical anesthetic and opioid such as lidocaine and tramadol has been shown to provide better analgesia after ovariohysterectomy in dogs (Farokhzad, Sabiza, Razi Jalali, & Baniadam, 2021). Also, tramadol added to bupivacaine administered intraperioneally led to appropriate pain relief after laparoscopic uterine ligation surgery in humans (Memis, Turan, & Karamanlioglu, 2005).

2.8.1.7 Opioid Free Anesthesia (OFA) and Opioid Sparing Anesthesia (OSA)

In the veterinary field there is evaluation of the analgesic efficacy of opioid-free and opioid sparing anesthesia protocols to prevent intraoperative and postoperative pain in animals (Lazzarini et al., 2024). The OFA consists of combining drugs and/or analgesic techniques, to achieve high quality general anesthesia without the need for opioids. OFA depends on the synergestic use of different medications, providing a balanced strategy to pain managementthat works at different levels along the pain pathways (Beloeil et al., 2018).

The drugs considered and used in these protocols are alfa-2 adrenoreceptor-agonists, NMDA antagonists, local anaesthetics or locoregional techniques and anti-inflamatory drugs (Lazzarini et al., 2024).

Paracetamol is also included in OFA protocols, that combine drugs such as medetomidine, ketamine, lidocaine, bupivacaine, carprofen and meloxicam. In humans the reasoning is to reduce the opioid dependency which is a serious clinical problem (White, Mair, & Martinez-Taboada, 2017).

2.8.1.8 Local anesthetics

Local anesthetics block the transmembrane neuronal channels, making an interruption in the nociceptive transmission to the spinal cord. Studies show that the use of local-regional anesthesia provides better intra-operative cardio-respiratory stability and lower post-surgery pain scores, which in turn reduces the perioperative use of opioids and its associated side effects (Becker & Reed, 2012; Fozzard, Lee, & Lipkind, 2005; Usunoff, Popratiloff, Schmitt, & Wree, 2006). These techniques have been associated with advantages In people such as reduced hospitalization time, less depression of the immune system, suppression of the neuro-endocrine stree response, less probability of central pain sensitization and better recovery (Romano, Portela, Breghi, & Otero, 2016; Usunoff et al., 2006).

The transverse abdominis plane block (TAP) consists of ultrasound-guided injection in the insterfascial plane between the internal oblique and the transverse abdominis muscles with local anesthetic (Paolini et al., 2022). In humans and small animals it provides intra and post-operative analgesia in patients undergoing celiotomy or laparoscopic procedures (Yu, Gao, Yuen, Choi, & Xu, 2021) (O'Donnell, McDonnell, & McShane, 2006) (McDonnell et al., 2008).

In a study a TAP block associated with intercostal blocks from T8 to T10, is valid for multimodal analgesia protocol for laparoscopic ovariectomy in bitches, allowing a reduction in the peri-operative opioid requirement (Paolini et al., 2022). In other study in laparoscopic ovariectomy in bitches the TAP block allowed for lower concentration of isoflurane needed to maintain the anesthetic depth and also the need for post-operative opioids, diminishing the signs of intra-operative nociception and postoperative pain (Espadas-González et al., 2022).
It has been shown that the hydrodissection associated with the TAP block does not increase the inflammatory response in laparoscopic ovariectomy (Espadas-González et al., 2024).
In humans the low pneumoperitoneum pressure is less than 10 mmHg in line with the described perfusion pressure in the distal segment of the capillary network of the parietal peritoneum (Solass et al., 2016). In cats the optimized intra-abdominal pressure (IAP) is 8 mmHg and they can tolerate until 12 mmHg (Park & Okano, 2015; Scott, Singh, & Valverde, 2020). For dogs minimally invasive surgeons should try to maintain the insufflation pressure under 12 mmHg or low enough to allow correct visualization and feasible working space (Fransson & Mayhew, 2022).
According to the Enhanced Recovery After Surgery (ERAS) guidelines in humans, the use of low pressure laparoscopy is recommended because studies show that it reduces early pain scores, postoperative nausea, vomiting, length of stay, the risk of mild postoperative complications, and it does not increase the rate of intraoperative complications (Reijnders-Boerboom et al., 2023).
There is evidence both in humans and small animals that the multimodal anaesthesia is the most adequate strategy to correctly manage the perioperative pain.

2.9 Acetazolamide as a part of multimodal analgesia protocol

In laparoscopic procedures, CO_2 diffuses into tissues. It is absorbed into the systemic circulation through the peritoneum, which results in a decrease in peritoneal and plasma pH from the liberation of hydrogen ions, which can be responsible for some degree of pain (Bergström, Falk, Park, & Holmdahl, 2008) (Liem et al., 1996) (Woehlck et al., 2003). Multiple techniques have been investigated to reduce or prevent the changes in intraperitoneal pH, such as the use of heating, humidification, bicarbonate, intraperitoneal lavage, gasless laparoscopy, and use of helium as a substitute for CO_2, all with mixed results

(Wong, Shah, Birkett, & Brams, 2004) (Neuhaus et al., 2000) (Neuhaus et al., 2001) (Dorrance, Oien, & O'Dwyer, 1999)(Farrell, Metreveli, Johnson, Smith, & Hunter, 2000)(Gupta, Watson, Ellis, & Jamieson, 2002)(Watson et al., 1997)
The enzyme carbonic anhydrase speeds up the generation of carbonic acid (Woehlck et al., 2003). Acetazolamide (2-acetylamino-1,3,4-thiadiazole-5-sulphonamide) is a carbonic anhydrase inhibitor utilised in both human and veterinary medicine (Alberts, Clarke, MacAllister, & Homer, 2000). Acetazolamide is used for glaucoma therapy since it reduces the aqueous humour production in the ciliary body (Maślanka, 2015); however, its uses in cats are limited due to its systemic toxicity (McLellan & Miller, 2011). The dose recommended for dogs is 5–10 mg/kg q8–12h. Acetazolamide has also been used to aid in the treatment of mild congestive heart failure in dogs (Shields, 2009), to reduce cerebrospinal fluid production, and consequently, as an adjuvant treatment of hydrocephalus, hydranencephaly and porencephaly (Kolecka, Ondreka, Moritz, Kramer, & Schmidt, 2015), to treat idiopathic intracranial hypertension in humans (Supuran, 2015), for prophylactic treatment of hyperkalaemic periodic paralysis, a heritable equine muscle disease (Alberts et al., 2000) and udder oedema in cattle (Vestweber, Al-Ani, & Johnson, 1989). Its adverse effects are: Toxicity is rare. Gastrointestinal disturbances, especially vomiting, may occur with oral administration. It is contraindicated in the presence of liver disease because it may precipitate hepatic coma by diverting ammonia produced in the kidney from the urine to the systemic circulation as a result of urine alkalinization; systemic acidosis and compensatory hyperpnea, GI disturbances, diuresis, and depression. Hypokalemia is rarely seen (Hsu, W., 2008)

Acetazolamide potentially has a role in inhibiting both the membrane-bound and the cytoplasmic forms of carbonic anhydrase, which slows down enzymatic catalysis's production of H+ ions (Bala, Bhatia, Mishra, Verma, & Kaman, 2015). By inhibiting the carbonic anhydrase, the carbonic acid is possibly removed by diffusion or blood flow at a speed able to decrease painful stimulation (Woehlck et al., 2003). Studies conducted in human medicine have concluded that acetazolamide reduces pain related to carbon dioxide insufflation following laparoscopic surgery (Bala et al., 2015) (Pourladian et al., 2016) (Figueroa-Balderas et al., 2013) (Nyerges, 1994). In dogs, the peritoneal fluid changes due to CO2 insufflation are similar to the response in human patients (Duerr, Twedt, &

Monnet, 2008). As a result, acetazolamide may reduce postoperative pain in dogs having laparoscopic surgery with CO_2 insufflation.

Chapter 3 - Results

Article 1 - Laparoscopic Castration Using Bipolar Forceps vs. Orchiectomy in Dogs: A Comparison of Two Techniques.

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1. Simple Summary

Castration of dogs is one of the most often performed surgeries in veterinary medicine. Minimally invasive techniques used in human medicine are now being used in animals. We compared the feasibility and effects on pain and inflammation of a new laparoscopic technique with the classical castration technique. The animals in which the new technique was applied showed less pain and inflammation then the other group. Our results suggest that this is a feasible alternative to classical castration.

2. Abstract

This paper aimed to study the feasibility of a new laparoscopic castration technique in male dogs, evaluate the pain associated with it, and compare it with the classical orchiectomy. Surgical times, pain scores, blood and salivary cortisol, and CRP were recorded and compared between the two groups. The use of high-frequency bipolar forceps allowed quick and uneventful laparoscopic procedures. The laparoscopic group had significantly lower pain scores, cortisol, and PCR values than the orchiectomy group. No complications were seen in any group. Our results suggest that this laparoscopic castration is a safe and beneficial surgical alternative to traditional orchiectomy in dogs.

Keywords: dog; laparoscopic castration; bipolar electrocoagulation; Pain; PCR; UMPS; salivary cortisol; serum cortisol electrocoagulation; pain; PCR; UMPS; salivary cortisol; serum cortisol

3. Introduction

Elective sterilization of dogs is one of the most performed procedures in veterinary practice, which is reported to increase dog's life expectancy [1,2]. It is used for contraception, to control dog overpopulation, to prevent diseases [2], and to reduce unwanted sexual and aggressive behaviors [3]. To date, an array of spaying procedures has been described, including castration by open or closed orchiectomy [4], scrotal ablation [5], vasectomy, vasal occlusion with calcium chloride [6], laparoscopic vasectomy and sterilization [7], and bilateral vasocystostomy [8].

In male dogs, the most often used technique is an orchiectomy, in which the testis is moved cranially and exposed with a pre-scrotal incision in order to be removed [9]. This conventional method for castration is associated with several postoperative complications such as scrotal swelling, wound dehiscence, infection, maggot infestation, and hemorrhage [10]. Hemorrhage can lead to scrotal hematoma or intra-abdominal hemorrhage. These may necessitate surgical repair by scrotal ablation and locating and ligating the spermatic cord [9]. Additionally, postoperative care of the animal is required to avoid infection [11].

In recent years, minimally invasive surgery has developed rapidly both in human and veterinary medicine, and many new techniques and instruments have been created. [12] Laparoscopic surgeries are becoming more common in veterinary medicine due to their benefits, such as reduced postoperative pain, reduced incidence of surgical site infection, shorter hospital stays [13,14,15,16], much smaller wounds that require no postoperative care dressings [10], and lower morbidity [17]. As pet owners become more aware of these techniques, they await a minimally invasive surgery to be an option for their pets [4].

So far, laparoscopic castration is considered to be a suitable choice if combined with another surgery with a laparoscopic approach, such as cystopexy [18], deferentopexy [19], colopexy [20], and gastropexy [21]. Besides this, it can be applied to criptorchidectomy and neoplastic testes in dogs [22] and as an option for mass sterilization programs in overpopulated urban and industrial areas [10].

To date, there are few references regarding laparoscopic sterilization (LS) of male dogs with descended testis. The first study on this used the occlusion of the ductus deferens, resulting in rapid azoospermia but maintained libido and mating behavior. The development of this technique had the goal of sterilizing the male animal without changing its physical characteristics or endogenous hormone secretion [23]. Other studies described a technique in which a segment of the vas deferens was cauterized and resected with monopolar grasping forceps [11] or a laparoscopic-assisted colopexy and sterilization, where both vessels and the deferens were double clipped and then coagulated, and the tissues in the area between the clips cut [20]. The most recent report of a laparoscopic sterilization technique was performed by Mahalingam et al., where besides cauterizing and resection of 2 to 3 cm of vas deferens, the spermatic artery–vein plexus was double clipped [7].

Thus, this study aimed to evaluate the feasibility of a new technique of LS in healthy dogs. We also intended to assess differences in time of surgery and pain responses between orchiectomy (OR) and the LS method presented here. We hypothesized that LS would be less painful than OR because of smaller incisions and decreased soft-tissue trauma. To test our hypothesis, we evaluated pain using biochemical responses and subjective measurements in two groups of dogs sterilized by either OR or LS.

4. Materials and Methods

4.1. Animals

A total of twenty healthy stray male dogs from a shelter of different breeds, mixed-breeds, and ages were enrolled. (see Table 2)

Number of Animals by Breed				
	Mixed-Breed	Miniature Poodle	Labrador	Yorkshire Terrier
OR	6	2	1	1
LS	10	-	-	-

Table 2- Number of animals by breed in groups OR and LS

Inclusion criteria were that the dogs had good body condition, suitable temperament, and were not receiving concurrent medications. Dogs were considered healthy based on history, complete physical examination, hematological examination, and serum biochemical analysis on admission, and therefore classified as American Society of Anesthesiologists (ASA) category ASA 1.

The Ethical Committee approved the study protocol of the Faculty of Veterinary Medicine of Lisbon University. Dogs were randomly assigned to two groups of ten animals, pre-scrotal orchiectomy (OR) or laparoscopic sterilization (LS). Food was withheld for 12 h and water for 8 h before surgery. The animals were admitted the day of the surgery. A consent document advising of the risks of each procedure, especially for laparoscopic sterilization and the possible need to convert to an open celiotomy for situations such as uncontrollable hemorrhage or iatrogenic injury, was signed by the dog's caretakers. The procedures were performed under the Portuguese Government for Animal Care Guidelines (DL No 260/2012).

4.2. Surgical Procedures

Before surgery, a 25 mm, 22-gauge catheter (Introcan-W; B. Braun, Lisbon; Portugal) was placed in the cephalic vein for drug administration and collection of blood samples. Peripheral venous blood and salivary samples were collected one hour before induction of anesthesia to obtain pre-operative samples (baseline values).

The anesthesia protocol was the same for both groups. The dogs were premedicated with 0.05 mg/kg acepromazine (Calmivet; Vetoquinol; Lisbon, Portugal) and 5 mg/kg intravenous tramadol (Tramadol; Labesfal; Lisbon, Portugal). Anesthesia was induced with 4 mg/kg intravenous propofol (Propofol

Lipuro; B. Braun; Lisbon, Portugal) and maintained with isoflurane (Vetflurane; Virbac; Portugal) in 100% oxygen (100% Medicinal Oxigen; Conoxia; Lisbon, Portugal) delivered through a non-rebreathing circuit. The dogs were closely monitored during the surgical procedure with pulse oximetry, electrocardiography, non-invasive blood pressure, capnography, and temperature.

4.2.1. Orchiectomy

The conventional open castration by the standard pre-scrotal method [5] was made using a 2-0 absorbable synthetic monofilament glyconate suture (Monosyn; Braun; Lisbon, Portugal). Recovery from anesthesia was uneventful.

4.2.2. Laparoscopic Castration

The laparoscopic castration used bipolar forceps with integrated scissors (5 mm, RobiPlus; Karl Storz; 78532 Tuttlingen, Germany). The technique applied was novel since we did not apply clips [7].

Dogs were placed in dorsal recumbency on the surgical table without being tied down. The bladder was emptied by catheterization. The pneumoperitoneum was established with a Veress needle (2.1 mm; Richard Wolf, 75438 Knittlingen; USA) inserted caudally to the xiphoid process for abdominal access. CO_2 administration was provided via automatic insufflator (Electronic Insuflator 2002; Cabot Medical; USA) with a gas flow of 9 L/min to a pressure set at 9 to 11 mmHg. Once the pneumoperitoneum was established, a stab incision was made through the skin and exposed the peritoneum. A perimeter mark was made with the cannula to achieve the incision length. The first cannula (threaded, 5.5 mm in all cases) was placed in the incision. Then, we inserted the telescope (5.3 mm; 0°; Panoview; Richard Wolf; 75438 Knittlingen, USA) using a standard clockwise rotation to evaluate the abdomen and iatrogenic injuries. Later, we positioned another cannula on the opposite side of the telescope port and placed the animals in Trendelenburg position. High-frequency bipolar forceps with integrated scissors (5 mm, RobiPlus; Karl Storz; 78532 Tuttlingen, Germany) were introduced through the left instrument port to handle the vessels and the deferens of the left testis without previous dissection (Figure 1). The structures were coagulated multiple times along 2–3 cm and cut (Figure 2 and Figure 3). We applied the same procedure to the right testicular vessels and deferens. Occlusion of the ductus deferens was considered complete when the structure was coiled and blanched (Figure 4). The portal valves were opened to evacuate the CO_2 partially, and with a lower pneumoperitoneum pressure, we inspected the abdominal to evaluate the presence of bleeding. After this verification, all the gas was evacuated, and the dogs were placed in normal recumbency. The cannulas were removed just before the suture application to guarantee maximum gas evacuation. Abdominal incisions were closed using a 2/0 absorbable synthetic monofilament glyconate suture (Monosyn; Braun; Lisbon, Portugal) in a simple interrupted suture pattern, and the skin was closed with an intradermal pattern with a single absorbable 3-0 suture (Monosyn; Braun; Portugal) (Figure 5). No dressings were applied.

After the surgical procedures, dogs received a single dose of meloxicam 0.2 mg/kg subcutaneously (Metacam; Boerhinger Ingelheim; Lisbon, Portugal) and were carefully monitored. The dog had a daily clinical examination, which included pain assessment and samples collection. Further meloxicam was administered the following two days (0.1 mg/kg subcutaneously). All the dogs were discharged home two days after surgery. One week after surgery, the animals returned to the veterinary hospital for a physical examination, evaluation of proper wound healing, and collection of blood samples.

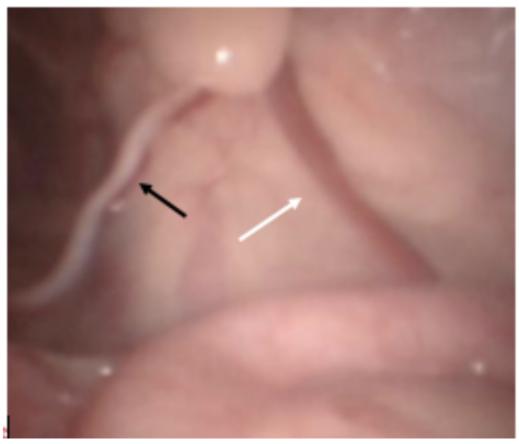


Figure 1- Laparoscopic anatomy of the vas deferens (black arrow), and the artery and vein plexus (white arrow) at the entrance of inguinal canal.

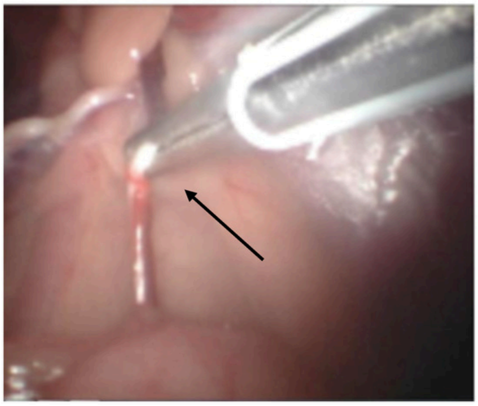


Figure 2- Coagulation of artery and vein with the high-frequency bipolar forceps with integrated scissors (arrow).

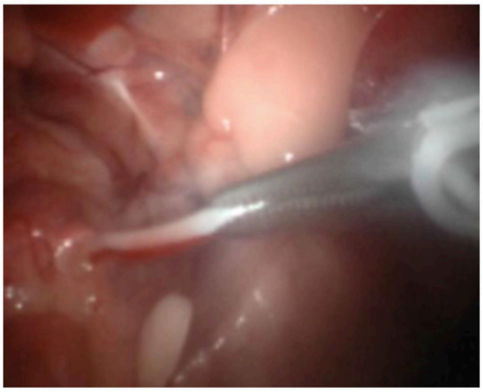


Figure 3.- Image of the laparoscopic surgery showing the coagulation of vas deferens.

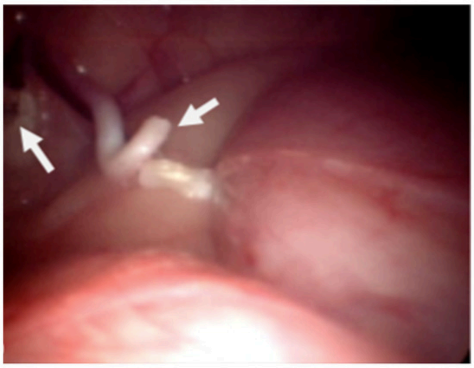


Figure 4- Image of the laparoscopic surgery to confirm that both structures were cut, and without the presence of active hemorrhage.

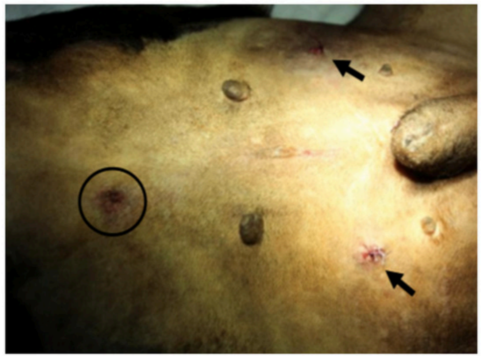


Figure 5- View of the sutures in both trocar sites (arrows) and Veress needle incision (circle)

4.3. Recorded Variables and Postoperative Pain Assessment

Data recorded included breed, age, body weight, duration of surgery, and occurrence of intraoperative bleeding or surgical complications.

The severity of pain was monitored 1, 12, and 24 h after surgery based on pain scores obtained with the UMPS (University of Melbourne Pain Scale). This is considered a validated method for assessment of postoperative pain in dogs [3]. It consists of multiple descriptors arranged in six categories which include behavioral and physiologic responses, and some observer bias can be eliminated by weighting of certain behaviors [4]. The score could vary between zero and twenty-seven (0–27). Pain was assessed by only one person familiar with the pain scoring system and semi-blind to the procedure. A pain score > 10 was considered justification for rescue analgesia, and methadone (0.5 mg/kg) would be administered intravenously if necessary. Nonetheless, we did not administer methadone in any of our animals since they did not reach a pain score of 10 in the UMPS.

Besides this, blood and salivary samples were collected to evaluate postoperative pain. Hence, we analyzed blood and salivary cortisol at 1, 12, 24 h; C-reactive protein (CRP) was evaluated in blood samples collected 24 and 168 h (7 days) after surgery to investigate the systemic inflammatory response to the surgical procedures. We also looked for a correlation between the blood and salivary cortisol values.

Since the surgical procedures were performed at night, dogs slept all night uneventfully. Therefore, a limited number of time points for blood and saliva samples and pain score acquisition were used. The objective was to limit disturbing the dogs and consequently limit potential confounding of the stress/pain variables, as others have previously limited [24].

4.4. Statistical Analysis

Data analysis was performed using SPSS statistical software (Version 22, SAS, Buckinghamshire, SL7 2EB, UK). As our data involved the analysis of repeated measurements, we used one-way repeated measures ANOVA to analyze all the variables evaluated and compared between groups for each data collection time point. Significance was set as $p < 0.05$.

5. Results

5.1. Surgical Procedures and Clinical Follow-Up

A total of 20 patients were randomized and allocated into one of the two groups (OR, LS). There were no significant differences in mean group ages: OR 3.3 years; LS, 1.04 years. There were no significant differences in mean group weights: OR 14 kg, LS 14.1 kg, or the duration of surgery between the OR and the SL groups (see Table 3)

Table 3 -Patient characteristics and intraoperative variables (values in mean, min, max)

Patient Characteristics and Intraoperative Variables	OR	LS
Age (years)	3.3 (0.6–7)	1.04 (0.4–2)
Weight (kg)	14 (4.5–27)	14.1 (5.7–24.4)
Duration of surgery (minutes)	41.4 (36–49.8)	42 (30–75)

Surgical complications were not found in the OR and LS groups. Conversion to an open approach was unnecessary in the LS group. All dogs recovered uneventfully from anesthesia and were bright, active, and with full appetite at discharge.

5.2. UMPS (University of Melbourne Pain Scale)

UMPS was scored by assigning a maximal score of 27 and a minimum score of 0. All dogs had scores of 0 preoperatively. None of the animals in either group had a pain score requiring additional postoperative analgesia (i.e., >10 of a possible 27) at any time after surgery.

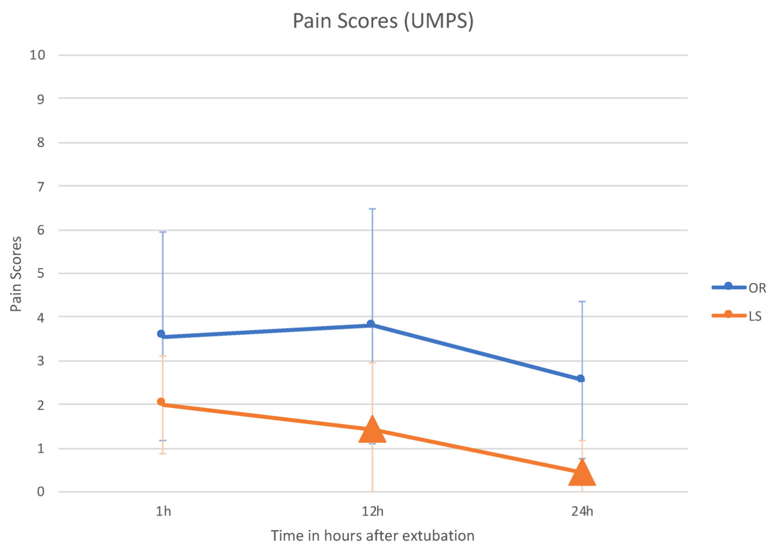
The LS group had the lowest pain scores at all postoperative time intervals. UMPS scores were significantly lower for the LS group compared with OR at 12 h (*p* = 0.010) and 24 h (*p* = 0.007) after surgery (see Table 4. and Fig.6).

Table 4-UMPS Scores (mean + SD) of dogs that underwent orchiectomy and laparoscopic sterilization.

Variable	Group	Time after Extubation		
		1 h	12 h	24 h
UMPS Score	OR	3.56 ± 2.4	3.8 ± 2.68	2.55 ± 1.8
	LS	2 ± 1.12	1.44 ± 1.5 *	0.44 ± 0.73 *

* significant values.

Figure 6 - University of Melbourne Pain Scale post-operative scores in groups OR and LS (triangles show the significant values).



5.3. Serum Cortisol

Preoperative serum cortisol concentrations were within the reference range of 20–250 mmol/L in the two groups and were not significantly different. The mean serum cortisol concentration peaked at 1 h after surgery and returned to baseline 12 h after surgery. Cortisol concentration was significantly lower in the LS group (119 nmol/L; *p* = 0.003) than in the OR group (234 nmol/L) one hour after surgery. No significant differences were found between groups at any other time (see Table 5).

Table 5- Mean ± SD values of serum and salivary cortisol recorded after extubation.

Variable	Group	Time after Extubation			
		0 h	1 h	12 h	24 h
Serum cortisol	OR	182 ± 133.67	234 ± 81.17	113.9 ± 51.99	110 ± 83.66
	LS	92.5 ± 36.02	119 ± 62.47 *	88.7 ± 36.18	95.1 ± 93.30
Salivary Cortisol	OR	8 ± 7.6	15 ± 4.1	5.6 ± 1.9	4.5 ± 1.77
	LS	4.9 ± 2.8	11.1 ± 3.8 *	5.2 ± 2.7	4.75 ± 2.71

* Serum cortisol concentration was significantly lower at 1 h in group LS. ($p < 0.05$) * Salivary cortisol concentration was significantly lower at 1 h in the LS group.

5.4. Saliva Cortisol

Preoperative mean saliva cortisol concentrations were not significantly different between groups. The mean saliva cortisol concentration peaked at 1 h after surgery and returned to baseline at 12 h after surgery, following the mean serum cortisol variation trend at the same time intervals. The mean saliva cortisol concentration was significantly lower for the LS group (11.1 ng/mL; $p = 0.039$) than the OR group (15 ng/mL) at one hour after surgery. A strong correlation ($r = 0.58$) was found between serum and saliva cortisol concentrations. No other significant differences between groups were found at any time (see Table 4).

5.5. C-Reactive Protein (PCR)

Preoperative mean PCR concentrations were not significantly different between groups. The mean PCR concentration peaked at 24 h after surgery and returned to baseline at 168 h. PCR concentration was significantly lower in the SL group (5.8 µg/mL; $p = 0.037$) than in the OR group (20.8 µg/mL) one week after surgery (168 h). No other significant differences between groups were found at any time (see Table 6).

Table 6- Mean ± SD values of C-Reactive Protein recorded at 0 h, 24 h and 168 h after extubation.

Variable	Group	Time after Extubation		
		0 h	24 h	168 h
C-reactive Protein	OR	17.8 ± 15.8	22.9 ± 11.2	20.8 ± 16.9
	LS	7.1 ± 4.1	18.7 ± 8.2	5.8 ± 3.3 *

* C-Reactive protein concentration was significantly lower at 168 h in group LS. ($p < 0.05$).

6. Discussion

To the best of the authors' knowledge, this laparoscopic sterilization technique for dogs is described for the first time in this study. Furthermore, a controlled study has not previously shown postoperative pain assessment after laparoscopic sterilization in dogs. Several complications are associated with laparoscopic surgery, such as viscera perforation, tissue damage caused by energy application, abdominal cavity access, and pneumoperitoneum [25]. Generally, the less experienced and trained the surgeon is, the more frequent the complications [26]. In this study, the surgeon's experience and the bipolar instrument used contributed to surgical procedures without complications.

Results of the present study show that the surgical technique was simple, feasible, and quick to perform without intraoperative complications or abnormalities requiring conversion to open laparotomy. The execution of both procedures did not differ significantly by time. The OR surgery requires several sutures, whereas, in LS, the most time-consuming step is the trocar placement and pneumoperitoneum establishment.

The Veress needle was used successfully to maintain the pneumoperitoneum through all the laparoscopic surgeries. It was introduced carefully into the abdominal wall without visceral damage due to a tight manual elevation before its introduction. Despite the risk of visceral damage, we consider using the Veress needle easy and less traumatic, and it avoids the difficulties associated with the modified Hasson technique. [27]

Trendelenburg positioning enabled the displacement of the digestive organs towards the diaphragm area, allowing precise observation of reproductive structures. The trocars placement permitted inspection of the abdominal cavity and a broad view of and access to the pelvic organs and tissues. The use of only two trocars had the following advantages: surgery was performed by a solo surgeon and with minor surgical trauma to the abdominal wall, which contributes to less post-operative pain [24].

The high-frequency bipolar forceps used were effective, user-friendly, and safe in coagulating and cutting vas deferens and artery and vein plexus. Since the scissors are integrated into the instrument, fewer surgical maneuvers and instruments were needed, reducing surgical time. Like other sealing and cutting instruments, the RobiPlus caused minimal smoke, resulting in a slight brief loss of observation in the surgical field [23].

Contrary to previous methods described for hemostasis of the spermatic cord, in this technique, no foreign material (metallic or plastic clips) was used and left in the animal. The sutures applied in the wounds from trocars proved to be entirely sufficient. The animals did not show interest in the wounds after surgery. The dog caretakers reported their well-being and high activity level, confirming the short period of convalescence needed due to low level of pain in the abdominal integument.

The aesthetical appearance was another advantage of laparoscopic sterilization. Although the evolution of testicular volume was not measured in detail in our study, we detected, as Mathon et al. observed, that the testicular volume was increased seven days after surgery and decreased by 90% at the end of 60 days [20]. This testicular atrophy happens due to ischemic degeneration [20], indicating a loss of function and a complete castration. This surgical technique allows the male dog to maintain the testis in the scrotum, although in a smaller size, which may pose a more attractive surgical option to male owners, who often avoid the castration of dogs [28].

Concerning postoperative pain, we found that LS was less painful in dogs when compared with OR surgery. Pain scores and cortisol measurements were considered effective in assessing pain levels. The use of multiple methods to assess pain in animals is accepted as the best way to avoid the unbalanced weighing of any single subjective or objective measurement [29,30]. Hence, no gold standard has been established for pain measurement in veterinary patients [31]. Objective physiologic (heart rate, respiratory rate, temperature) and biochemical (cortisol) measurements are usually used as indirect measures of pain, but they give inconsistent assessments of pain if not used with simultaneous evaluation of behavioral changes [32,33,34,35,36].

The measurement of cortisol values has been helpful as an objective indicator of animal pain [34,37,38,39], and it has been described as significantly increasing due to surgical stress in dogs and cats [33,34,35]. Similar results were observed in our study, where a correlation was found between serum and salivary cortisol concentrations. As salivary cortisol is highly correlated with serum cortisol in dogs, and its sampling is non-invasive [40], it has been chosen to measure stress response in these animals [40,41,42]. According to other laparoscopic sterilization studies in dogs [15,43], the first and highest postoperative peak of cortisol in this study occurred one hour after the surgery. Hence, the mean salivary and serum cortisol concentrations in the LS group were significantly lower than in the OR group. Since the anesthesia protocol was the same for every group, anesthesia alone apparently would not explain the difference in cortisol concentrations at 1 h postoperatively. Thus, it is possible that the low cortisol concentrations at hour one in the LS group may genuinely reflect the lower level of pain from the less traumatic surgical procedure.

In dogs, increases in serum CRP concentrations have been described after different surgical procedures; the more severe the tissue trauma made, the higher the magnitude of CRP increase [44,45]. Data confirm that canine serum CRP can be used to differentiate variable degrees of inflammatory activity after elective surgical procedures [46]. At the time of suture removal—usually seven days after surgery—CRP concentration is considered a more useful tool to monitor postsurgical progress than WBC counts in surgical procedures without clinical complications [44,47]. In this study, the use of meloxicam (non-steroidal anti-inflammatory drugs—NSAIDs) could also influence changes in serum CRP. However, it is known that CRP production is mainly dependent on pro-inflammatory cytokines, and NSAID only regulates prostaglandin synthesis [46].

As seen in another study [48], the maximum peak CRP concentration was 24 h in our groups. The CRP concentrations were lower at all times in the LS group, but only at 168 h after surgery, did we observe a significant difference between the CRP concentrations. Other studies comparing inflammation between open and laparoscopic procedures also found significantly lower concentrations of CRP for laparoscopic procedures [46,49,50].

The present data confirm that laparoscopic castration in dogs induces less inflammation, as it is a surgical procedure that produces less tissue trauma.

7. Conclusions

In conclusion, the novel laparoscopic sterilization technique was minimally invasive and allowed excellent visualization of the reproductive structures. Significant differences were found in pain-associated variables in the laparoscopic group at one hour postoperative, suggesting that this group had less pain. The surgeries did not have any complications, and the animals recovered uneventfully. Therefore, the authors believe that this laparoscopic castration is a safe and beneficial surgical alternative to traditional orchiectomy in dogs. In the future, it would be interesting to perform further studies, including more time points for the pain analysis—both with UMPS score and cortisol.

8. Author Contributions

I.T.T. executed all the surgical procedures; analyzed and interpreted data for the work and drafted the manuscript. R.R.B. and J.P.S.-L. made substantial contributions to the conception of the work and revised the manuscript critically for important intellectual content. C.G.V. assisted in all the surgical procedures and made a substantial contribution to the acquisition of data for the work. J.R.J. revised the manuscript critically for important intellectual and scientific content. All authors have read and agreed to the published version of the manuscript.

9. Funding

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10. Institutional Review Board Statement

The Ethical Committee of the Faculty of Veterinary Medicine of Lisbon University approved the study protocol. The procedures were performed under the Portuguese Government for Animal Care Guidelines (DL No 260/2012).

11. Informed Consent Statement

Not applicable.

12. Data Availability Statement

Not applicable.

13. Conflicts of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Article 2 - Premedication with acetazolamide: Is its use for postoperative pain and stress control after laparoscopic ovariectomy in dogs ruled out?

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1. Abstract

1.1. Background

Studies in human medicine have concluded that acetazolamide reduces pain associated with carbon dioxide insufflation during laparoscopic surgery. However, there are no published reports regarding the use of acetazolamide for this purpose in companion animals, despite the increasing popularity of laparoscopic techniques in veterinary medicine due to their advantages over open surgeries.

1.2. Objectives

Thirty mixed-breed female dogs were included in the study and randomly assigned to one of three groups: OVE (median celiotomy ovariectomy; *n* = 10), OVEL (laparoscopic ovariectomy, *n* = 10) and OVELA (laparoscopic ovariectomy with acetazolamide preoperative administration; *n* = 10). Experienced surgeons performed all procedures, and the anaesthetic and analgesic protocols were identical for all animals. Acetazolamide was administered orally (at a dose of 25 mg/kg) 2 h prior to induction in the OVELA group. Postoperative pain was evaluated using serum cortisol, salivary cortisol, and the University of Melbourne Pain Scale (UMPS) Score.

1.3. Results

Any statistical differences were observed in the UMPS scores when the OVELA group was compared to the OVEL group at 1 h after surgery ($p = 0.515$), 12 h ($p = 0.375$) and 24 h ($p = 0.242$). Animals undergoing open surgery (OVE group) had at all times significantly higher pain scores after surgery when compared with OVEL and OVELA groups. A high positive correlation ($r = 0.792$; $p = 0.01$) was found between serum and saliva cortisol concentrations. Mean saliva cortisol concentration was not significantly lower for the OVELA group compared to the other groups.

1.4. Conclusions

This study found evidence that preoperative administration of acetazolamide may be beneficial in managing postoperative pain in dogs after laparoscopic surgeries. However, further research with a larger sample size is needed to confirm this and to determine if acetazolamide should be included in a multimodal postoperative analgesia protocol for laparoscopic ovariectomy in dogs.

Keywords: acetazolamide, carbon dioxide, dog, laparoscopic, ovariectomy, multimodal analgesia, pain assessment, pain scale, pneumoperitoneum, University of Melbourne Pain Scale

2. Introduction

One of the most frequent procedures carried out in veterinary practice is elective sterilisation of dogs (Mayhew & Brown, 2007). Laparoscopic ovariectomy is a technique with several benefits and is safe and reliable in dogs (Van Goethem et al., 2003; Van Nimwegen et al., 2005; Wenkel et al., 2005; Cicirelli et al., 2022; Radford et al., 2021; Leonardi et al., 2020; Tavares et al., 2021).

Laparoscopic postoperative pain is associated with phrenic nerve neurapraxia, which is secondary to abdominal distension and the intraabdominal temperature, humidity, and amount of residual gas (Mouton et al., 1999). The number of cannulas used, the type of surgical procedure, and the surgeon's experience also influence the severity of postoperative pain (Leggett et al., 2000). The recommended approach to postoperative pain management in small animals is multimodal and uses several medications to act in different modes and sites (Epstein et al., 2015; Lamont, 2008). In laparoscopic procedures, carbon dioxide (CO_2) diffuses into tissues. It is absorbed into the systemic circulation through the peritoneum, which results in a decrease in peritoneal and plasma pH from the liberation of hydrogen ions, which can be responsible for some degree of pain (Bergström et al., 2008; Liem et al., 1996; Woehlck et al., 2003). Multiple techniques have been investigated to reduce or prevent the changes in intraperitoneal pH, such as the use of heating, humidification, bicarbonate, intraperitoneal lavage, gasless laparoscopy, and use of helium as a substitute for CO_2, all with mixed results (Wong et al., 2004; Neuhaus et al., 2000; Neuhaus et al., 2001; Dorrance et al., 1999; Farrell et al., 2000; Gupta et al., 2002; Watson et al., 1997).

The enzyme carbonic anhydrase speeds up the generation of carbonic acid (Woehlck et al., 2003). Acetazolamide (2-acetylamino-1,3,4-thiadiazole-5-sulphonamide) is a carbonic anhydrase inhibitor utilised in both human and veterinary medicine (Alberts et al., 2000). Acetazolamide is used for glaucoma therapy since it reduces the aqueous humour production in the ciliary body (Maślanka, 2015 Mar 1); however, its uses in cats are limited due to its systemic toxicity (McLellan & Miller, 2011). Acetazolamide has also been used to aid in the treatment of mild congestive heart failure in dogs (Shields, 2009), to reduce cerebrospinal fluid production, and consequently, as an adjuvant treatment of hydrocephalus, hydranencephaly and porencephaly (Kolecka et al., 2015), to treat idiopathic intracranial hypertension in humans (Supuran, 2015), for prophylactic treatment of hyperkalaemic periodic paralysis, a heritable equine muscle disease (Alberts et al., 2000) and udder oedema in cattle (Vestweber et al., 1989).

Acetazolamide potentially has a role in inhibiting both the membrane-bound and the cytoplasmic forms of carbonic anhydrase, which slows down enzymatic catalysis's production of H^+ ions (Bala et al., 2015). By inhibiting the carbonic anhydrase, the carbonic acid is possibly removed by diffusion or blood flow at a speed able to decrease painful stimulation (Woehlck et al., 2003). Studies conducted in human medicine have concluded that acetazolamide reduces pain related to carbon dioxide insufflation following laparoscopic surgery (Bala et al., 2015; Pourladian et al., 2016; Figueroa-Balderas et al., 2013; Nyerges, 1994). In dogs, the peritoneal fluid changes due to CO_2 insufflation are similar to the response in human patients (Duerr et al., 2008). As a result, acetazolamide may reduce postoperative pain in dogs having laparoscopic surgery with CO_2 insufflation.

Therefore, this study aimed to assess the effect of acetazolamide in postoperative pain after laparoscopic ovariectomy in dogs and compare those results with the traditional celiotomy ovariectomy. We hypothesised that variables associated with postsurgical pain/stress – serum and salivary cortisol – and pain scores from the University of Melbourne Pain Scale (UMPS) (Firth & Haldane, 1999) would be lower for the animals receiving perioperative acetazolamide and laparoscopy ovariectomy.

3. Materials And Methods

3.1. Ethical considerations

The study protocol has been approved by the Ethical Committee of the Faculty of Medicine of the University of Lisbon. The procedures were performed under the Portuguese Government for Animal Care Guidelines (DL No 260/2012). A consent document advising of the risks of each procedure, especially for laparoscopic ovariectomy and the possible need to convert to an open celiotomy for situations such as uncontrollable haemorrhage or iatrogenic injury, was signed by the dog's owners.

3.2. Study populations

A randomised parallel-group study design was conducted. Thirty healthy adult queens from different breeds, weighing between 4.8 and 31.4 kg, with ages between 4 months and 8 years old, were used in the study. Inclusion criteria included dogs with ideal body condition scores (Freeman et al., 2011), appropriate behaviour b, and not taking any drug. Dogs were deemed healthy based on clinical history, complete physical examination, and complete blood work (complete blood counts – CBC – and serum biochemistry parameters) on admission (t = –1 h). Presurgical blood biochemistry included glucose, fibrinogen, C-reactive protein, serum cortisol, and salivary cortisol. Animals' physical status was classified using the American Society of Anesthesiologists Classification (ASA) (Portier & Ida, 2018).

G* Power for Windows V. 3.1.6 was used to calculate sample size (Heinrich Heine Universität, Düsseldorf, Germany) (Faul et al., 2007; Erdfelder et al., 2009). The power analysis indicated that the number of animals included in the present study would allow detecting 1.0-point differences between groups for the Simple Descriptive Scale (SDS) and 3.0-point differences between groups for Numeric Rating Scale (NRS) scores with a power of 80% at 5% of the significance level.

A total of 32 dogs were assessed for eligibility. At enrolment, we excluded one dog for abnormalities in the blood analysis and another dog due to aggressive behaviour. Animals were randomly and blindly assigned to one of three groups of ten animals each: ventral median celiotomy ovariectomy (OVE group), laparoscopic ovariectomy (OVEL group) and laparoscopic ovariectomy with acetazolamide administration (OVELA group). Food was withheld for 12 h before surgery. Also, water was withheld for 8 h before the procedure. On the day of the procedure, the animals were admitted.

The OVE group was composed of one Border Collie and nine mixed-breed dogs. The OVEL group comprised one Pitbull, one Yorkshire Terrier and eight mixed-breed dogs. Finally, the OVELA group consisted of one Miniature Poodle, one Golden Retriever, one Husky, and seven mixed-breed dogs.

3.3. Anaesthesia

Before each surgery, a 25-mm, 22-gauge catheter (Introcan-W; B. Braun) was inserted into the cephalic vein for blood sample collection and drug administration. Venus peripheral blood (2.6 mL per animal) and the collection of salivary samples were collected 1 h (t = –1 h) prior to induction of the anaesthesia

to obtain baseline values. In the OVELA group, 2 h before to induction, 25 mg/kg of acetazolamide in tablets at a dose of was administered orally.
For all the three groups, the identical anaesthesia protocol was used. The animals were premedicated with acepromazine (Calmivet; Vetoquinol) 0.05 mg/kg and tramadol (Tramadol; Labesfal) 5 mg/kg iv. Anaesthesia was induced with 4 mg/kg intravenous propofol (Propofol Lipuro; B. Braun) to allow dogs intubation and maintained with isoflurane (Vetflurane; Virbac) in 100% oxygen (100% Medicinal Oxigen; Conoxia). A semiclosed circuit was used. Before surgery, a dose of fentanyl (Fentanilo, Sandoz) 5 mcg/kg was added intravenously and then a constant rate infusion of 5 mcg/kg/h was started. During the anaesthesia, we used mechanical ventilation to improve intraoperative gas exchange. Dogs were properly clipped and moved to the surgical room, placed in dorsal recumbency, disinfected and draped for surgery. NaCl 0.9 % solution (B. Braun) was infused during surgery and recovery period (5 mL/kg/h) (Davis et al., 2013). At the moment of the induction, 8.75 mg/kg (7.0 mg amoxicillin, 1.75 mg clavulanic acid) (Synulox; Zoetis) was administered. The same antibiotic was prescribed for 7 days. A General Electric Anaesthesia equipment (Datex-Ohmeda 9100c NXT) was used with a semiclosed circuit with ventilation rate of 1 L per minute (0.5 L of oxygen and 0.5 L of air) and a tidal volume of 5–15 mL/kg. The anaesthetic machine incorporated the ventilator and animals were continuously monitored. Pulse oximetry, oscillometric noninvasive blood pressure, spirometry, electrocardiogram, capnography, oesophageal temperature, and an end-tidal fraction of anaesthetic gases was continuously monitored using a multiparameter monitor (B125 General Electric Medical Systems Information Technologies GmbH, Freiburg, Germany).

3.4. Surgical procedures

3.4.1. Ovariectomy

The open surgery was performed using the classic technique (Williams, 2013).

3.4.2. Laparoscopic ovariectomy

Animals were positioned in dorsal recumbency. The urine bladder was emptied by catheterisation. For abdominal access, the pneumoperitoneum was established with a Veress needle (2.1 mm; Richard Wolf) inserted caudally into the xiphoid process. The CO_2 was provided via an automatic insufflator (Electronic Insufflator 2002; Cabot Medical) with a gas flow of 9 L/min to a pressure set at 9–11 mmHg. The first cannula (threaded, 5.5 mm in all cases) was placed 2 cm caudal to the umbilicus. We made a perimeter mark with the cannula to achieve the incision length through the skin and linea alba incision. The laparoscope (5.3 mm; 0°; Panoview) was inserted to evaluate the abdomen using standard clockwise rotation to avoid possible iatrogenic injuries.

Additionally, two cannulas were placed, each 3 cm cranial and caudal, to the first cannula, under direct vision of the laparoscope. The animal was rotated manually by an assistant into right lateral recumbency for identification of the left ovary. In all cases, forceps (5 mm; Robi; Karl Storz) was used to grasp, expose the ovary and suspend it to allow its removal. With high-frequency bipolar forceps (5 mm, RobiPlus; Karl Storz), the ovariectomy was completed by cauterising and transecting the proper ovarian ligament at the level of the uterine horn then the mesovarium and the suspensory ligament. The resected ovary was grasped by the proper ligament, elevated, and tacked to the body wall by passing a 40 mm, ½ circle curved cutting needle and sutured percutaneously through the body wall. The dog was manually positioned in left lateral recumbency to expose the right ovary. The ovariectomy was performed using the same technique described, except for the ovary not being tacked into the abdominal wall. The right ovary was grasped and secured with forceps. The dog was tilted into the right lateral recumbency, then the cannula with the forceps was pulled out, the 1-cm incision was extended to 1.5 cm, and the right ovary was removed from the abdomen. The pneumoperitoneum was partially lost, the cannula was inserted again, a clamp helped close the incision, and the pneumoperitoneum was reestablished. The left ovary was removed. The dog was turned into dorsal recumbency to inspect the abdominal cavity to evaluate for the presence of bleeding. Ovaries were checked to ensure complete excision; the two portals were removed, and the pneumoperitoneum was released. Abdominal incisions were closed in three layers: the first layer was muscular with the abdominal fascia and the second was the subcutaneous layer. A 2/0 absorbable synthetic monofilament glyconate suture (Monosyn; Braun) was used for suturing and a simple interrupted suture pattern was completed. A single absorbable 3-0 suture (Monosyn; Braun) was used for suturing the skin.

Once finalised the surgical procedure, dogs in all groups received one dosage of meloxicam 0.2 mg/kg SC (Metacam; Boehringer Ingelheim) (Hernández-Avalos et al., 2020; Cicirelli et al., 2021). Meloxicam was administered the following 2 days (0.1 mg/kg SC). All the dogs were bright, alert and responsive and were discharged home 2 days after surgery. One week after surgery, the animals returned to the veterinary hospital for a physical examination, evaluation of proper wound healing and collection of blood samples for a blood count (2.6 mL per animal).

3.5. Recorded variables and assessment of postoperative pain

Breed, age, body weight, length of surgery and occurrence of intraoperative haemorrhage or surgical complications were among the data collected. Physiological data and behavioural response variables included in the UPMS were assessed preoperatively (baseline) and 1, 12 and 24 h after surgery to monitor the presence and severity of pain (Firth & Haldane, 1999). The UMPS score varies between 0 and 27 (Shields, 2009). In order to reduce the variability of this parameter, only one person who was familiar with the pain scoring system and was not aware of the procedure's group assignment evaluated the patient's level of pain. If the patient's pain level was higher than 10, rescue analgesia was justified, and methadone (0.5 mg/kg) was given as necessary (Cicirelli et al., 2021). Immediately after extubating (t = 0 h), blood samples (1.3 mL per animal) for measuring serum biochemistry parameters (glucose, cortisol, C-reactive protein and fibrinogen) and salivary cortisol and were collected at 1, 12 and 24 h. Blood was collected directly from the catheter placed in the cephalic vein at t = –1 h, t = 0 h and t = 24 h. CBC was measured 24 h (1.3 mL per animal) and 7 days (2.6 mL per animal) after surgery. C-reactive protein and fibrinogen were also measured 7 days after surgery.

Thus, the total of blood required from each animal was 7.8 mL (2.6 mL, t = –1 h), (1.3 mL, t = 0 h), (1.3 mL, t = 24 h) and (2.6 mL, t = 7 days), with an estimate of a maximum of 2.2 mL discarded per animal for the entire blood sampling process. CBC was analysed immediately after blood collection, and sera were stored at –18°C until the day of evaluation.

3.6. Expression of the results and statistical analysis

Data with the normal distribution obtained from the study were expressed as means ± standard deviation (SD). For this purpose, the Kolmogorov-Smirnov test was used. Data obtained with nonnormal distribution or with high standard deviations were expressed as median because is a more appropriate average and absolute range (min and max) as an indicator of dispersion. Student *t*-tests to compare the differences in the mean values between groups were used. The interdependence of the variables was determined using Pearson's correlation coefficient. Parametric and nonparametric tests were conducted using Student's, Kruskal–Wallis and Friedman tests for intergroup and intragroup comparisons, respectively. Statistical analyses were performed using SPSS v.27.0 (SPSS Inc. Chicago IL). Significance was set as $p < 0.05$ for rejecting the null hypothesis.

4. Results

Previous studies used similar size groups for the sedation assessment and laparoscopic procedures (Van Goethem et al., 2003; Van Nimwegen et al., 2005; Wenkel et al., 2005; Mouton et al., 1999; Monteiro et al., 2016). All the animals included in the present study were classified in ASA 1 category (Portier & Ida, 2018). The animal caretakers gave their consent to participate in this survey, including the surgical procedures and blood sample collection.

Animals included in the OVE group weighed (kg) between 8.1 and 22.7 (mean = 15.4); those included in the OVEL group weighed between 4.8 and 31.4 (mean = 15.9); and those included in the OVELA group weighed between 5.0 and 23.0 (mean = 12.2). There were no significant differences in the mean weight among the three groups.

Animals included in the OVE group were between 4 months and 6 years of age (mean = 1.62); those included in the OVEL group were between 4 months and 8 years of age (mean = 1.82); and those included in the OVELA group were between 5 months and 3 years of age (mean = 1.23). No significant age differences were observed between the OVEL and OVELA groups.

However, the average surgical time (in minutes) was significantly longer for laparoscopic procedures in the OVEL group (mean = 102.6; min 60; max 108.0; $p = 0.001$) and in the OVELA group (mean = 99.6; min = 75; max = 120; $p = 0.003$) compared to the OVE group (mean = 72.6; min = 60; max = 84). No significant differences were observed in the duration of surgery between the OVEL and OVELA groups.

Intraabdominal bleeding was not documented in OVEL and OVELA groups and was minimal and self-limited in the OVE group. No significant intraabdominal bleeding occurred in the laparoscopic procedures. Moreover, neither the OVE nor OVELA groups had any surgical complications. Therefore, conversion to an open surgery was not required for any of the animals included in the OVEL and OVELA groups. No side effects potentially attributable to acetazolamide were observed in the OVELA group.

4.1. UMPS scores

All dogs had UMPS scores of 0 preoperatively. None of the animals in either group had a pain score requiring additional postoperative analgesia (i.e., >10 of a possible 27) after surgery. The OVELA group had the lowest pain scores at all postoperative time intervals. UMPS scores were statistically significantly lower in the OVEL group compared to the OVE group at 1 h after surgery (p = 0.024), 12 h (p = 0.011) and 24 h (p = 0.04). Also, UMPS scores were statistically lower in the OVELA group compared to the OVE group at 1 h (p = 0.009), 12 h (p = 0.001) and 24 h (p = 0.002). Then, animals undergoing open surgery (OVE group) had significantly higher pain scores at all time points after surgery compared to the OVEL and OVELA groups (see Table 7.)

Table 7- UMPS scores median (min and max) of dogs that underwent ovariectomy (OVE), laparoscopic ovariectomy (OVEL) and laparoscopic ovariectomy + acetazolamide (OVELA).

	Time after extubation		
Group	**1 h**	**12 h**	**24 h**
OVE	5.5 (1–10)	4.5 (1–8)	4.0 (2–8)
OVEL	1.0 (0–3)	1.0 (0–2)	1.0 (0–1)
OVELA	1.0 (0–3)	0.0 (0–2)	0.0 (0–0)

However, our results on UMPS scores did not show any statistical differences when the OVELA group was compared to the OVEL group at 1 h after surgery (p = 0.515), 12 h (p = 0.375) and 24 h (p = 0.242).

4.2. Serum and salivary cortisol

All three groups' preoperative serum cortisol levels were within the standard range of 20–250 mmol/L. There were no statistically significant differences between groups preoperatively. Mean serum cortisol concentration peaked 1 h after surgery, began a trend towards the baseline and only returned to baseline 12 h after surgery. We found statistical differences between 1 h and the rest of the hours (p = 0.0001) in all three groups. Median serum cortisol concentrations for the OVE group for each time interval ranged from 90.9 nmol/L to 382.5 nmol/L; from 60.7 nmol/L to 477.0 nmol/L for the OVEL group; and from 55.9 nmol/L to 457.0 nmol/L for the OVELA group. (see Table 8)

Table 8 - Median and range of serum cortisol levels (nmol/L); in each of the study groups measured throughout the duration of the study.

	Time after extubation			
Group	**0 h***	**1 h**	**12 h**	**24 h**
OVE	117.1 (37.8–216.0)	382.5 (219.0–629.0)	106.5 (30.6–272.0)	90.9 (37.8–234.0)
OVEL	60.7 (29.0–167.0)	477.0 (144.0–590.0)	61.0 (19.5–222.0)	62.600 (20.3–129.0)
OVELA	55.9 (32.3–133.0)	457.0 (123.0–806.0)	90.5 (35.0–259.0)	74.0 (30.6–208.0)

*No statistical differences were found between groups at any time point in all the three groups.

Related to cortisol concentrations shown in Table 8, we did not find statistical differences between the groups (t = 0 h, p = 0.340; t = 1 h, p = 0.303; t = 12 h, p = 0.146; t = 24 h, p = 0.651).

Median preoperative salivary cortisol concentrations were not statistically significantly different between groups (see Table 9). Like serum cortisol results, mean salivary cortisol concentration peaked 1 h after surgery and returned to baseline 12 h after surgery, following the mean plasma cortisol fluctuation trend at intervals. A strong positive connection between serum and salivary cortisol levels was discovered (r = 0.792; p = 0.01). The median salivary cortisol concentration was significantly higher in the OVEL group (61.9 ng/mL) compared to the OVE group (27.6 ng/mL) 1 h after surgery (p = 0.007). On the other hand, the median salivary cortisol concentration in the OVELA group (36.2 ng/mL) was not significantly lower (p = 0.075) compared to the OVEL group (61.9 ng/mL). At any other time, no more significant differences between the groups were discovered (0, 12 or 24 h).

Table 9 - Median and range of salivary cortisol levels (nmol/L); in each of the study groups measured throughout the duration of the study.

	Time after extubation			
Group	**0 h**	**1 h**	**12 h**	**24 h**
OVE	6.1 (1.3–24.9)	27.6 (1.5–45.5)*	3.6 (1.8–18)	3.9 (1.9–12.3)
OVEL	4.8 (3.1–17.2)	61.9 (22.9–86.4) *	7.0 (4.1–11.3)	5.2 (2.9–11.1)
OVELA	6.1 (3.5–12.3)	36.2 (5.8–93.7)	7.6 (3.5–15.2)	5.2 (2.4–15.6)

- *Statistical differences were found between OVE and OVEL group at 1 h time point (p = 0.007).

4.3. Complete blood work

Samples were acquired at baseline, at 24 h and 7 days following extubation. White blood cell concentrations increased from baseline value in all the groups at 24 h. These differences were not statistically significant between groups (t = 0 h, p = 0.087; t = 24 h, p = 0.139; t = 7 days, p = 0.659). (see Table10.)

Time

Group	0 h	24 h	7 d
OVE	11.32 ± 0.73	15.90 ± 1.03	14.05 ± 1.72
OVEL	13.95 ± 0.75	19.94 ± 1.25	13.63 ± 0.74
OVELA	13.74 ± 2.28	20.97 ± 2.68	14.79 ± 2.85

Table 10. - White blood cells concentrations (WBC) (× 10^9/L; mean ± SE) in the different groups at any time point studied. No significant differences were observed in any group from preoperative concentration.

No abnormal variation was observed in other parameters included in the complete blood work. Results of the red blood cell (RBC) counts and serum biochemistry parameters (glucose, C-reactive protein and fibrinogen) studied at all the time points in all three groups were within the reference range.

5. Discussion

5.1. UMPS

For evaluating canine postoperative pain, the UMPS is considered a valid method (Firth & Haldane, 1999; Reid et al., 2018; Farokhzad et al., 2021; Okur & Polat, 2021; Costa et al., 2019). Six categories of multiple descriptors make up UMPS, including behavioural and physiological responses. By weighing particular behaviours, some observer bias can be removed (Mich & Hellyer, 2009). We chose this scale as previous studies showed its utility in laparoscopic postoperative pain assessment (Hancock et al., 2005; Freeman et al., 2009; Brad Case et al., 2011; Devitt et al., 2005; Haraguchi et al., 2017).

Using a variety of methods for pain assessment in veterinary medicine is considered the greatest method to prevent the unbalanced weighing of a single subjective or objective measurement (Mich & Hellyer, 2009; Mathews, 2000). Nonetheless, a gold standard has not been approved for measuring pain in veterinary patients (Hancock et al., 2005).

Objective measures such as clinical parameters (e.g. heart rate, respiratory rate and temperature) or biochemical test results (e.g. cortisol) are often used as indirect measures of pain. If they are not employed in conjunction with a concurrent assessment of behavioural changes, these metrics could result in inconsistent pain assessments (Hansen et al., 1997; Fox et al., 1998; Smith et al., 1996; Smith et al., 1999; Conzemius et al., 1997).

It was intended to cause the dogs as little discomfort as possible by restricting the amount of time points for blood and saliva sampling and measuring pain levels and, consequently, limiting potential interference in the studied variables motivated by stress/pain. Recent studies have used similar intervals for pain assessment (Hu et al., 2021; Hou et al., 2019).

Unlike other studies in which the pain relief duration lasted for a limited time of 6 h (Woehlck et al., 2003; Bala et al., 2015), we observed lower pain scores in the OVELA group compared to the OVEL group 12 h after surgery. However, these differences were not statistically significant in our study. Some reports have described that patient position is a factor that may contribute to CO_2 elimination in minimally invasive surgery in humans (Eaton et al., 2009). Thus, the rotation of dogs during the procedure and the sternal or lateral recumbency in the postoperative period, which could facilitate the rapid displacement of the residual CO_2 in the abdomen and its elimination, should be confirmed. Nevertheless, further studies with more animals are required to confirm this hypothesis on dogs.

Although pain scores in the open surgery (OVE) group were significantly higher throughout compared with the OVEL and OVELA groups, none of the dogs in the OVE group again required additional pain medication. Our results suggest that the anaesthetic and analgesic protocols were appropriate for the procedures used. However, different anaesthesia and analgesia protocols for routine ovariohysterectomies have been published (Gates et al., 2020). Further research is needed to compare these protocols and improve the safety and efficacy of the most used protocols and, finally, determine whether there is potential to improve the level of patient comfort.

This study could not find that the preoperative oral administration of acetazolamide reduced postoperative pain after laparoscopic ovariectomy in dogs. Specifically, pain scores for dogs in which acetazolamide was administered were lower at all postoperative time points compared to OVE and OVEL groups, nevertheless, these differences were not statistically significant.

5.2. Serum and salivary cortisol

Cortisol levels can be measured and used as an accurate objective indicator of pain in animals (Smith et al., 1996; Benson et al., 1991; Popilskis et al., 1993; Lin et al., 1993). Different studies on dogs and cats reported a significant increase in cortisol due to surgical stress (Fox et al., 1998; Smith et al., 1996; Smith et al., 1999).

Since blood samples were only collected at baseline, the effect of stress on cortisol levels at this time was observed. However, our results conclude that the blood collection procedure was not enough to produce changes in the baseline value for each dog since we did not identify significant differences at baseline within groups. These findings have also been reported in a previous study (Kobelt et al., 2003).

The variation of cortisol concentrations followed a similar path to salivary cortisol. Therefore, a high positive correlation ($r = 0.792$; $p = 0.001$) was found between serum and salivary cortisol concentrations. These results agree with previous studies on dogs (Vincent & Michell, 1992; Giannetto et al., 2014).

Due to the fact that noninvasive salivary cortisol sampling and serum cortisol in dogs have a strong correlation (Beerda et al., 1996), it has been increasingly used for measuring stress response in this species (Vincent & Michell, 1992; Beerda et al., 1996; Beerda et al., 1998).

According to other laparoscopic ovariohysterectomy studies in dogs (Devitt et al., 2005; Ko et al., 2000), 1 h following surgery, cortisol experienced its first and highest postoperative peak. Thus, the mean salivary cortisol concentration in the OVELA group was not significantly lower than in the OVEL group. The anaesthesia protocol was the same for each group, therefore, it appears that anaesthesia by itself would not explain the variation in saliva cortisol concentrations at 1-h after surgery (see Table 4). On the other hand, the similar salivary cortisol concentration at hour 1 in the OVELA and OVE groups did not reflect a lower stress response from the effect of acetazolamide.

5.3. Complete blood count

Besides stress and pain, tissue damage from surgical procedures can stimulate, as soon as 12–36 h after surgery, the hypothalamus–hypophysis–adrenal axis to liberate glucocorticoids (cortisol), which increase the production and migration of neutrophils (Katz et al., 2012).

Laparoscopic surgery can prevent or reduce alterations such as leucocytosis (Katz et al., 2012). Our study showed an increase in WBC concentrations from baseline in all the groups 24 h after surgery; however, these differences were not statistically significant. One week after surgery, the WBC concentrations returned to baseline. Thus, our findings corroborate previous results (Alves et al., 2010), suggesting that WBC concentrations may be helpful for the evaluation of inflammatory response induced by surgery.

5.4. Acetazolamide

The duration of peritoneal CO_2 after laparoscopic surgery in people resolves within 6 h in most patients, leaving some people with small amounts of residual gas (Woehlck et al., 2003).

After a single dose of acetazolamide, peak effects occur within 2–4 h and last for 4–6 h in small animals (Plumb, 2011). Another report suggested that treatment of postoperative pain derived from CO_2 insufflation could be necessary 6 h after the procedure (Woehlck et al., 2003). This period coincided with the lasting time effect of acetazolamide (Plumb, 2011; Roberts, 1985). In light of these studies, a single dose of 25 mg/kg was administered in the OVELA group 2 h before anaesthesia induction, which corresponds to the maximal effects of acetazolamide in the dog. We decided on acetazolamide administration time based on pharmacokinetics (Plumb, 2011; Roberts, 1985). Then, administration 2 h before induction of anaesthesia allowed the peak effects of acetazolamide to be 2–4 h after its administration. Since we did not find differences between the groups in blood and salivary cortisol values at baseline, we assumed that oral administration of acetazolamide before sample collection did not interfere with these values, allowing adequate comparison of their variations postoperatively.

Although the specimens in the present study were of different breeds, they represented healthy dogs, which normally come to veterinary hospitals for elective neutering. We believe that the differences detected truly represent those observed between groups since there were no significant differences in mean group age and weight.

On the other hand, acetazolamide-induced metabolic acidosis, which reaches its maximum within 24 h in humans (Schmickl et al., 2021), could be a valuable issue. Overdose of acetazolamide (625 mg/kg) can produce severe hyperchloremic metabolic acidosis and hypokalaemia in dogs (Johnston et al., 2021). The animals included in this study received 25 mg/kg acetazolamide, and none of the animals developed any signs indicative of metabolic acidosis. Future studies should include programmed serial blood tests to monitor blood pH, arterial PCO_2 and PaO_2 to assess the relationship of these parameters to the effects of acetazolamide on acid–base status. In any case, adequate ventilation of animals during anaesthesia should always be monitored to avoid adverse side effects.

Several studies reported that laparoscopic procedures take longer than the traditional midline open technique (Culp et al., 2009; Davidson et al., 2004). In our study, the celiotomy (OVE) approach was the fastest, and surgical time was significantly longer for laparoscopic ovariectomy (OVEL and OVELA groups). We attributed the longer time of surgery to the recumbency changes produced in each laparoscopic procedure in both OVEL and OVEL groups. Using high-frequency bipolar electrocoagulation, we did not register any intraabdominal bleeding in the laparoscopic procedures.

6. Limitations

This study showed limitations because the sample size was not as significant as other studies evaluating pain. However, it is essential to highlight that pet owners do not easily choose this procedure. Then, it is challenging to obtain groups with a large animal's number. On the other hand, the number of evaluation times could have been higher to give a more detailed evaluation of the evolution of the variables throughout the postoperative period. Further studies should be performed to identify electrolyte disturbances caused by acetazolamide diuretic effects and arterial blood gas alterations that CO_2 can induce.

7. Conclusions

Although the results of the present study do not demonstrate statistically significant differences between the groups studied, there is evidence that a benefit of preoperative administration of acetazolamide may exist for the management of postoperative pain in dogs after laparoscopic surgeries. Then, we suggest that further studies with a larger number of animals are required to demonstrate that acetazolamide could be considered helpful as an adjuvant in a multimodal postoperative analgesia protocol for laparoscopic ovariectomy in dogs.

8. Author Contributions

Conceptualisation: I.T.T., R.R., J.P.S.L., C.G.V. and S.D.C. Methodology: I.T.T., R.R., J.P.S.L., C.G.V. and S.D.C. Software: J.A.C. Validation: I.T.T., R.R., J.P.S.L., C.G.V., S.D.C., J.A.C. and J.R.J. Formal analysis: I.T.T., R.R., J.P.S.L., C.G.V., S.D.C., J.A.C. and J.R.J. Investigation: I.T.T., R.R., J.P.S.L., C.G.V., S.D.C., J.A.C. and J.R.J. Resources: I.T.T., R.R., J.P.S.L., C.G.V. and S.D.C. Data curation: I.T.T. and J.A.C. Writing – original draft preparation: I.T.T., R.R., J.P.S.L., C.G.V., S.D.C., J.A.C. and J.R.J. Writing – review and editing: I.T.T., J.A.C. and J.R.J. Visualisation: I.T.T., R.R., J.P.S.L., C.G.V., S.D.C., J.A.C. and J.R.J. Supervision: I.T.T., R.R., J.P.S.L., C.G.V., S.D.C., J.A.C. and J.R.J. Project administration: I.T.T., R.R., J.P.S.L., C.G.V. and S.D.C. All authors have read and agreed to the published version of the manuscript.

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10. Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

11. Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

12. Ethics statement

The study protocol has been approved by the Faculty of Veterinary Medicine's Ethical Committee at the University of Lisbon. The procedure was carried out following the Portuguese Government´s Guidelines for Animal Care (Decreto-Lei N° 260/2012).

13. Peer review

The peer review history for this article is available at https://publons.com/publon/10.1002/vms3.1115.

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Article 3 - Ovarian teratoma removed by laparoscopic ovariectomy in a dog

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1. Abstract

An elective laparoscopic ovariectomy on a healthy dog revealed a cystic structure in the left ovary. The surgical procedure was successful. Histopathological examination showed the presence of a teratoma adjacent to the ovary. To the best of the authors' knowledge, this is the first reported case of an ovarian teratoma removed by laparoscopic ovariectomy in a dog by using a multiport laparoscopic ovariectomy technique.

Keywords: dogs, laparoscopy, ovariectomy, teratoma

Ovarian diseases are rare in female dogs [2], but the most common ovarian diseases seem to be cystic ovaries and ovarian tumors [1]. The types of tumors that have originated from the primordial germ cells of the ovary, dysgerminoma, teratoma, and embryonal carcinoma, are rare, observed in 6–12% [4] or 20% [8] of canine ovarian neoplasms. Ovariectomy is therapeutic under most conditions and is suggested by many authors [3,7]. Being less invasive and requiring less hospitalization time [6] than other procedures, laparoscopic procedures are becoming more frequently applied in veterinary and human medicine [10]. The aim of this report is to describe a single successful case of a laparoscopic ovariectomy in which a teratoma was found and removed. We show that, in the patient presented in this report, the procedure was feasible and there was no need to convert to an open procedure. A mixed-breed dog of an estimated age of approximately 2 years, clinically healthy, and weighing 23.45 kg, was admitted to the Oeiras Veterinary Hospital to undergo an elective laparoscopic ovariectomy. The dog had been adopted from a dog shelter and its previous history was unknown. The dog's owner signed a consent document advising of the risks of laparoscopic ovariectomy and the possible necessity of converting to an open celiotomy in case of an emergency situation such as hemorrhage or iatrogenic injury. The animal was kept and the procedure was performed in accordance with the Portuguese government's Animal Care Guidelines (Decree-Law No. 260/2012).

Physical examination of the dog during a pre-surgical appointment was unremarkable; the dog's respiratory and cardiac functions were considered normal and the animal was very active. Hematological and biochemical parameters were within normal limits. The dog was premedicated with acepromazine (Calmivet; Vetoquinol, Portugal) 0.05 mg/kg and tramadol (Tramadol Labesfal; Labesfal, Portugal) 5 mg/kg intravenous (IV), and anesthesia was induced with 4 mg/kg IV propofol (Propofol Lipuro; B. Braun Medical, Portugal). General anesthesia was maintained with isoflurane (Vetflurane; Virbac, Portugal) in oxygen. At induction, 8.75 mg/kg Synulox (7.0 mg amoxicillin and 1.75 mg clavulanic acid; Zoetis, Portugal) was administered subcutaneously and administration was continued postoperatively as a part of a standard protocol. The dog was closely monitored during the surgical procedure via pulse oximetry and electrocardiography. IV fluid therapy (NaCl 0.9%, Soro Fisiológico Braun Vet; B. Braun Medical) was administered during the procedure and through the subsequent 8 h. For abdominal access, the pneumoperitoneum was established with a Veress needle (2.1 mm; Richard Wolf, USA) inserted after the xiphoid process. CO_2 was provided via automatic insufflator (Electronic Insufflator 2002; Cabot Medical, USA) with a gas flow of 9 L/min at a pressure set to 9 to 11 mmHg. The first cannula (threaded, 5.5 mm in all cases) was placed 2 cm caudal to the umbilicus. A perimeter mark was made with the cannula to achieve the length of the incision and a number 11 scalpel blade was used to make the skin and linea alba incisions. The telescope (5.3 mm, 0°, Panoview; Richard Wolf) was inserted and the abdomen was evaluated using a standard clockwise rotation to access the possibility of iatrogenic injury. Additionally, two cannulas were placed 5 cm cranial and 5 cm caudal to the first cannula, under direct vision of the telescope. The dog was positioned in right lateral recumbency and the telescope, which was placed in the cranial port, was handled by an assistant, while the surgeon maneuvered the dissecting/grasping forceps in the middle port and the bipolar forceps in the caudal port.

With the subject in right lateral recumbency, observation of the left ovary area revealed the presence of a cystic structure with a hard consistency and a grossly normal non-gravid uterus. The left ovarian pedicle, proper ligament, and suspensory ligament were sealed and transected by using a high-frequency bipolar forceps with an integrated blade (RoBi plus forceps; Karl Storz, Germany). Then the proper ligament of the ovary was grasped, elevated, and tacked to the body wall by passing a 40 mm, half circle, curved cutting needle, and sutured percutaneously through the body wall. The dog was then positioned in left lateral recumbency and the instruments placed in inverted order in the three ports. The ovarian structures had no macroscopic abnormalities and the ovariectomy was performed using the same techniques as that just described. No additional masses were detected in the abdominal viscera or the abdominal wall, and no intraabdominal or peripheral lymph nodes were enlarged. There were no additional abnormalities noted during examination of the abdomen. The dog was then tilted in right lateral recumbency, the caudal cannula pulled out, the 1 cm incision extended to 2 cm and ovaries removed from the abdomen. The ovaries were checked to ensure complete removal and the pneumoperitoneum was released. The three portals were then removed and the abdominal incisions closed in two layers using a 2/0 absorbable synthetic monofilament glyconate suture (Monosyn; B. Braun) in a simple interrupted suture pattern. When the surgical procedure was completed the dog received a 0.2 mg/kg subcutaneous dose of meloxicam (Metacam; Boehringer Ingelheim, Portugal). All surgically removed structures were fixed in a 10% formalin solution and processed for histopathological evaluation. One day after the surgery, the dog was discharged home with 0.1 mg/kg meloxicam per orally for two days. On gross examination, both ovaries were enclosed within an ovarian bursa. The right and left ovaries had a regular appearance, with hard consistency and a dark brown color. Adjacent to the left ovary there was a cystic structure, measuring 2.5 cm × 2 cm × 2 cm, with a whitish, fibrous wall as well as hairs and sebaceous content (Fig. 7 and 8). Histopathological examination showed both ovaries to have no changes of pathological significance. The cystic structure next to the left ovary was surrounded by epidermis and had a lumen filled with keratin and hair. Adjacent to the cyst, there were well defined sebaceous glands, hair follicles, and several inflammatory infiltrates populated with macrophages and ceroid pigment (Fig.9). A diagnosis of a dermoid cyst (teratoma) was made.

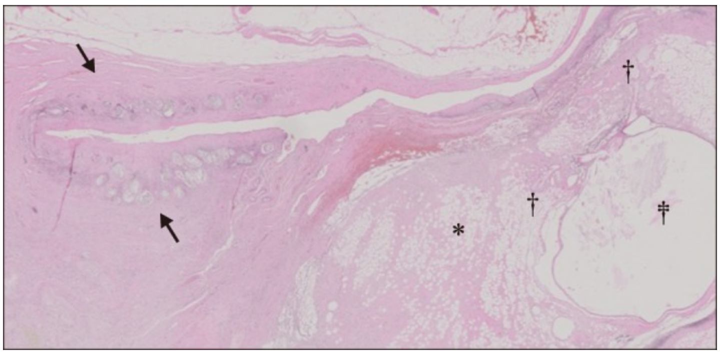

Figure 7-. Left ovary and teratoma. The arrows indicate atresic follicles. Indicated by symbols are abundant adipose tissue (*), multiple hair follicles and sebaceous glands (†), and a cystic follicle with stratified epithelium and keratin inside it (‡). H&E stain. 40×.

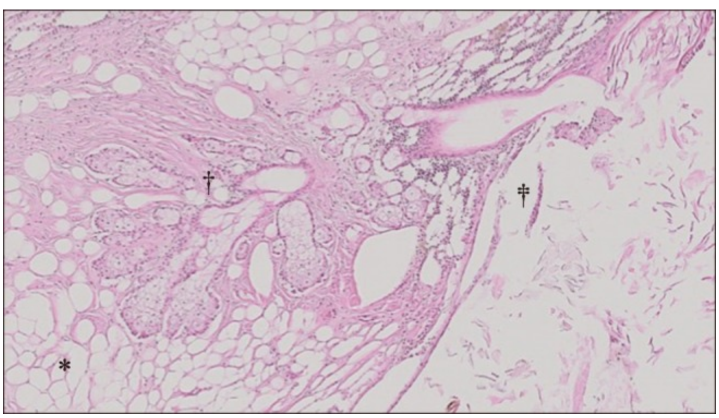

Figure 8- Left ovary and teratoma. The symbols indicate sebaceous glands and hair follicles (*), cystic follicle with stratified epithelium (†), and keratin (‡). H&E stain. 100×.

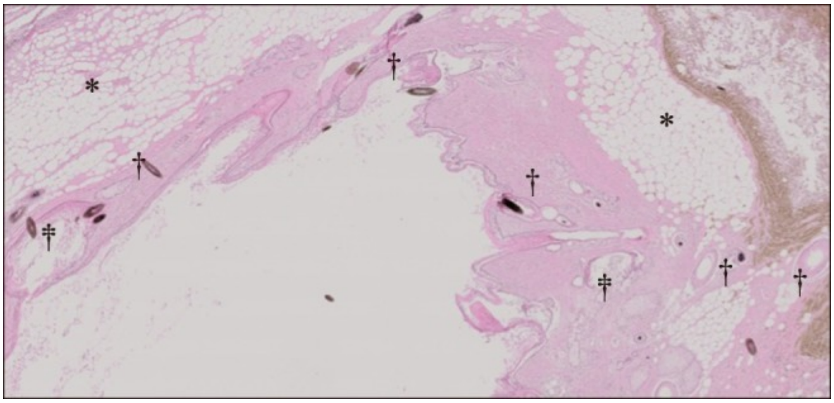

Figure 9- Left ovary and teratoma. Symbols indicate abundant adipose panicle (*) and epidermis and dermis with several hair follicles (†) and keratin (‡). H&E stain. 40×.

The cyst was lined by well-differentiated skin with keratinization, sebaceous glands, and hair follicles. On the basis of the morphological observations, the cyst was considered a teratoma attached to the left ovary. As described in previous studies, teratomas seem to occur more frequently on the left side of dogs, and generally, ovarian tumors have a predilection for the left ovary, as observed in this dog, although the reason remains unknown [5,9,11].

At follow-up, one year after surgery, the dog was clinically normal and abdominal ultrasound did not indicate the presence of anatomical alterations in the abdominal organs nor the presence of free abdominal fluid.

Laparoscopic surgery may be particularly relevant to ovarian teratoma removal, where detailed examination of the ovarian structures is required and where an open laparotomy approach would most likely require a larger abdominal incision. Based on a recent case report in which a larger teratoma was successfully removed by a single incision laparoscopic-assisted ovariohysterectomy [5], and on our experience in the present case, we suggest that laparoscopic techniques for the removal of ovarian teratomas can be used as a viable minimally invasive treatment for ovarian neoplasia.

To the authors' knowledge, this is the first reported case of a teratoma removed by laparoscopic ovariectomy in a dog by using a multiport laparoscopic ovariectomy technique. Further study involving a large sample size is required to evaluate the benefits and risks associated with laparoscopic surgery for the treatment of ovarian neoplasia in dogs.

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Chapter 4 - Discussion, Conclusion and Future Perspectives

1. Discussion

This project had the objective to contribute with laparoscopic techniques for the reproductive tract surgery in dogs. The results obtained demonstrate valid contributions that can be considered useful and applicable in the clinical surgery context that may improve the dogs experience and recovery after these surgical procedures.

In the male laparoscopic sterilization study, to the best of the authors' knowledge, at the time of publication this laparoscopic sterilization technique for dogs was described for the first time. Recently this technique of devascularization was considered a valid alternative to orchiectomy for dogs (Libermann et al., 2020) regarding the inhibition of male fertility and sexual behavior (Fransson & Mayhew, 2022). Furthermore, no other controlled study has previously shown postoperative pain assessment after laparoscopic sterilization in dogs. Several complications are associated with laparoscopic surgery, such as viscera perforation, tissue damage caused by energy application, abdominal cavity access, and pneumoperitoneum (McClaran & Buote, 2009). Generally, the less experienced and trained the surgeon is, the more frequent the complications (Tapia-Araya et al., 2015). In this study, the surgeon's experience and the bipolar instrument used contributed to surgical procedures without complications.

Results show that the surgical technique was simple, feasible, and quick to perform without intraoperative complications or abnormalities requiring conversion to open laparotomy. The execution of both procedures did not differ significantly by time. The OR surgery requires several sutures, whereas, in LS, the most time-consuming step is the trocar placement and pneumoperitoneum establishment.

The Veress needle was used successfully to maintain the pneumoperitoneum through all the laparoscopic surgeries. It was introduced carefully into the abdominal wall without visceral damage due to a tight manual elevation before its introduction. Despite the risk of visceral damage, we consider using the Veress needle easy and less traumatic, and it avoids the difficulties associated with the modified Hasson technique (Granados, Usón, Martínez, Sánchez Margallo, & Perez, 2017).

Trendelenburg positioning enabled the displacement of the digestive organs towards the diaphragmatic area, allowing precise observation of reproductive structures. The trocars placement permitted inspection of the abdominal cavity and a broad view of and access to the pelvic organs and tissues. The use of only two trocars had the following advantages: surgery was performed by a solo surgeon and with minor surgical trauma to the abdominal wall, which contributes to less post-operative pain (Case et al., 2011).

The high-frequency bipolar forceps used were effective, user-friendly, and safe in coagulating and cutting vas deferens and artery and vein plexus. Since the scissors are integrated into the instrument, fewer surgical maneuvers and instruments were needed, reducing surgical time. Like other sealing and cutting instruments, the RobiPlus caused minimal smoke, resulting in a slight brief loss of observation in the surgical field (Mayhew & Brown, 2007).

Contrary to previous methods described for hemostasis of the spermatic cord, in this technique, no foreign material (metallic or plastic clips) was used and left in the animal. The sutures applied in the wounds from trocars proved to be entirely sufficient. The animals did not show interest in the wounds after surgery. The dog caretakers reported their well-being and high activity level, confirming the short period of convalescence needed due to low level of pain in the abdominal integument.

The aesthetical appearance was another advantage of laparoscopic sterilization. Although the evolution of testicular volume was not measured in detail in our study, we detected, as Mathon et al. observed, that the testicular volume was increased seven days after surgery and decreased by 90% at the end of 60 days (Mathon et al., 2011). This testicular atrophy happens due to ischemic degeneration (Mathon et al., 2011), indicating a loss of function and a complete castration. This surgical technique allows the male dog to maintain the testis in the scrotum, although in a smaller size, which may pose a more attractive surgical option to male owners, who often avoid the castration of dogs (Wildt et al., 1981).

Concerning postoperative pain, we found that LS was less painful in dogs when compared with OR surgery. Pain scores and cortisol measurements were considered effective in assessing pain levels. The use of multiple methods to assess pain in animals is accepted as the best way to avoid the unbalanced weighing of any single subjective or objective measurement (K a Mathews, 2000) (Mich & Hellyer, 2008). Hence, no gold standard has been established for pain measurement in veterinary patients (Hancock et al., 2005). Objective physiologic (heart rate, respiratory rate, temperature) and biochemical (cortisol) measurements are usually used as indirect measures of pain, but they give inconsistent assessments of pain if not used with simultaneous evaluation of behavioral changes. (Bernard D. Hansen, Hardie, & Carroll, 1997) (Fox, Mellor, Lawoko, Hodge, & Firth, 1998)(J. D. Smith, Allen, Quandt, & Tackett, 1996)(Conzemius, Hill, Sammarco, & Perkowski, 1997)(BENSON et al., 1991).

The measurement of cortisol values has been helpful as an objective indicator of animal pain (J. D. Smith et al., 1996)(Benson & Wheaton, 1991) (Popilskis, Kohn, Laurent, & Danilo, 1993)(Lin et al., 1993), and it has been described as significantly increasing due to surgical stress in dogs and cats (Fox et al., 1998) (JD Smith, Allen, & Quandt, 1996) (J. D. Smith et al., 1999). Similar results were observed in our study, where a correlation was found between serum and salivary cortisol concentrations. As salivary cortisol is highly correlated with serum cortisol in dogs, and its sampling is non-invasive (Beerda, Schilder, Janssen, & Mol, 1996), it has been chosen to measure stress response in these animals (Beerda et al., 1996) (Beerda, Schilder, Van Hooff, De Vries, & Mol, 1998) (Vincent & Michell, 1992). According to other laparoscopic sterilization studies in dogs (Devitt et al., 2005) (Ko, Mandsager, Lange, & Fox, 2000), the first and highest postoperative peak of cortisol in this study occurred one hour after the surgery. Hence, the mean salivary and serum cortisol concentrations in the LS group were significantly lower than in the OR group. Since the anesthesia protocol was the same for every group, anesthesia alone apparently would not explain the difference in cortisol concentrations at 1 h postoperatively. Thus, it is possible that the low cortisol concentrations at hour one in the LS group may genuinely reflect the lower level of pain from the less traumatic surgical procedure.

In dogs, increases in serum CRP concentrations have been described after different surgical procedures; the more severe the tissue trauma made, the higher the magnitude of CRP increase (Yamamoto et al., 1993)(Martinez-Subiela, 2003). Data confirm that canine serum CRP can be used to differentiate variable degrees of inflammatory activity after elective surgical procedures (Kjelgaard-Hansen et al., 2013). At the time of suture removal—usually seven days after surgery—CRP concentration is considered a more useful tool to monitor postsurgical progress than WBC counts in surgical procedures without clinical complications (Yamamoto et al., 1993)(JE Smith, 1997). In this study, the use of meloxicam (non-steroidal anti-inflammatory drugs—NSAIDs) could also influence changes in serum CRP. However, it is known that CRP production is mainly dependent on pro-inflammatory cytokines, and NSAID only regulates prostaglandin synthesis (Kjelgaard-Hansen et al., 2013).

As seen in another study (CONNER, ECKERSALL, FERGUSON, & DOUGLAS, 2018), the maximum peak CRP concentration was 24 h in our groups. The CRP concentrations were lower at all times in the LS group, but only at 168 h after surgery, did we observe a significant difference between the CRP concentrations. Other studies comparing inflammation between open and laparoscopic procedures also found significantly lower concentrations of CRP for laparoscopic procedures (Kjelgaard-Hansen et al., 2013)(S.-X. Zhang, Zhang, Zhang, Wang, & Pan, 2013)(Stedile et al., 2009).

The present data confirms that laparoscopic castration in dogs induces less inflammation, as it is a surgical procedure that produces less tissue trauma.

On a young animal, the recent recommendations are to practice traditional orchiectomy and reserve the laparoscopic sterilization for animals where there is a high risk of complications such as chronic dermatological conditions, self-multilation, from a prescrotal wound (Fransson & Mayhew, 2022).

In the second study for evaluating canine postoperative pain, the UMPS is considered a valid method (Firth & Haldane, 1999; Reid et al., 2018; Farokhzad et al., 2021; Okur & Polat, 2021; Costa et al., 2019). Six categories of multiple descriptors make up UMPS, including behavioural and physiological responses. By weighing particular behaviours, some observer bias can be removed (Mich & Hellyer, 2009). We chose this scale as previous studies showed its utility in laparoscopic postoperative pain assessment (Hancock et al., 2005; Freeman et al., 2009; Brad Case et al., 2011; Devitt et al., 2005; Haraguchi et al., 2017).

Using a variety of methods for pain assessment in veterinary medicine is considered the greatest method to prevent the unbalanced weighing of a single subjective or objective measurement (Mich & Hellyer, 2009; Mathews, 2000). Nonetheless, a gold standard has not been approved for measuring pain in veterinary patients (Hancock et al., 2005).

Objective measures such as clinical parameters (e.g. heart rate, respiratory rate and temperature) or biochemical test results (e.g. cortisol) are often used as indirect measures of pain. If they are not employed in conjunction with a concurrent assessment of behavioural changes, these metrics could result in inconsistent pain assessments (Hansen et al., 1997; Fox et al., 1998; Smith et al., 1996; Smith et al., 1999; Conzemius et al., 1997).

It was intended to cause the dogs as little discomfort as possible by restricting the amount of time points for blood and saliva sampling and measuring pain levels and, consequently, limiting potential interference in the studied variables motivated by stress/pain. Recent studies have used similar intervals for pain assessment (Hu et al., 2021; Hou et al., 2019).

Unlike other studies in which the pain relief duration lasted for a limited time of 6 h (Woehlck et al., 2003; Bala et al., 2015), we observed lower pain scores in the OVELA group compared to the OVEL group 12 h after surgery. However, these differences were not statistically significant in our study. Some reports have described that patient position is a factor that may contribute to CO_2 elimination in minimally invasive surgery in humans (Eaton et al., 2009). Thus, the rotation of dogs during the procedure and the sternal or lateral recumbency in the postoperative period, which could facilitate the rapid displacement of the residual CO_2 in the abdomen and its elimination, should be confirmed. Nevertheless, further studies with more animals are required to confirm this hypothesis on dogs.

Although pain scores in the open surgery (OVE) group were significantly higher throughout compared with the OVEL and OVELA groups, none of the dogs in the OVE group again required additional pain medication. Our results suggest that the anaesthetic and analgesic protocols were appropriate for the procedures used. However, different anaesthesia and analgesia protocols for routine ovariohysterectomies have been published (Gates et al., 2020). Further research is needed to compare these protocols and improve the safety and efficacy of the most used protocols and, finally, determine whether there is potential to improve the level of patient comfort.

This study could not find that the preoperative oral administration of acetazolamide reduced postoperative pain after laparoscopic ovariectomy in dogs. Specifically, pain scores for dogs in which acetazolamide was administered were lower at all postoperative time points compared to OVE and OVEL groups, nevertheless, these differences were not statistically significant.

Cortisol levels can be measured and used as an accurate objective indicator of pain in animals (Smith et al., 1996; Benson et al., 1991; Popilskis et al., 1993; Lin et al., 1993). Different studies on dogs and cats reported a significant increase in cortisol due to surgical stress (Fox et al., 1998; Smith et al., 1996; Smith et al., 1999).

Since blood samples were only collected at baseline, the effect of stress on cortisol levels at this time was observed. However, our results conclude that the blood collection procedure was not enough to produce changes in the baseline value for each dog since we did not identify significant differences at baseline within groups. These findings have also been reported in a previous study (Kobelt et al., 2003).

The variation of cortisol concentrations followed a similar path to salivary cortisol. Therefore, a high positive correlation ($r = 0.792$; $p = 0.001$) was found between serum and salivary cortisol concentrations. These results agree with previous studies on dogs (Vincent & Michell, 1992; Giannetto et al., 2014). Due to the fact that noninvasive salivary cortisol sampling and serum cortisol in dogs have a strong correlation (Beerda et al., 1996), it has been increasingly used for measuring stress response in this species (Vincent & Michell, 1992; Beerda et al., 1996; Beerda et al., 1998).

According to other laparoscopic ovariohysterectomy studies in dogs (Devitt et al., 2005; Ko et al., 2000), 1 h following surgery, cortisol experienced its first and highest postoperative peak. Thus, the mean salivary cortisol concentration in the OVELA group was not significantly lower than in the OVEL group. The anaesthesia protocol was the same for each group, therefore, it appears that anaesthesia by itself would not explain the variation in saliva cortisol concentrations at 1-h after surgery (see Table 4). On the other hand, the similar salivary cortisol concentration at hour 1 in the OVELA and OVE groups did not reflect a lower stress response from the effect of acetazolamide.

Besides stress and pain, tissue damage from surgical procedures can stimulate, as soon as 12–36 h after surgery, the hypothalamus–hypophysis–adrenal axis to liberate glucocorticoids (cortisol), which increase the production and migration of neutrophils (Katz et al., 2012).

Laparoscopic surgery can prevent or reduce alterations such as leucocytosis (Katz et al., 2012). Our study showed an increase in WBC concentrations from baseline in all the groups 24 h after surgery; however, these differences were not statistically significant. One week after surgery, the WBC concentrations returned to baseline. Thus, our findings corroborate previous results (Alves et al., 2010), suggesting that WBC concentrations may be helpful for the evaluation of inflammatory response induced by surgery.

After a single dose of acetazolamide, peak effects occur within 2–4 h and last for 4–6 h in small animals (Plumb, 2011). Another report suggested that treatment of postoperative pain derived from CO_2 insufflation could be necessary 6 h after the procedure (Woehlck et al., 2003). This period coincided with the lasting time effect of acetazolamide (Plumb, 2011; Roberts, 1985). In light of these studies, a single dose of 25 mg/kg was administered in the OVELA group 2 h before anaesthesia induction, which corresponds to the maximal effects of acetazolamide in the dog. We decided on acetazolamide administration time based on pharmacokinetics (Plumb, 2011; Roberts, 1985). Then, administration 2 h before induction of anaesthesia allowed the peak effects of acetazolamide to be 2–4 h after its administration. Since we did not find differences between the groups in blood and salivary cortisol values at baseline, we assumed that oral administration of acetazolamide before sample collection did not interfere with these values, allowing adequate comparison of their variations postoperatively.

Although the specimens in the present study were of different breeds, they represented healthy dogs, which normally come to veterinary hospitals for elective neutering. We believe that the differences detected truly represent those observed between groups since there were no significant differences in mean group age and weight.

On the other hand, acetazolamide-induced metabolic acidosis, which reaches its maximum within 24 h in humans (Schmickl et al., 2021), could be a valuable issue. Overdose of acetazolamide (625 mg/kg) can produce severe hyperchloremic metabolic acidosis and hypokalaemia in dogs (Johnston et al., 2021). The animals included in this study received 25 mg/kg acetazolamide, and none of the animals developed any signs indicative of metabolic acidosis. Future studies should include programmed serial blood tests to monitor blood pH, arterial PCO_2 and PaO_2 to assess the relationship of these parameters to the effects of acetazolamide on acid–base status. In any case, adequate ventilation of animals during anaesthesia should always be monitored to avoid adverse side effects.

Several studies reported that laparoscopic procedures take longer than the traditional midline open technique (Culp et al., 2009; Davidson et al., 2004). In our study, the celiotomy (OVE) approach was the fastest, and surgical time was significantly longer for laparoscopic ovariectomy (OVEL and OVELA groups). We attributed the longer time of surgery to the recumbency changes produced in each laparoscopic procedure in both OVEL and OVEL groups. Using high-frequency bipolar electrocoagulation, we did not register any intraabdominal bleeding in the laparoscopic procedures. This study showed limitations because the sample size was not as significant as other studies evaluating pain. However, it is essential to highlight that pet owners do not easily choose this procedure. Then, it is challenging to obtain groups with a large animal's number. On the other hand, the number of evaluation times could have been higher to give a more detailed evaluation of the evolution of the variables throughout the postoperative period. Further studies should be performed to identify electrolyte disturbances caused by acetazolamide diuretic effects and arterial blood gas alterations that CO_2 can induce.

Taking into consideration the review on chapter II, at this point in time after the compilation if the final work, there is the necessity to debate some methods chosen.

The evaluation of pain in these studies was fundamental to assess the efficacy of the multimodal anesthetic and analgesic protocol.

In the past several years the most used validated pain scale has been the Glasgow Composite Measures Pain Scale (CMPS). To increase the practicality of this scale in the clinical setting, a shortened form was developed. This has been tested in veterinary hospital universities and showed significant and consistent statistical differences in scores across different hospital sites (Jacky Reid et al., 2007)(Murrell, Psatha, Scott, Reid, & Hellebrekers, 2008). It remains one of the most used pain scales in dogs (Murrell et al., 2008) (Testa, Reid, Scott, Murison, & Bell, 2021). This scale allows the quantification of individual pain, assesses pain management, provides an intervention score to allow the determination of the necessity of additional analgesia (Jacky Reid et al., 2007). Is has been also been validated to be used in studies to evaluate the effectiveness of a new drug in a postoperative period (Sharkey, 2013).

Contrary to the CMPS that evaluates the animal’s behaviour, response to touch, posture and activity, the UMPS assess alterations in several objective physiological measurements such as heart rate, respiratory rate, pupil dilatation, salivation and rectal temperature. As seen in humans, the variation in physiologic measurements can be related to pain but also be related simply to physical exertion, fever and anxiety (Drendel, Kelly, & Ali, 2011). This can explain why we did not find significantly changes in pain assessments with the UMPS in our groups.

Concerning the anesthetic protocol, the premedication was equal in the castration and ovariectomy, acepromazine and tramadol with propofol as an anesthesia inductor.

Tramadol is a centrally acting analgesic with a dual mechanism of action (weak mu opioid agonist and inhibition of serotomin and norepinephrine reuptake) among other mechanisms. The level of evidence for using tramadol in dogs is low, due to species inhability to produce the M1 metabolite that has mu opioid agonist effects (B. P. Monteiro et al., 2023). Both acepromazine and propofol have hypotensive effects. Acepromazine decreases arterial blood pressure due to its alpha-antagonism and consequent vasodilatation (W. W. Muir & Hubbell, 1985) (Wagner & Brodbelt, 1997). Similarly, propofol causes significant decreases in heart rate, mean arterial pressure and a direct ionotropic effect leading to hypotension (Short & Bufalari, 1999). The fluidtherapy administered during anesthesia maintained normotension in the face of anesthetic-induced vasodilation (Bolaji-Alabi, Solanke, & Adetunji, 2018).

It is known that sodium channels, opioid receptors and NMDA receptors play a crucial role in the long-term potentiation and the central sensitization as mentioned before in this tesis. Taken into consideration the knowledge stated the premedication protocol could have incorporated dexmedetomidine, an alpha-2 adrenoreceptor agonist that unlike acepromazine produces analgesia, sedation, hypnosis and muscle relaxation (B. P. Monteiro et al., 2023). Methadone, contrary to tramadol is syntetic full mu opioid receptor agonist and reported to be an antagonist at the NMDA receptor and inhibites norepinephrine reuptake (Bradbrook & Clark, 2018). Methadone's and dexmedetomidine's properties combined can act in several mechanisms to prevent the development of central sensitization with less pain in the postoperative period. And for reference it was the premedication protocol chosen in a recent study of postoperative pain evaluation by owners in dogs undergoing either laparoscopic or open ovariectomy (Fuertes-Recuero et al., 2024).

In the female study the anesthetic protocol included a fentanyl bolus followed by a fentanyl constant rate infusion (CRI). CRI of fentanyl has shown to be efficient in suppressing the nociceptive response during surgery, with low requirements for intraoperative analgesic rescues (Marques et al., 2023). We believe this administration managed the short comings of the premedication protocol because there was no necessity of intraoperative analgesic rescues.

In the third paper laparoscopic surgery may be particularly relevant to ovarian teratoma removal, where detailed examination of the ovarian structures is required and where an open laparotomy approach would most likely require a larger abdominal incision. Based on a recent case report in which a larger teratoma was successfully removed by a single incision laparoscopic-assisted ovariohysterectomy [5], and on our experience in the present case, we suggest that laparoscopic techniques for the removal of ovarian teratomas can be used as a viable minimally invasive treatment for ovarian neoplasia.

2. Conclusion

In the first study, the novel laparoscopic sterilization technique was minimally invasive and allowed excellent visualization of the reproductive structures. Significant differences were found in pain-associated variables in the laparoscopic group at one hour postoperative, suggesting that this group had less pain. The surgeries did not have any complications, and the animals recovered uneventfully. Therefore, the authors believe that this laparoscopic castration is a safe and beneficial surgical alternative to traditional orchiectomy in dogs.

In the second study although the results do not demonstrate statistically significant differences between the groups studied, there is evidence that a benefit of preoperative administration of acetazolamide may exist for the management of postoperative pain in dogs after laparoscopic surgeries. Then, we suggest that further studies with a larger number of animals are required to demonstrate that acetazolamide could be considered helpful as an adjuvant in a multimodal postoperative analgesia protocol for laparoscopic ovariectomy in dogs. In the third paper to the authors' knowledge, this is the first reported case of a teratoma removed by laparoscopic ovariectomy in a dog by using a multiport laparoscopic ovariectomy technique.

Some final considerations concerning the neutering decision on dogs nowadays. Negative consequences on canine health after spaying have become more known as more studies show its implications with diseases, behavior and welfare. Joint disorders, cancers, male aggression are more prevalent in animals neutered before one year of age. The recent paradigm is to individually assess the lifestyle, the breed, age, genetic predispositions before deciding whether or not to neuter , and when to neuter a specific dog (Hart, L.A., 2024).

3. Future Perspectives

In the future, it would be interesting to perform further studies, including more time points for the pain analysis—both with CMPS-SF score and cortisol respecting to the first study.

In the second study however, further research with a larger sample size is needed to confirm this and to determine if acetazolamide should be included in a multimodal postoperative analgesia protocol for laparoscopic ovariectomy in dogs.

In the third paper, further study involving a large sample size is required to evaluate the benefits and risks associated with laparoscopic surgery for the treatment of ovarian neoplasia in dogs.

Since long-term follow-up concerning the effects of leaving the devascularized testicle *in situ* in the scrotum is lacking, it would be usefull to accompany the evolution of these patients.

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