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OVARIECTOMIA EM CADELAS POR NOTES HÍBRIDA OU TOTAL: ESTUDO DE VIABILIDADE TÉCNICA, ANÁLISES ÁLGICA E DE CORTISOL PLASMÁTICO

DISSERTAÇÃO DE MESTRADO

Marcella Teixeira Linhares

Santa Maria, RS
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OVARIECTOMIA EM CADELAS POR NOTES HÍBRIDA OU TOTAL: ESTUDO DE VIABILIDADE TÉCNICA, ANÁLISES ÁLGICA E DE CORTISOL PLASMÁTICO

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Orientador: Prof. Maurício Veloso Brun

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elaborada por
Marcella Teixeira Linhares

como requisito parcial para obtenção do grau de
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"Dias inteiros de calmaria, noites de ardentia, dedos no leme e olhos no horizonte,
descobri a alegria de transformar distâncias em tempo.
Um tempo em que aprendi a entender as coisas do mar,
a conversar com as grandes ondas e não discutir com o mal tempo.
A transformar o medo em respeito, o respeito em confiança.
Descobri como é bom chegar quando se tem paciência.
E para se chegar onde quer que seja,
aprendi que não é preciso dominar a força, mas a razão.
É preciso antes de mais nada querer."

(Amyr Klink)

RESUMO

Dissertação de Mestrado
Programa de Pós-Graduação em Medicina Veterinária
Universidade Federal de Santa Maria, RS, Brasil

OVARIECTOMIA EM CADELAS POR NOTES HÍBRIDA OU TOTAL: ESTUDO DE VIABILIDADE TÉCNICA, ANÁLISES ÁLGICA E DE CORTISOL PLASMÁTICO

AUTOR: MARCELLA TEIXEIRA LINHARES
ORIENTADOR: MAURÍCIO VELOSO BRUN
Data e Local da Defesa: Santa Maria, 20 de janeiro de 2017.

O presente estudo busca comparar duas novas técnicas de ovariectomia (OVE) por NOTES transvaginal em cadelas quanto a viabilidade técnica, tempo cirúrgico e incidência de complicações trans e pós-operatórias, bem como quanto à concentração de cortisol plasmático e escores de dor no período pós-operatório. Para tanto, uma amostra de 16 cadelas foi separada em dois grupos, sendo os pacientes do GNH (n=8) submetidos à OVE por NOTES transvaginal híbrida e os do grupo GNT (n=8) submetidas à OVE por NOTES transvaginal total. Os tempos cirúrgicos não diferiram entre os grupos experimentais. Nenhum dos cães requereu resgate analgésico nos períodos trans ou pós-operatório. Quanto ao escore de dor, os grupos não diferiram significativamente entre si na maioria dos tempos estudados, com exceção da avaliação nas 72 horas após extubação, na escala visual analógica (EVA), onde o GNH demonstrou índices mais elevados de dor que o grupo GNT. Os valores do cortisol plasmático não diferiram entre os grupos na maioria dos tempos, exceto no basal, onde os do grupo GNT foram superiores. Os valores mais elevados de cortisol para ambos os grupos experimentais foram encontrados no pós-operatório imediato, porém considerados significativos apenas para o grupo GNH. Ambas as técnicas de NOTES propostas se mostraram viáveis e seguras na realização de OV em cadelas, desde que executadas em pacientes selecionados quanto às condições anatômicas. As duas técnicas apresentaram resultados semelhantes para os parâmetros avaliados, com baixas taxas de complicações e reduzido estímulo álgico nos períodos trans e pós-operatório.

Palavras-chave: Cão. Cirurgia sem incisão. Cirurgia laparoscópica. Cirurgia por orifícios naturais. Dor.

ABSTRACT

Master's Dissertation
Postgraduate Program in Veterinary Medicine
Federal University of Santa Maria – RS – Brazil

OVARIECTOMY IN BITCHES BY HYBRID OR PURE NOTES: TECHINICAL VIABILITY, ALGIC AND PLASMA CORTISOL CONCENTRATIONS STUDY

AUTHOR: MARCELLA TEIXEIRA LINHARES
ADVISER: MAURÍCIO VELOSO BRUN
Date and Place of Defense: Santa Maria, January 20th, 2017

The purpose of this study was to compare two transvaginal NOTES ovariectomy (OVE) techniques in bitches regarding technical issues, surgical time, complications, as well as plasma cortisol concentration and postoperative pain scores. A sample of 16 dogs was divided into two groups: GNH patients (n=8) underwent transvaginal hybrid-NOTES OVE; and GNT dogs (n=8) were submitted to transvaginal total-NOTES OVE. The surgical time was not different between groups. None of the dogs required rescue analgesic during or after surgery at any time point. Groups did not differ significantly from each other regarding pain scores, except for 72 hours after extubation on visual analogue scale (EVA) assessment. GNH group presented higher pain score than GNT at 72 hours. Plasma cortisol did not differ between groups in most time points. However, GNT group presented higher plasma cortisol at the baseline. Cortisol peaked at the immediate postoperative period in both groups, but was significantly raised only in the GNH group. Both NOTES OVE techniques were feasible and safe in dogs. However, proper patient selection is advised. Techniques showed similar results for all assessment. Both techniques presented low complications rates and reduced pain during and after surgery.

Keywords: Dog. Scarless surgery. Laparoscopic surgery. Natural orifice surgery.Pain.

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INTRODUÇÃO

Desde o início da era moderna da cirurgia minimamente invasiva, tem ocorrido uma lenta, porém constante, evolução e o refinamento das técnicas laparoscópicas aplicadas a pequenos animais (MAYHEW, 2011). Tais técnicas alcançaram aceitação em razão de suas vantagens em relação à cirurgia convencional que incluem menor lesão tecidual, menor risco de deiscência ou outras complicações relacionadas às feridas cirúrgicas, menos estresse e dor pós-operatória, menor morbidade, melhor cosmese, mais curto período de hospitalização e um rápido retorno a atividade normal (DAVIDSON et al., 2004; DEVITT et al., 2005; HANCOCK et al., 2005).

Uma progressão natural para técnicas laparoscópicas mais complexas tem ocorrido de forma mais recente, nas quais a redução no tamanho e número de portais é uma tendência (MAYHEW, 2011). Dentro desta evolução destaca-se a modalidade da *Natural Orifice Translumenal Endoscopic Surgery* (NOTES), que pode ser entendida como o conjunto de intervenções diagnósticas ou terapêuticas, aplicadas às cavidades torácica e abdominal, a partir de orifícios naturais (SHAFI et al., 2006; BARON, 2007; BERGMAN e MELVIN et al., 2008).

Na NOTES, o acesso à cavidade abdominal pode ser realizado através das abordagens transgástrica, transcolônica, transvesical e transvaginal, enquanto que toracoscopias podem ser realizadas via transesofágica (BERGMAN e MELVIN et al., 2008). Quanto a sua classificação, a abordagem total ou pura, refere-se a procedimentos realizados apenas com ferramentas flexíveis ou rígidas colocados transluminalmente, sem assistência laparoscópica, enquanto o termo híbrido é usado para descrever os procedimentos que envolvem qualquer tipo de assistência laparoscópica transabdominal (MORIS et al., 2012).

Dentre os acessos descritos para NOTES, a via transvaginal detém a maior popularidade, correspondendo a até 84% dos acessos relatados para procedimentos em humanos (AUYANG et al., 2011), incluindo colecistectomia, apendicectomia, reparação de hérnia, nefrectomia, esplenectomia e gastrotomia (LIU et al., 2013). Esta via parece ser menos relacionada a complicações, como fístulas e vazamento de conteúdo, por ser de fácil acesso e descontaminação e por apresentar oclusão segura, sob visão direta (MORIS et al., 2012).

Os benefícios potenciais da abordagem endoscópica transluminal incluem a ausência de cicatrizes cutâneas, eliminando a possibilidade de infecção, formação de hérnia ou aderências na parede abdominal, além da redução na dor pós-operatória, no tempo de internação hospitalar

e período de convalescença (SHAFI et al., 2006; WAGH et al., 2006; PEARL e PONSKY, 2008; MARTÍNEK et al, 2012). A despeito de todos os benefícios supracitados e do crescente número de estudos envolvendo cirurgia transluminal em animais, observa-se que poucas são as pesquisas que visam a aplicação prática dessa modalidade na rotina de animais de companhia e a maioria se concentra no desenvolvimento de modelos experimentais para aplicação em humanos.

A ovariectomia (OVE), com finalidade de esterilização eletiva de pequenos animais, ainda é uma técnica pouco realizada no Brasil em contraste com a ovariohisterectomia (OVH). A predileção pela OVH, baseia-se principalmente na preocupação com futuras alterações uterinas, embora não exista nenhuma evidência científica que suporte tal receio (DETORA e MCCARTHY, 2011). Sabe-se que a hiperplasia endometrial cística e piometra não se desenvolvem em cadelas ovariectomizadas, a menos que sejam administrados progestágenos (OKKENS et al., 1997) e que a chance de desenvolvimento de tumores uterinos malignos é tão baixa quanto 0,003% (BRODEY, 1967). Ademais, a ovariectomia potencialmente induz menor trauma cirúrgico (incisões menores, uma melhor visibilização do pedículo ovariano e menos risco de complicações associadas manipulação cirúrgica do útero) e reduz o tempo cirúrgico e anestésico (OKKENS et al., 1997; VAN GOETHEM et al., 2006).

Em relação às técnicas de ovariectomia laparoscópica em cães, vários acessos são descritos e o número de portais varia de acordo com os diferentes autores, podendo-se empregar três (TAPIA-ARAYA et al., 2015), dois (DUPRÉ et al., 2009) ou mesmo um único portal de acesso (DUPRÉ et al., 2009; MANASSERO et al., 2012; TAPIA-ARAYA et al., 2015). Ainda que já descritas as técnicas de OVH eletiva em cães nas modalidades NOTES transvaginal total (SILVA et al., 2012) e NOTES transvaginal híbrida (BRUN et al., 2011), no que concerne à OVE via NOTES em cães, encontram-se apenas estudos utilizando a abordagem transgástrica, com o uso de endoscopia flexível (FREEMAN et al., 2010; FREEMAN et al., 2011).

Os benefícios da ovariectomia laparoscópica já são bem estabelecidos, incluindo excelente visão cirúrgica perioperatória, diminuição do estresse cirúrgico, dor pós-operatória e morbidade e um retorno mais rápido à atividade normal (CULP et al., 2009). Embora benefícios semelhantes já tenham sido descritos para a OVH por NOTES transvaginal nestas duas modalidades (BRUN et al., 2011; BASSO et al., 2014; SILVA et al., 2012), se faz necessário avaliar as técnicas de OVE propostas quanto a sua exequibilidade, segurança e reduzido estímulo álgico para que se possa realmente incluí-las como opção no conjunto de procedimentos laparoscópicos disponíveis.

A avaliação da dor em animais pode ser bastante difícil, porque diferente do paciente humano, os animais não podem verbalizar e manifestam sua percepção dolorosa predominantemente por alterações nos parâmetros fisiológicos e nos padrões comportamentais (HELLYER et al., 2007). Neste sentido, numerosas escalas unidimensionais ou multidimensionais têm sido utilizadas para avaliação da dor em animais, embora até o presente momento não exista qualquer padrão ouro estabelecido para esta finalidade (HOLTON et al., 1998). Por este motivo, é bem aceito que esta avaliação seja melhor executada associando-se várias escalas, a fim de aumentar a sensibilidade e evitar ponderação indevida de qualquer medida subjetiva ou objetiva única (MATHEWS, 2000; HELLYER et al., 2007).

A maioria das escalas utilizadas atualmente são adaptações de sistemas desenvolvidos para seres humanos (HOLTON et al., 1998). A escala visual analógica (EVA) (ANEXO A) é um sistema de pontuação subjetivo e unidimensional, que avalia apenas a intensidade da dor, e consiste basicamente em uma linha reta horizontal de 100mm de comprimento, que descreve a intensidade dolorosa em cada uma das extremidades da linha (GAYNOR e MUIR, 2009). A escala de dor da Universidade de Melbourne (FIRTH e HALDANE, 1999) (Anexo B) é uma escala multidimentsional, baseada em respostas comportamentais e fisiológicas específicas, incluindo descritores múltiplos em seis categorias.

A secreção de hormônios hipofisários, dentre os quais o hormônio adrenocorticotrófico (ACTH), que estimula a secreção de corticosteróides, tende a ser alterada em muitas situações estressantes e dolorosas (SACKMAN, 1991). A concentração de cortisol plasmático tem sido muito utilizada para medir dor induzida e estresse em cães após cirurgias (HANSEN et al., 1997; KO et al., 2000). Este parece ser um bom indicador no modelo canino e seu rápido declínio após procedimentos laparoscópicos em comparação com a cirurgia aberta pode indicar um grau menor, ou uma resolução mais rápida, de estresse cirúrgico (KO et al., 2000; ZANELLA et al., 2009).

Deste modo, este estudo foi conduzido com o objetivo de avaliar a viabilidade e segurança de duas novas técnicas de ovariectomia laparoscópica por NOTES total e NOTES híbrida pelo acesso transvaginal em cães, com base nos tempos cirúrgicos, dificuldades na execução das técnicas e complicações observadas nos períodos trans e pós-operatório. Além disso, busca-se comparar as duas técnicas quanto ao escore de dor e variação na concentração de cortisol plasmático induzidos no período pós-operatório.

ARTIGO

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OVARIECTOMY IN DOGS BY HYBRID OR TOTAL NOTES: TECHNICAL FEASIBILITY STUDY, PAIN ASSESSMENT, AND SERUM CORTISOL MEASUREMENT

Ovariectomy in dogs by hybrid or total NOTES: technical feasibility study, pain assessment, and serum cortisol measurement

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Abstract

Objective: To compare the technical feasibility, surgical time, surgical complications, postoperative pain, and serum cortisol concentration in two new ovariectomy (OVE) methods in dogs: (1) hybrid NOTES (HNG) and (2) total NOTES (TNG).

Study design: Prospective randomized clinical trial.

Animals: Healthy and sexually intact bitches (n = 16).

Methods: The selected patients were randomly assigned to two groups: HNG (n=8) and TNG (n=8). Pain was assessed before the surgical procedure (baseline) and at 2, 4, 6, 12, 24, and 72 hours after endotracheal extubation, using the visual analog scale (VAS) and Melbourne pain scale. Serum cortisol was measured before the surgery (baseline), immediately after extubation, and at 6, 12, 24, and 72 hours after extubation.

Results: Surgical time did not differ across the experimental groups. Exteriorization of the ovaries through the vaginal wound was the major difficulty. Intraoperative complications included splenic puncture (1) and subcutaneous emphysema (1), both in the HNG, and postoperative complications included mild vulvar edema in all patients and mild vaginal bleeding (2) and dysuria (1) in the TNG. No dogs required rescue analgesia in the intraoperative and postoperative periods. Regarding the pain score, there were no differences between the groups at most surgical times assessed. Serum cortisol levels were not different between groups at most surgical times, except for baseline, when higher levels were observed for the TNG.

Conclusions: Both NOTES techniques proved feasible and safe for OVE in bitches, with weak pain stimuli in the intraoperative and postoperative periods.

1. INTRODUCTION

The development of laparoscopic procedures indicates the beginning of a modern era in minimally invasive surgery, radically changing how abdominal procedures are performed.[1] The advantages of laparoscopic procedures over conventional ones are widely accepted currently,[2] and include mainly smaller tissue damage, weaker intensity of postoperative pain, and lower risk of wound dehiscence or of other complications related to small surgical wounds, in addition to shorter hospital stays and quicker return to normal activities.[3-6]

In laparoscopy, surgical trauma is mitigated by reduction in port number or size.[7] Given the constant paradigm shifts that result from the evolution of surgery towards less invasive procedures and smaller tissue damage, the concept of Natural Orifice Transluminal Endoscopic Surgery (NOTES) is of special relevance.[8] This technique allows for several diagnostic or therapeutic interventions through transgastric, transcolonic, transvesical, transvaginal, and transesophageal procedures.[9] Potential benefits include absence of skin incisions and of complications associated with surgical wounds, shorter hospital stay, in addition to less intense postoperative pain.[9-11]

Despite the growing number of studies into transluminal surgery in animals, few seek to apply this technique to the treatment of small animals, and most of them have the objective of developing experimental models for later use in humans. While elective ovariohysterectomy (OVH) by hybrid transvaginal NOTES[12] and total transvaginal NOTES[13] have been described for OVE by NOTES in dogs, only studies that use the transgastric approach (flexible endoscopy) are available.[2,14]

Accordingly, the aim of the present study was to apply OVE by total transvaginal NOTES and hybrid transvaginal NOTES to dogs and to compare them in terms of technical feasibility, surgical time, complications, pain score, and serum cortisol levels in the postoperative period. We assume that, akin to previously described OVH by NOTES, the

proposed OVE modalities are feasible and safe and will reduce serum cortisol levels and pain stimuli in the postoperative period.

2. MATERIALS AND METHODS

2.1 Dogs

Sixteen adult, mixed-breed female dogs weighing 15 to 30 kg were selected from veterinary hospitals and animal shelters after written authorization by their tutors and after approval by the local Animal Research Ethics Committee. All dogs were anestrous and were deemed to be healthy based on their medical history, clinical examination, bloodwork, and abdominal ultrasound scan results. Those bitches with ovarian, uterine or vaginal findings such as neoplasms or infections, as well as those which showed an aggressive behavior or were too agitated, were excluded from the study.

The patients were admitted 3 days before the surgery, kept in cages, and in contact with the researchers for their adaptation to the experimental environment. All of them were fasted from solid food for 12 hours and from water for 4 hours. The dogs were randomly assigned to two groups: HNG (n=8), in which they were submitted to OVE by hybrid transvaginal NOTES, and TNG (n=8), in which they underwent OVE by total transvaginal NOTES.

2.2 Anesthesia

Fifteen minutes after premedication (0.05 mg/kg of acepromazine + 4 mg/kg of tramadol hydrochloride + 0.5 mg/kg of ketamine given intramuscularly [IM]), Ringer's lactate solution (10 mL/kg/h given intravenously [IV]) was administered as fluid therapy, and propofol (4 mg/kg IV) was used for anesthetic induction. Anesthetic maintenance consisted of inhaled isoflurane using 100% oxygen in a circuit without rebreathing. Antibacterial prophylaxis was obtained with single-dose ampicillin sodium (20 mg/kg IV).

The dogs were placed in dorsal recumbency and the dorsalis pedis artery was punctured using a 24G catheter connected to a pressure transducer for invasive measurement of systolic blood pressure (SBP), mean arterial pressure (MAP), and diastolic blood pressure (DBP) in a multiparameter monitor. The parameters were recorded every 10 minutes. If SBP and heart rate increased by more than 20% of the baseline value, fentanyl sulfate (5 µg/kg IV) was used as rescue analgesia.

2.3 Surgical procedures

All patients were placed in dorsal recumbency and the abdomen, vulva, and perineum were disinfected with 0.05% chlorhexidine-alcohol. The vaginal access was prepared by antisepsis of the mucosa with 0.5% PVP-I, in the proportion of 10 mL/kg and by bladder catheterization with a urethral probe # 8, with complete emptying of the bladder. The surgical procedures were performed always by the same surgeon (M.V.B.), with expertise in laparoscopy.

2.4 OVE by hybrid transvaginal NOTES

The first trocar (6 mm) was inserted into the umbilical scar using the open technique, allowing for insufflation of the abdominal cavity with carbon dioxide (CO_2) at a rate of 1.5 L/min until a pressure of 12 mmHg was reached. A 25-degree rigid endoscope measuring 4.8 mm in diameter and 29 cm in length was introduced into the cannula for visualization of the abdomen. The second trocar, measuring 11 mm in diameter and 11 cm in length, was introduced ventrally to the entrance of the cervix (at 12 h, in this perspective) under laparoscopic visualization, being used as working trocar. The patients were then rotated laterally to the right for exposure of the left ovarian pedicle. A 5-mm Kelly laparoscopy forceps was used for clamping the ovary in the proper ligament region and for its suspension in the abdominal wall,

for application of a transparietal suture, with a polypropylene 2 thread and a 4-cm needle, and for exposure of the ovarian pedicle, as described earlier.[4,12,13,15] At this stage or at the end of the transection of the pedicle and of the suspensory ligament of the ovary, a long (100 cm) nylon thread # 0 was additionally applied to the transparietal suture, coupled to an 18G catheter needle, transecting the proper ligament of the ovary.

A laparoscopy forceps with bipolar coagulation and with a blade measuring 5 mm in diameter and 42 cm in length (Lina Tripol Powerblade™, LINA Medical Inc., Denmark, distributed by WEM Electronic Equipment Ltd., Ribeirão Preto, SP, Brazil), was used for hemostasis of OAVC and uterine vascular branches, approximately 1 cm caudally to the proper ligament of the ovary. After resection of the ovary, the vessels were examined to confirm hemostasis. The ovary was grasped with a Kelly forceps close to the proper ligament of the ovary and brought by traction into the vaginal port, transected by the long nylon thread, applied additionally to the transparietal suture, thus preventing it from being inadvertently dropped within the cavity during the procedure. The ovary was then removed from the abdominal cavity through the vaginal wound. After exteriorization of the left ovary, the animal was placed once again in dorsal recumbency and the trocar was reinserted into the abdominal cavity through the same vaginal wound. The animals were rotated to the right and the same procedure was performed in the contralateral ovary. After exteriorization of the right ovary, CO_2 was aspirated. The abdominal wall wound was sutured in the muscle and cutaneous planes, respectively with polyglactin 910 2-0 in a cruciate mattress pattern and with nylon 4-0 in a simple interrupted pattern.

2.5 OVE by total transvaginal NOTES

By using a vaginal speculum, the mucous membrane of the vaginal floor, adjacent to the cervical region, was grasped with a long, curved Crile hemostatic forceps, moving it caudally for exposure of the incision site. A tightening suture was made in the vaginal mucosa

for posterior colporrhaphy. The mucosa was sectioned, followed by blunt dissection of the submucosa using a Halsted-Hartmann delicate forceps (approximately at 12 h, in this perspective). Thereafter, a disposable, transparent, blunt-tipped trocar measuring 12 mm in diameter and 10 cm in length (Endopath Xcel Optical Trocar®, Ethicon Endosurgery, São Paulo, SP, Brazil) was inserted through the vaginal wound for access to the abdominal cavity. As the trocar was inserted by external abdominal palpation, the colon was dislodged to the contralateral side. A 10-mm, 27-cm-long and zero-degree rigid endoscope with a working channel for 6-mm tools (26038 AA – Karl Storz™, Germany, distributed by H. Strattner, São Paulo, SP, Brazil) was used for checking the abdominal access. Subsequently, pneumoperitoneum was established by insufflation with CO_2 through the trocar at a rate of 1.5 L/min until an intra-abdominal pressure of 12 mmHg was reached. The surgical sequence was similar to that described for hybrid NOTES; however, instrumentation and visualization of the cavity occurred through the vagina without any puncture for trocar insertion into abdominal muscles. At the end of the procedure, the vagina was sutured with polyglactin 910 2-0 using a simple continuous pattern. The vagina was repositioned by the surgeon's finger and a plug made of sterile gauze was placed in the vaginal canal for temporary preventive hemostasis, and was removed 2 hours after extubation.

2.6 Data recording and postoperative pain assessment

Both intraoperative and postoperative complications were recorded for all procedures. The length of surgery was defined as the time elapsed between the first skin incision up to the last suture of the skin or vagina. Venous blood collection, for serum cortisol measurement, occurred before pre-anesthetic medication (baseline), immediately after orotracheal extubation and at 6, 12, 24 and 72 hours after extubation.

Pain was assessed by three blinded observers. Therefore, identical abdominal bandages were used after the surgeries so that the specific surgical procedure performed on each patient could not be identified. Pain scores were obtained from the visual analog scale (VAS) and Melbourne pain scale, before pre-anesthetic medication (baseline) and at 2, 4, 6, 12, 24 and 72 hours after orotracheal extubation.

Oral tramadol hydrochloride (4 mg/kg) was used as postoperative medication 6 hours after the administration of the pre-anesthetic, and was given every 8 hours for 48 hours in the postoperative period. In case of scores greater than 50 mm in the VAS, rescue analgesia with morphine sulfate (0.3 mg/kg IM) was administered.

2.7 Statistical analysis

One-way ANOVA with Dunnett's post-hoc test was used to compare the means within each group to the baseline values. Unpaired Student's t test was used for comparison between the groups at each time point. The level of agreement between the three observers was calculated for both pain scores using Pearson's correlation coefficient. Statistical significance was obtained when $p<0.05$.

3. RESULTS

3.1 Dogs

Initially, 19 bitches were assessed, but three were excluded from the study: 1 for being pregnant, 1 for presenting with a uterine disorder (pyometra), and 1 for being too agitated, thereby not cooperating during physical examination or blood collection. None of the assessed dogs had vulvar or vaginal abnormalities.

No statistical difference ($p>0.05$) was observed between the groups regarding mean age (TNG 2.2±2.3 years; HNG 3.5±1.6 years) and average weight – 21.72±3.08 kg (range, 18.3-27.3 kg) for TNG and 19.12±4.1 kg (range, 15.9-28.4 kg) for HNG. The analyzed groups were also quite homogenous in terms of animal temperament and initial clinical findings.

3.2 Surgical time and technical difficulties

Surgical time did not differ statistically between the two groups ($p>0.05$), yielding an average of 54.625±31.07 min (range, 30-125 min) for TNG and 46.25±18.46 min (range, 20-70 min) for HNG.

Regardless of the technique used, exteriorization of the ovaries through the vaginal wound was the major difficulty, especially in the animals with larger deposition of ovarian adipose tissue. Another limitation concerns the lack of size compatibility for the laparoscopic tools used and the anatomic features of the animals.

3.3 Complications

The only intraoperative complications were one case of splenic puncture and one case of subcutaneous emphysema, both in patients from the HNG, associated with the abdominal surgical access. Splenic puncture did not have to be managed and the emphysema was aspirated with a 40x12mm needle in the postoperative period without any intercurrent events.

Mild vulvar edema was observed in all patients in the postoperative period and two patients from the TNG presented with mild vaginal bleeding, which ceased spontaneously within 12 and 72 hours in the postoperative period. One patient from the TNG had dysuria in

the first 48 postoperative hours. No signs of systemic infection or problems related to the surgical wounds were observed.

3.4 Postoperative pain assessment

None of the animals in either group required rescue analgesia in the intraoperative period. All patients received food and water within 2 to 12 hours after extubation (TNG 4.5±4.62 h; HNG 3.25±3.53 h) and started urinating and defecating again within 24 hours after extubation.

Taking into account the pain scores, VAS (Figure 1A) did not reveal any differences ($p>0.05$) between surgical times and between the groups, except for the assessment at 72 hours after extubation ($p<0.05$), in which the HNG (1.11±0.29) obtained higher values than the TNG (0.68±0.37). The Melbourne pain scale (Figure 1B) did not show any statistical difference between the groups ($p>0.05$) at any of the assessment time points, but baseline values were significantly higher. The level of agreement between the three observers, according to Pearson's correlation coefficient, ranged from moderately to strongly positive both in the Melbourne pain scale and in the VAS.

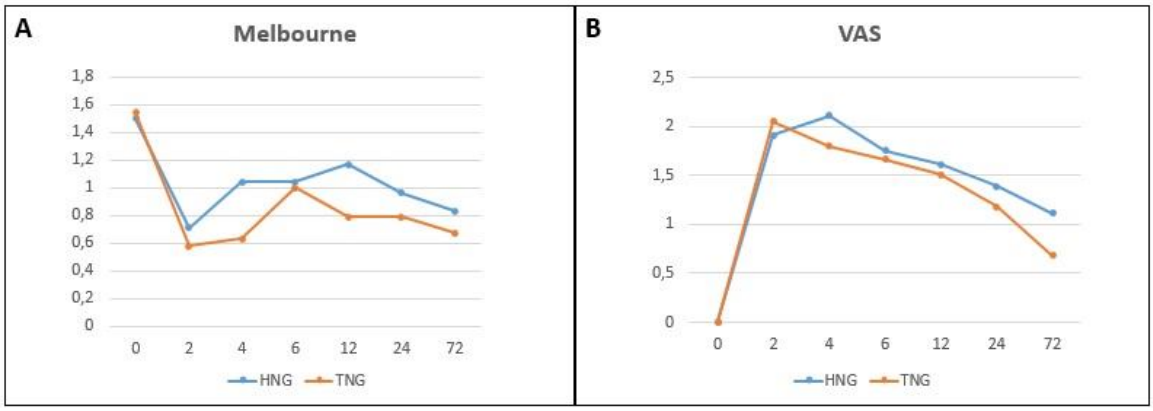

Figure 1 – Results for postoperative pain scores. (A) Values obtained (dots) in the assessment of postoperative pain based on the Melbourne pain scale, relative to the groups and surgical

times (hours after extubation); (B) Values obtained (cm) in the assessment of postoperative pain based on the visual analog scale (VAS) relative to the groups and surgical times (hours after extubation).

3.5 Serum cortisol levels

Serum cortisol levels (Figure 2) did not differ between the groups at most assessment time points, except for baseline, in which the TNG (9.59±5.69) showed higher values ($p<0.05$) than the HNG (3.63±1.94). Although the higher values in both experimental groups refer to the period immediately after extubation, they were significant ($p<0.0001$) only for the HNG, since baseline values in the TNG were already quite higher than those described for the species.

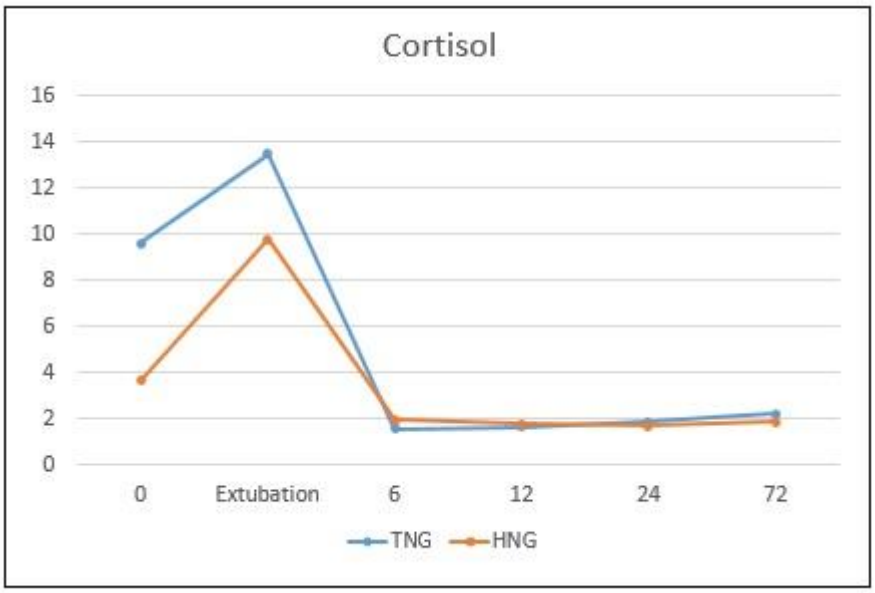

Figure 2 – Serum cortisol concentration (µg/dL) in relation to the groups and surgical times (hours after extubation).

4. DISCUSSION

Both OVE techniques by transvaginal NOTES proved to be feasible and safe for bitches, demonstrating few technical difficulties or associated complications. Surgical times were

similar between the groups, as well as the pain scores in the postoperative period and serum cortisol concentrations. Limitations of the techniques have to do with the need to select patients based on anatomic features (distance between ovary and vulva), amount of ovarian adipose tissue, and absence of ovarian, uterine, and vaginal disorders.

The similar surgical times for both techniques are at odds with those reported for the same techniques applied to OVH,[8] for which the average time for OVH by hybrid NOTES (74.28±34.21 min) was significantly higher than that observed for the TNG (30.42±7.61 min). The average time for the HNG was shorter than that described (104.6±30.2 min[16] and 74.6±35.9 min)[17] for this same technique applied to OVH in bitches. These differences in surgical time seen in the HNG can be explained by poorer visualization with a 2.7-mm arthroscope,[8] difficulties during the exteriorization of structures through the 5-mm vaginal port,[16] as well as by the initial learning curve.[8,16,17]

The surgical time observed in the TNG, however, is similar to that described for OVH by total transvaginal NOTES (52.1±11.5 min)[13] and longer than that reported for this same technique, performed by the same surgeon, at a more advanced stage of the learning curve (25.7±6.8 min),[18] suggesting a tendency towards shorter surgical times as soon as the learning curve for the proposed procedures is reached. This tendency was observed in a study that assessed the evolution of the learning curve for OVE by transgastric NOTES.[14]

Notwithstanding, remarkably shorter surgical times for OVE were expected due to its smaller technical complexity and lower volume of tissue to be excised when compared to OVH. However, owing to difficulties in exteriorization of the ovary through the vagina, short uterine segment, adipose tissue, and associated ligaments of larger animals in this experiment, time was eventually longer than expected for both groups.

In a study that compared OVE by transgastric NOTES, two-port video-assisted surgery, and open surgery in two bitches, surgical times averaged 76, 44, and 35 min, respectively.[2] For other laparoscopic OVE, surgical times averaged 47 min (range, 27-110 min),[19] 32.0±3.0 min (range, 28-35 min),[20] and 19.3±3.4 min[21] for three-port surgery; 30 min (range, 24-48 min),[6] 19±6 min,[22] and 18.2±4.4 min[21] for two-port surgeries, and 36.6±3.5 min (range, 34-42 min),[20] 29.7±5.6 min,[21] and 21±6 min[22] for single-port OVE. These surgical times are quite similar to those found in our study and even though prospective studies still need to be conducted to compare NOTES with other OVE methods in dogs, we deem these findings to be promising for both of the proposed techniques.

Regardless of the method used, exteriorization of the ovaries turned out to be technically challenging, as also pointed out by other authors.[8, 13,14,18,19,22] Most studies involving different laparoscopic OVE methods that established a correlation between body condition score and ovarian adipose tissue as a function of each surgical step demonstrate that such factors affect and significantly increase surgical time;[19,22,23] however, other studies have not found this correlation.[20,24] While this correlation was not carefully evaluated in the present study, we assume it can be established for both techniques, but further investigation should be made.

On some occasions, because of the technical difficulty described above, the ovaries slipped off the forceps during exteriorization, requiring adaptation of the transvaginal NOTES previously developed for OVH,[12,13] considering that, in OVE, the ovaries could not be retrieved from the cavity by visualization and traction of uterine horns. This difficulty was also observed in OVE by transgastric NOTES,[14] in which, more than once, the ovary slipped off the forceps and could not be visualized in the cavity. Thus, the use of a long nylon thread in the transparietal suture and the partial release of this safety suture after section of the ovaries were aimed at keeping the organ clamped during its traction, allowing for its quick retrieval if it slipped off the forceps. This technique is similar to that described for single-port laparoscopic OVE,[22] even

though the transparietal suture thread was used in that case. This was not possible in the present study due to the short length of the polypropylene thread used in the transparietal suture, which did not allow reaching the vaginal port.

The lack of compatibility of laparoscopic tools used in our study and the anatomic features of the animals, as previously described,[13] are limitations of both techniques and, therefore, bitches in which the distance between ovarian pedicles and the vulva exceeded 30 cm are not good candidates for OVE by transvaginal NOTES, at least not with the tools at hand. Likewise, the described techniques are not suitable for very small-sized dogs. While OVH by hybrid transvaginal NOTES has been reported for a bitch weighing 4.2 kg,[17] difficulty in transvaginal access due to the small diameter of the vaginal canal should be taken into consideration.

Owing to anatomic proximity, lesions in the bladder, urethra, small intestine, colon, and rectum have already been reported for transvaginal NOTES.[25] However, the only complications observed in the intraoperative period in this study, both in patients from the HNG, were not related to the transvaginal surgical access, but rather to the abdominal surgical access for the laparoscopic approach. Both complications were properly managed with conservative treatment. Other studies also mention subcutaneous emphysema as complication from OVH by hybrid transvaginal NOTES,[12] OVE by transgastric NOTES,[14] and three-port laparoscopic OVE.[21] Splenic puncture was also reported in single-port laparoscopic OVE[21,22] and two-port OVE,[22] attributed, in these cases, to the use of Verres needles, which were not utilized in the present study.

The mild vulvar edema seen in all patients, in addition to mild vaginal bleeding, has also been described as self-limiting postoperative complications in OVH by total NOTES.[13,18] Although the vaginal bleeding observed in 33.3% of the patients submitted to OVH by total

NOTES[18] has been attributed to the absence of vaginal wound suture, in our study, this type of bleeding was detected in two patients from the TNG, despite suture of the wound.

Notwithstanding the complications mentioned above, it should be underscored that the major advantage of total NOTES concerns the surgical wound, as a single incision is made in the vaginal canal, avoiding complications such as self-injury, dehiscence, and evisceration,[13] in addition to sparing tutors from cleansing the wounds, having their pets wear after-surgery clothing or protective collars, and returning for suture removal.

None of the animals from either group required rescue analgesia in the intraoperative period, as described for OVH by transvaginal NOTES,[8] corroborating the weaker intensity of pain stimuli associated with NOTES in bitches.

Rapid return to a normal diet and to normal activities in the postoperative period, as well as absence of rescue analgesia in patients from both groups within 72 hours, has also been described for OVE by transgastric NOTES.[2] On the other hand, during pain assessment in another study[8] carried out by the same research team, 2 out of 7 dogs submitted to OVH by hybrid NOTES required additional analgesia within 2 hours in the postoperative period, which was probably associated with longer surgical time and with larger tissue damage compared with the present study, as the surgical team involved in OVH by hybrid NOTES had a less advanced learning curve at the time.

When comparing the pain scores obtained from the postoperative period for both groups, results are different from those described for OVH by NOTES,[8] as pain scores for OVH by hybrid NOTES were statistically higher than those for OVH by total NOTES. Conversely, the similarity of pain scores between the HNG and TNG demonstrates that both procedures cause small pain stimuli, which could be related to the minimally invasive approach used in both procedures, as highlighted by other authors.[2]

While some studies regard serum cortisol concentration as a good marker of surgical stress in canine models,[26,27] some others suggest it is not a reliable marker.[28] In the present study, serum cortisol was not a sensitive marker because, despite the adaptation period, as described by other authors,[8] baseline levels were already high before the surgical procedure, which could be ascribed to other causes of stress such as change of environment, transportation, and patient handling, factors that are difficult to exclude in experiments with dogs referred from the hospital setting.

Both of the proposed NOTES techniques proved feasible and safe for OVE in bitches, characterized by weak pain stimuli in the intraoperative and postoperative periods. However, it is necessary to select patients based on the available size of surgical tools and exclude those with ovarian, uterine, or vaginal disorders.

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DISCLOSURE

The authors declare no conflict of interest related to this report.

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CONSIDERAÇÕES FINAIS

Ambas as técnicas de NOTES híbrida e NOTES total via transvaginal se mostraram viáveis e seguras para a realização de ovariectomia em cadelas, cursando com reduzido estímulo álgico nos períodos trans e pós-operatório. Destaca-se, no entanto, a necessidade de considerar alguns critérios na seleção dos pacientes, tais como a condição anatômica e ausência de alterações ovarianas, uterinas ou vaginais.

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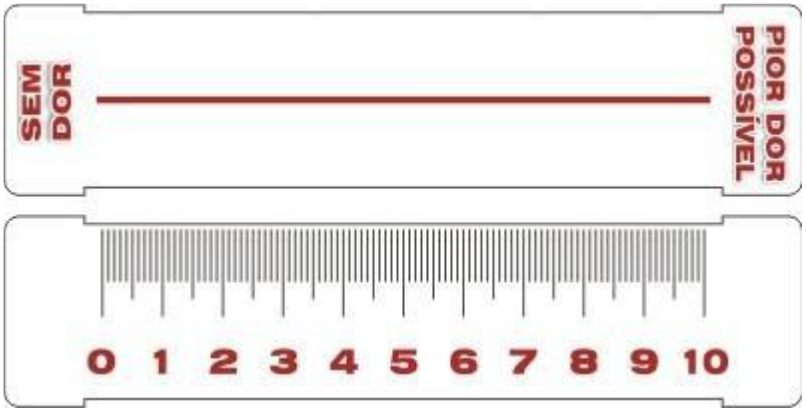
ANEXOS

Anexo A - Escala Visual Analógica (EVA). Linha reta horizontal de 100mm de comprimento, que descreve a intensidade dolorosa, variando entre nenhuma dor e pior dor possível, em cada uma das extremidades



Anexo B - Escala da Universidade de Melbourne, adaptada por Firth & Haldane (1999)

Observação	Score	Características
FC	1	> 20% valor basal
	2	> 50% valor basal
	3	> 100% valor basal
F	1	> 20% valor basal
	2	> 50% valor basal
	3	> 100% valor basal
PAS	1	> 20% valor basal
	2	> 50% valor basal
	3	> 100% valor basal
Temperatura retal	1	Acima do valor basal
Salivação	2	
Pupilas dilatadas	2	
Resposta à palpação	0	Normal
	2	Reage/ Protege a ferida no momento do toque
	3	Reage/ Protege a ferida antes do toque
Atividade	0	Dormindo
	0	Semiconsciente
	1	Acordado
	0	Alimenta-se
	2	Agitado
	3	Mudanças contínuas de posição, mutilação
Status mental	0	Dócil
	1	Amigável
	2	Cauteloso
	3	Alerta
Postura	2	Protege a área afetada
	0	Decúbito lateral
	1	Decúbito esternal
	1	Sentado ou em pé, cabeça elevada
	2	Em pé, cabeça baixa
	1	Movimenta-se
	2	Postura anormal
Vocalização	0	Não vocaliza
	1	Vocaliza quando tocado
	2	Vocalização intermitente
	3	Vocalização contínua

Anexo C – Figura 1

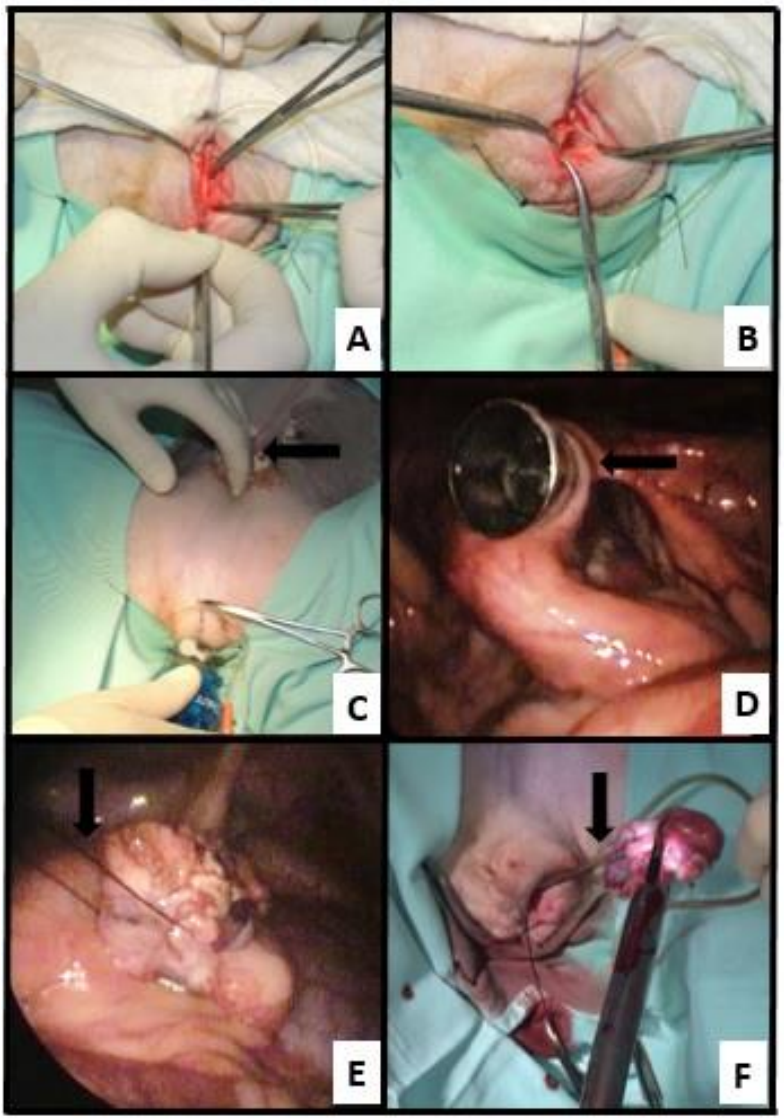


Figura 1 – Imagens fotográficas representando momentos do transoperatório referentes ao estabelecimento do acesso vaginal nas técnicas NOTES total (A, B, C) e NOTES híbrida (D), bem como à manobra de fixação do ovário com fio de náilon longo (E, F). (A) Secção da mucosa vaginal, mantida tracionada caudalmente com pinças hemostáticas e uma sutura de arrimo, posicionada para posterior vaginorrafia. (B) Mucosa vaginal seccionada, após dissecção romba da camada submucosa. (C) Posicionamento do trocarte descartável através da ferida vaginal, enquanto, por palpação abdominal externa (seta), o cólon era direcionado para o lado contralateral. (D) Imagem do portal vaginal, introduzido ventralmente à entrada da cérvix (seta), sob visão laparoscópica na técnica NOTES híbrida. (E) Ovário transfixado por fio de náilon longo (seta), após sua completa secção, manobra realizada para evitar perda inadvertida do mesmo dentro da cavidade abdominal durante a etapa de exteriorização. (F) Ovário ainda transfixado por fio de náilon longo (seta), após sua exteriorização pela ferida vaginal.

Anexo D – Tabela 1 - Idade, peso médio e tempo cirúrgico dos cães, distribuídos nos dois grupos experimentais

Grupo	Cão	Idade (anos)	Peso (Kg)	Tempo (min)
GNT	C1	0,66	21,3	46
GNT	C2	0,66	24,7	45
GNT	C3	0,66	22,7	30
GNT	C5	6	18,5	52
GNT	C7	6	27,3	125
GNT	C8	2	18,3	69
GNT	C15	1	20	40
GNT	C16	1	21	30
GNH	C4	3	18,3	40
GNH	C6	5	28,4	70
GNH	C9	2	18	70
GNH	C10	6	21,4	60
GNH	C11	3	17	40
GNH	C12	2	16,3	20
GNH	C13	2	17,7	40
GNH	C14	5	15,9	30