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Letícia Reginato Martins

PRÉ-CONDICIONAMENTO COM OXIGENOTERAPIA HIPERBÁRICA EM GATAS SUBMETIDAS A OVARIOHISTERECTOMIA ELETIVA VIDEOASSISTIDA: TEMPERATURA CORPÓREA, ANALGESIA E BIOMARCADORES OXIDATIVOS

Santa Maria, RS

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Tese apresentada ao Programa de Pós-Graduação em Medicina Veterinária, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do título de **Doutora em Medicina Veterinária.**

Orientador: Prof. Dr. Maurício Veloso Brun

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Aprovada em 24 de fevereiro de 2022:

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Maurício Veloso Brun, Dr. (UFSM)
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Santa Maria, RS
2022

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A memória do meu padrinho Nardeli, por ser um grande incentivador do meu crescimento profissional. Saudosamente, com todo o meu amor e gratidão.

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“Gatos amam mais as pessoas do que elas permitiriam, mas eles têm sabedoria suficiente para manter isso em segredo.”

(Mary Eleanor Wilkins Freeman)

RESUMO

PRÉ-CONDICIONAMENTO COM OXIGENOTERAPIA HIPERBÁRICA EM GATAS SUBMETIDAS A OVARIOHISTERECTOMIA ELETIVA VIDEOASSISTIDA: TEMPERATURA CORPÓREA, ANALGESIA E BIOMARCADORES OXIDATIVOS

AUTORA: Letícia Reginato Martins
ORIENTADOR: Maurício Veloso Brun

Esse estudo objetivou avaliar a influência do uso de oxigenoterapia hiperbárica (HBOT) em felinos submetidos à ovariohisterectomia (OVH) quanto ao estímulo álgico pós-operatório, a ação sobre temperatura central (TC), periférica (TP) e variação da temperatura central e periférica (ΔT_{c-p}) além da influência da HBOT em biomarcadores de estresse oxidativo. Para tal, 45 gatas foram aleatoriamente alocadas em: Grupo Hiperbárica (GH): (n= 15), HBOT por 45 minutos na pressão de duas atmosferas absolutas (ATA) previamente à OVH videoassistida; Grupo Hiperbárica Controle (GHC): (n=15) mesmo regime de HBOT, sem procedimento cirúrgico; Grupo Sham (SHAM) (n=15) animais submetidos apenas à cirurgias, sem pré-tratamento. O primeiro artigo avaliou os efeitos do pré-tratamento com HBOT na temperatura e na dor pós-operatória. Para avaliação analgésica, foi utilizada a Feline Grimace Scale (FGS) e Escala Multidimensional da UNESP-Botucatu (EUNESP) em tempos determinados. Cinco animais receberam resgate analgésico, quatro do SHAM um do GH. As TC finais dos grupos GH e SHAM foram menores que as TC iniciais, porém sem diferença entre os grupos. O ΔT_{c-p} mostrou efeito do tratamento (GH x SHAM) ($p<0,038$) e do tempo (inicial x final) ($p<0,0001$). GH e SHAM apresentaram diferença no ΔT_{c-p} inicial e final ($p< 0,001$ para ambos). Concluímos que não houve influência positiva da HBOT na analgesia pós-operatória. Não houve diferença entre a TC, TP ou ΔT_{c-p} em animais submetidos ou não a HBOT. A redução da temperatura em gatas hígidas após HBOT não teve significância clínica. O segundo artigo buscou determinar como a HBOT pré-cirúrgica altera biomarcadores de estresse oxidativo durante OVH videoassistida, para tanto se quantificou os valores séricos de Substâncias Reativas ao Ácido Tiobarbitúrico (TBARS), as Espécies Reativas ao Oxigênio (ROS), Superóxido Dismutase (SOD), Catalase (CAT), Acetilcolinesterase (AChE) e Butirilcolinesterase (BChE) nas 45 gatas selecionadas. Amostras de sangue foram obtidas para os grupos GH e SHAM: T1 = Imediatamente antes da cirurgia, na estabilização anestésica; T2 = Na extubação; T3 = 24 horas após o término do procedimento cirúrgico. Para o GHC as coletas foram realizadas em T1 = após a sedação; T2 = Após a reversão (30 minutos depois) e T3 = 24 horas após a reversão. Não houve diferença quanto ao aumento sérico de ROS, AChE ou BChE. No T3 o grupo SHAM apresentou aumento das TBARS quando comparado ao grupo GH ($p=0,043$). Na CAT, houve redução da atividade em SHAM T2 e Sham T3, em relação ao Sham T1 ($p=0,012$ e $p<0,001$, respectivamente). Sham T2 apresentou menor atividade da CAT em relação ao GHC T2 ($p=0,05$). Também o Sham T3 foi menor que GH T3 ($p=0,030$) e GHC T3 ($p=0,050$). Houve redução da SOD em Sham T2 em relação ao GH T2 ($p=0,033$) e GHC T2 ($p=0,027$). Similarmente, no Sham T3 a SOD estava reduzida em relação ao GH T3 ($p=0,039$) e GHC T3 (0,019). Esse estudo demonstrou que a HBOT tem influência favorável no metabolismo oxidativo de pacientes felinos submetidos à OVH eletiva.

P**alavras-chave**: Hiperbárica. Biomarcadores. Medicina felina. Videocirurgia. Temperatura. Analgesia.

ABSTRACT

HYPERBARIC OXYGEN THERAPY PRECONDITIONING ON CATS UNDERGOING VIDEO-ASSISTED ELECTIVE OVARYHYSTERECTOMY: BODY TEMPERATURE, ANALGESIA AND OXIDATIVE BIOMARKERS

AUTHOR: Letícia Reginato Martins
ADVISOR: Maurício Veloso Brun

This study aimed to evaluate the influence of hiperbaric oxygen therapy (HBOT) in cats undergoing ovariohysterectomy (OHE) regarding postoperative pain stimulus, central temperature (CT), peripheral (PT) and the variation between central temperature and peripheral temperature (ΔT_{c-p}), in addition to the HBOT's influence in oxidative stress biomarkers. For such matter, 45 female cats were divided randomly in: Hiperbaric Group (HG): (n=15) 45 minutes of HBOT under the pressure of 2 absolute atmospheric pressure (AAP) before video assisted OHE; Control Hiperbaric Group (CHG): (n=15) same HBOT regimen, without surgical procedure; Sham Group (SHAM) (n=15) submitted only to elective OHE. The first article evaluated the effects of the treatment with HBOT before the surgery on temperature and post operatory pain. For analgesic evaluation, was used the Feline Grimace Scale (FGS) and the Multidimensional UNESP – Botucatu Scale (UNESPS) in predetermined times. Five received analgesic rescue, four of them belonging to SHAM and one to HG. Final CTs of groups SHAM and HG were smaller than the initial CTs, but there was no difference between the groups. ΔCT-P showed treatment effect (HG x SHAM) ($p<0,038$) and time (initial x final) ($p<0,0001$). HG and SHAM presented difference in initial and final ΔCT-P (<0,001 for both). It was concluded that there was no HBOT's positive influence on post operative pain. There was no difference between CT, PT or ΔT_{c-p} in animals submitted or not to HBOT. The temperature reduction in healthy cats after HBOT had no clinic significance. The second article aimed to determinate how pre surgical HBOT changes oxidative stress biomarkers during video assisted OHE, for such matter it was quantified serum values of Thiobarbituric Acid Reactive Substances (TBARS), Reactive Oxygen Species (ROS), Superoxide Dismutase (SOD), Catalase (CAT), Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) in all the 45 selected cats. Blood samples were obtained to SHAM and HG groups: T1 = immediately before the beginning of the surgery, during anesthetic stabilization; T2 = in the extubation moment; T3 = 24 hours after the end of the surgical procedure. To the CHG the collections were done in T1 = after sedation; T2 = after reversion (30 minutes after) and T3 = 24 hours after reversion. There was no difference regarding the serum generation of ROS, AChE or BChE. There was a rise in TBARS in T3 SHAM when compared to HG ($p=0,043$). There was an activity reduction in CAT in T2 and T3 SHAM in relation to T1 SHAM ($p=0,012$ and $p<0,001$, respectively). T2 SHAM presented less CAT activity in comparison to T2 CHG ($p=0,05$). Also, T3 SHAM was smaller than T3 HG ($p=0,030$) and T3 CHG ($p=0,050$). There was a SOD's reduction in T2 SHAM in comparison to T3 HG ($p=0,039$) and T3 CHG ($p=0,019$). This study showed that HBOT seems to have a favorable influence at oxidative stress reduction and at serum levels of these biomarkers.

Keywords: Hyperbaric. Biomarkers. Feline Medicine. Videosurgery. Temperature. Analgesia.

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1 INTRODUÇÃO

1.1 APRESENTAÇÃO

A presente tese dispõe de um estudo prospectivo randomizado em 45 fêmeas da espécie felina, encaminhadas por médicos veterinários para realização de ováriohisterectomia (OVH) videoassistida eletiva, com o consentimento prévio dos tutores.

O estudo oportunizou a elaboração de dois artigos, nos quais foram apresentados os resultados relativos à avaliação do impacto do pré-tratamento com oxigenoterapia hiperbárica (HBOT) em diferentes biomarcadores do metabolismo oxidativo, assim como a influência dessa nova abordagem terapêutica na manutenção da termorregulação fisiológica e redução do estímulo álgico nas pacientes submetidas à OVH videoassistida. O primeiro artigo abordou os resultados obtidos no controle da dor pelo tratamento pré-operatório com HBOT e avaliação por dois profissionais proficientes nos parâmetros estabelecidos por duas escalas de dor nos intervalos pós-cirúrgicos pré-determinados e cegos aos procedimentos executados. No mesmo artigo foram avaliadas as alterações de temperatura central (TC), periférica (TP), assim como a variação entre ambas ($\Delta T_{c\text{-}p}$); as temperaturas foram mensuradas antes e depois da HBOT nos grupos submetidos à terapia, após a indução anestésica e ao fim da anestesia, em animais submetidos à cirurgia. O segundo artigo discorreu sobre a avaliação de biomarcadores séricos do metabolismo oxidativo e o impacto da HBOT na concentração desses. Foram analisadas no sangue total coletado em tubos contendo citrato de sódio a superóxido dismutase (SOD) e a catalase (CAT). Amostras de soro foram coletadas para dosagem das espécies reativas de oxigênio (ROS), as substâncias reativas ao ácido tiobarbitúrico (TBARS), a acetilcolinesterase (AChE) e a butirilcolinesterase (BChE).

Ambos os estudos foram desenvolvidos no Laboratório de Cirurgia Experimental (LACE), situado no Hospital Veterinário Universitário (HVU) da Universidade Federal de Santa Maria (UFSM) e as análises dos biomarcadores realizadas no Laboratório de Enzimologia e Toxicologia (ENZITOX) da mesma instituição. O estudo teve orientação do Professor Doutor Maurício Veloso Brun. Esta tese foi elaborada de acordo com as diretrizes do Manual de dissertações e teses da Universidade Federal de Santa Maria de 2015.

Dentre os capítulos iniciais, encontra-se uma seção destinada à revisão da literatura consultada sobre o tema, denominada REFERENCIAL TEÓRICO, seguida da exposição dos objetivos gerais e específicos propostos, na seção intitulada PROPOSIÇÃO. A metodologia aplicada para o desenvolvimento deste estudo encontra-se no item MATERIAL E MÉTODOS.

Os artigos estão estruturados de acordo com as normas das revistas para as quais foram submetidos para publicação (*Journal of Feline Medicine and Surgery* e *Journal of Veterinary Medical Science*).

Nos últimos capítulos a seção intitulada DISCUSSÃO busca estabelecer uma conectividade entre os dois artigos apresentados, estabelecer demonstrando o vínculo entre a temática abordada e os resultados apresentados. A elaboração de uma explanação abrangente integrando os temas debatidos e resultados deste ensaio encontram-se no item CONCLUSÃO. As REFERÊNCIAS reportam ao referencial consultado para a elaboração das seções REFERENCIAL TEÓRICO e DISCUSSÃO.

1.2 REFERENCIAL TEÓRICO

A oxigenioterapia hiperbárica (HBOT) consiste em um método relativamente antigo de tratamento (EDWARDS, 2010a). Embora os primeiros registros do uso de terapia hiperbárica em humanos datem de 1662, somente em 1887 foram relatados os primeiros resultados da aplicação da técnica na Medicina Veterinária, os quais demonstraram a capacidade de redução do status febril de coelhos em sepse induzida após sessões em câmara hiperbárica (BEAN, 1945).

Com mais câmaras sendo disponibilizadas e pesquisas que demonstram o benefício do seu uso, a HBOT tem se destacado cada vez mais como opção de terapia tanto na Medicina Humana quanto Veterinária. Além disso, consiste em uma técnica com diversas aplicações, como em situações emergenciais tais como intoxicação por monóxido de carbono, síndrome compartimental, acidentes ofídicos e lesões nervosas centrais. Ademais, pode também ser empregada em afecções de evolução crônica, como nos casos de lesões com cicatrização retardada, queimaduras e flaps ou enxertos cutâneos (EDWARDS, 2010b, 2010a).

Segundo Jain (2004), os princípios da HBOT são baseados em como os gases de diferentes solubilidades se comportam nos tecidos e fluidos corporais sob diferentes pressões e volumes, fenômeno este determinado pelas leis do comportamento gasoso descritas por Henry, Fick e Boyle. A lei de Henry descreve como a pressão do gás afeta sua concentração no interior dos tecidos, enquanto a de Fick refere-se à taxa de difusão de um gás através destes tecidos, e a de Boyle diz respeito ao comportamento dos gases sob pressão. Tendo isso em vista, a HBOT pode ser considerada uma modalidade terapêutica, na qual o paciente é inserido em uma câmara com oxigênio a 100% e submetido a pressões acima de uma atmosfera absoluta (ATA) (YANAGISAWA et al., 2011).

O oxigênio (O_2) é necessário para prover energia e possibilitar a respiração celular, portanto um aporte deficitário leva à morte das células. Um animal doente possui menor capacidade de transporte de O_2 ao passo que aumenta a necessidade tecidual deste gás, levando ao colapso do sistema e gerando estresse oxidativo (EDWARDS, 2010a).

O organismo dos humanos e animais possui um estado oxidativo, sendo esse totalmente dependente do equilíbrio entre reagentes oxidantes (estresse oxidativo) e defesas antioxidantes (CASTILLO et al., 2013).

Há diversos mecanismos propostos para justificar os benefícios da HBOT fisiologicamente, tais como a disponibilidade plasmática do oxigênio, hiperoxigenação tecidual, efeito barométrico, imunomodulação e redução do estresse oxidativo (BIRNIE; FRY; BEST, 2018). O estresse oxidativo pode ser quantificado por meio de diferentes biomarcadores, pela medição direta dos radicais livres, dos produtos finais de danos dos radicais livres, ou dos níveis de antioxidantes específicos e totais (ARSALANI-ZADEH et al., 2011). Estas medidas podem ser realizadas em tecidos, sangue e outros fluidos.

As espécies reativas de oxigênio (ROS) são geradas como subprodutos naturais do metabolismo e abrangem uma variedade de espécies químicas, incluindo superóxido, peróxido de hidrogênio, ácido hipocloroso e hidroxila (THOM, 2009). Elas possuem duplo papel no organismo, podendo ser benéficas, através do envolvimento na defesa contra agentes infecciosos, sinalização celular e indução de resposta mitogênica, dentre outras (POLI et al., 2004). Em contrapartida, o excesso deste pode ser prejudicial, levando à oxidação de proteínas, lipídeos e ácidos nucléicos, podendo ser gerados exogenamente ou produzidos pelas células a partir de diferentes fontes (FINKEL; HOLBROOK, 2000).

Uma das consequências do estresse oxidativo é o aumento da intensidade da peroxidação dos lipídios. Isso ocorre em reação em cadeia, na qual os radicais livres oxidam ácidos graxos poli-insaturados. A membrana celular é um dos componentes mais afetados, acarretando em alterações em sua estrutura e permeabilidade com consequente liberação do conteúdo de organelas, como o malondialdeido (MDA). Esse marcador pode ser mensurado através da quantificação de espécies reativas ao ácido tiobarbitúrico (TBARS) (BASSO et al., 2014; DALMOLIN et al., 2016; FINKEL; HOLBROOK, 2000).

As enzimas AChE e BChE séricas são utilizadas como indicadores da inflamação local ou sistêmica de baixo grau. A acetilcolina (Ach) tem importante ação supressora inflamatória, no entanto ela é rapidamente hidrolisada pela AChE e BChE. Portanto, um aumento nessas enzimas poderia levar à diminuição dos níveis de Ach, reduzindo os seus efeitos anti-inflamatórios (DAS, 2012).

A ovariohisterectomia (OVH) é amplamente utilizada tanto para esterilização eletiva quanto para tratamento de afecções do trato reprodutivo ou auxílio na estabilização de doenças sistêmicas, sendo, na década passada, o procedimento cirúrgico mais realizado na casuística veterinária de pequenos animais (HEDLUND, 2008). Além disso, pode diminuir o risco de desenvolvimento de neoplasias mamárias e piometrites (DAVIDSON; MOLL; PAYTON, 2004).

Muitas variações da técnica cirúrgica de OVH têm sido descritas, desde a abordagem convencional via celiotomia mediana, até abordagens minimamente invasivas laparoscópicas ou videocirúrgicas, nas diferentes modalidades. Estas vêm cada dia mais ganhando popularidade por apresentarem diversas vantagens quando comparada as cirurgias convencionais. Dentre elas, podemos citar o menor estímulo álgico pós-operatórios devido ao menor dano tecidual somático, reduzido período de hospitalização pós-operatória, menor risco de deiscência e hemorragia, redução na formação de aderências e melhores resultados estéticos (DUQUE; MORENO, 2015; FERREIRA et al., 2011; MALM et al., 2005).

Os anestésicos voláteis, como o isoflurano, produzem hipotermia principalmente por inibição de tremores e perda de calor por vasodilatação. A combinação deste com a exposição a um ambiente fresco da sala de operações aliado à facilidade com que os felinos perdem temperatura corporal, aproximando a temperatura central da periférica contribuem para a grande ocorrência de hipotermia pós-operatória (MARTINEZ TABOADA; MURISON, 2010; POSNER et al., 2010; REDONDO et al., 2012). Um estudo realizado por (SHMALBERG & JUFFE, 2015) em cães submetidos ao tratamento em câmara hiperbárica a dois ATA por 45 minutos demonstrou perda de 0,07 ºC ao fim da sessão, porém ainda não há dados que avaliem este dado em felinos.

De acordo com MORTON et al. (2005) a dor nos animais pode ser definida como uma experiência sensorial e emocional negativa, produtora de ações motoras protetoras, resultando em aversão condicionada, a qual modifica os traços de comportamento específicos para a espécie, incluindo o comportamento social. Esta é uma inevitável consequência da cirurgia. Nesse sentido, estudos já demonstram que o pré-tratamento com oxigenoterapia hiperbárica pode reduzir o estímulo álgico, reduzir a inflamação local e aliviar dor neuropática no período pós-operatório (THOMPSON et al., 2010).

O manejo da dor em pacientes incapazes de autorrelato é um desafio, pois a capacidade de tratá-la e diagnosticá-la efetivamente tornam-se subjetivas. Sendo assim, o reconhecimento da dor tem papel fundamental e a utilização de métodos que possam auxiliar na avaliação desta é crucial para identificação e acompanhamento da eficácia do tratamento analgésico. Uma

abordagem com múltiplos aspectos para a avaliação da dor é geralmente aceita como a melhor opção, oferecendo melhores resultados (HELLYER et al., 2007; MATICIC et al., 2010).

Para gatos, duas escalas de avaliação de dor estão devidamente validadas: Escala Multidimensional da UNESP-Botucatu para avaliação de dor aguda pós-operatória em gatos (EUNESP) e a Feline Grimace Scale (FGS). A EUNESP-Botucatu combina tanto fatores comportamentais (grau de atividade, resposta à palpação, postura, entre outros) como parâmetros fisiológicos (frequências cardíaca e respiratória, pressão arterial, temperatura, dilatação pupilar) enquanto a FGS interpreta a dor por meio de expressões faciais felinas. A EUNESP, criada e validada em gatas submetidas à OVH eletiva, permite o máximo de 30 pontos, considerado dor máxima, e a classificação do paciente como tendo dor leve (0 – 8 pontos), dor moderada (9 – 21 pontos) e dor intensa (22 – 30 pontos). Pontuações acima de oito indicam necessidade de analgesia de resgate (BRONDANI et al., 2013). Já a FGS, faz uso de cinco alterações de ação facial indicativas de dor em gatos: posição da orelha, aperto orbital, tensão do focinho, posição do bigode e posição da cabeça. Uma pontuação total de quatro ou mais significa que o gato está com dor e precisa de analgesia de resgate. A pontuação total máxima desta escala é 10. (EVANGELISTA et al., 2019).

1.3 PROPOSIÇÃO

O objetivo geral desse estudo foi avaliar as consequências do uso da oxigenoterapia hiperbárica pré-operatória sobre a o metabolismo oxidativo e estímulo álgico de gatas submetidas à OVH videoassitida com dois portais, bem como a influência sobre a temperatura central e variação da temperatura central e periférica (ΔT_{c-p}).

Especificamente, tivemos o objetivo no primeiro estudo determinar se o tratamento com oxigenoterapia hiperbárica pré-cirúrgica seria capaz de reduzir a resposta inflamatória tecidual e o consequente estímulo álgico causados pela cirurgia e identificar possíveis alterações de temperatura (central, periférica ou ΔT_{c-p}) causadas pela HBOT, bem como comparar as alterações provocadas por este tratamento com outros animais não submetidos ao tratamento. Quanto aos objetivos específicos da segunda pesquisa científica, procurou-se determinar se o tratamento com oxigenoterapia hiperbárica pré-cirúrgica alteraria os biomarcadores de estresse oxidativo durante OVH videoassistida com dois portais.

1.4 MATERIAL E MÉTODOS

1.4.1 Modelo Experimental

O experimento proposto foi submetido à apreciação pela Comissão de Ética no Uso de Animais (CEUA) da UFSM e aprovado sob o protocolo de nº 5134560820.

As cirurgias foram executadas por equipe consolidada no que se refere à realização de videocirurgias, sempre pelo operador da câmara hiperbárica, mesmo cirurgião, câmera e anestesista. Para tanto, foram selecionadas 45 gatas hígidas, de variadas raças não braquicefálicas, com idade de 1,89±1,06 anos e peso 3,05±0,52 kg, provenientes da rotina hospitalar, encaminhadas por médico veterinário para OVH eletiva.

As gatas participantes tiveram higidez confirmada em exame clínico e laboratorial, não tendo alterações com relevância clínica. Também foram consideradas a avaliação hematológica pré-operatória, incluindo hemograma, dosagem sérica de creatinina, albumina e alanina aminotransferase (ALT), aspartato aminotransferase (AST), fosfatase alcalina (FA), ureia e frutosamina. Todas as gatas realizaram teste de imunodeficiência viral felina e leucemia viral felina, sendo negativas para inclusão no estudo.

As mesmas foram internadas três dias antes dos procedimentos, a fim de se aclimatar com o local e com a equipe. Em quatro horários durante o dia, os animais foram soltos, um a um, para explorar o ambiente do gatil. Nesse ambiente foi realizada a difusão de um análogo sintético do ferormônio facial felino (F3) com antecedência de 24 h antes de cada hospitalização, visando auxiliar na adaptação das pacientes, proporcionando um ambiente acolhedor e agradável aos felinos. Os animais ficaram internados em box individuais com caixas de papel, almofadas e cobertores, vasilhas para água e alimentos e caixa com areia sanitária para gatos. Toalhas e itens dos tutores foram disponibilizados. As gatas receberam água *ad libitum* e ração superpremium seca e úmida.

No terceiro dia, para a obtenção dos valores basais, os animais foram avaliados pelas escalas de dor Escala multidimensional da UNESP-Botucatu quanto à dor aguda pós-operatória em gatos (EUNESP) e pela Feline Grimace Scale (FGS) por dois avaliadores não envolvidos e cegos aos tratamentos. Após isso, foi instituído jejum sólido e hídrico de oito e duas horas, respectivamente, previamente à cirurgia. No dia do procedimento as gatas foram alocadas por meio de sorteio, em 3 grupos, os quais receberam, diferentes tratamentos, a saber:

1. Grupo Hiperbárica (GH): 15 animais pré-tratados com HBOT na pressão de 2 ATA durante 45 minutos e posteriormente submetidos à OVH eletiva videoassistida com dois portais;

2. Grupo Hiperbárica Controle (GHC): 15 animais pré-tratados com HBOT na pressão de 2 ATA durante 45 minutos, sem procedimento cirúrgico. A OVH eletiva foi realizada somente ao final das avaliações e não foi parte da avaliação deste estudo

3. Grupo SHAM (SHAM): 15 gatas submetidas à OVH eletiva videoassistida com dois portais sem pré-tratamento com HBOT.

1.4.2 Procediementos pré-cirúrgicos

As pacientes dos grupos GH e GHC foram submetidas à HBOT com pressão de 2 ATA por 45 minutos previamente à aplicação da medicação pré-anestésica. Todos os animais receberam o mesmo protocolo anestésico, sendo pré-medicados com cloridrato de dexmedetomidina (20 µg/kg) aplicada via intramuscular (IM). Após 20 minutos em sala escura e silenciosa, todos os animais foram igualmente tricotomizados. Os animais do grupo GHC tiveram a sedação revertida com atipamezol (10 µg/kg) e foram encaminhados para as avaliações de dor, que será detalhada posteriormente.

Nos demais pacientes que foram submetidos à cirurgia, foram realizadas a indução anestésica com propofol ao efeito administrado via intravenosa (IV) e a intubação orotraqueal após anestesia da glote, com a aspersão de 1 mg/kg de lidocaína 1% sem vasoconstritor. A manutenção anestésica foi realizada com isoflurano em oxigênio a 100% em concentração necessária para manter os animais em plano adequado de anestesia.

Após estabilização anestésica, previamente à antissepsia cirúrgica, os animais receberam atipamezol (10 µg/kg), buscando-se a reversão total dos efeitos da dexmedetomidina, para que não houvesse interferência na avaliação analgésica posterior. Visando analgesia transoperatória, todos os animais foram mantidos em infusão contínua de cloridrato de remifentanila (10 µg/kg/h). A fluidoterapia de suporte foi promovida com a infusão de Ringer com lactato na taxa de 3 ml/kg/h, IV. Os animais foram monitorados de forma contínua com a utilização de monitor multiparamétrico e mantidos em ventilação mecânica, mantendo-se a pressão inspiratória de 10 cmH_2O e a concentração expirada de CO_2 ($ETCO_2$) entre 30-45 mmHg. Para isso, a frequência respiratória foi ajustada conforme a necessidade.

1.4.3 Procedimento cirúrgico

Para as cirurgias videolaparoscópicas as gatas foram posicionadas em decúbito dorsal. O primeiro portal, de 11mm, foi inserido na linha alba na região pré-púbica, em ponto

estratégico para a exteriorização do trato reprodutor, considerando a topografia da bifurcação dos cornos uterinos. Em seguida, foi introduzido no interior da cânula um endoscópio rígido de 10mm e 0°, conectado ao sistema de vídeo. A cavidade abdominal foi insuflada com CO_2 na pressão de 6mmHg com a utilização do insuflador Endoflator Karl Storz. O segundo portal (6mm) foi inserido sob visibilização da óptica, na linha alba na região da cicatriz umbilical.

As gatas foram rotacionadas para a direita, para a visibilização do ligamento suspensor ovariano e ovário esquerdos. Esses, suspensos por meio de pinça Kelly, sendo o ovário fixado na parede abdominal temporariamente através de uma sutura transparietal com fio PGA 2-0. A hemostasia e secção do complexo arterio-venoso ovariano (CAVO) foi realizada com pinça bipolar munida de lâmina de corte. Obtida secção do mesovário e ligamento suspensor, a sutura transparietal foi removida da parede abdominal liberando o ovário, sendo essas mesmas manobras repetidas do lado direito.

Os ovários e o útero foram expostos pela ferida do primeiro portal, permitindo a aplicação da técnica das três pinças imediatamente cranial à cérvix. Foi realizada a secção entre a primeira e a segunda pinça, aplicando-se na sequência, duas suturas transfixantes com fio poliglactina 910 2-0, junto à cérvix. O coto uterino foi devolvido à cavidade abdominal sob visibilização direta e as feridas cirúrgicas suturadas em três planos. Afim de padronizar o procedimento, a insuflação foi mantida por 30 minutos.

1.4.4 Pós-operatório

Imediatamente após o fim da cirurgia, os animais receberam dipirona sódica (25 mg/kg) via subcutânea a cada 12 horas, durante três dias. Os animais que não passaram pelo procedimento cirúrgico (grupo GHC) receberam o mesmo volume de solução de cloreto de sódio a 0,9% nos mesmos tempos.

Após realização de todas as análises as pacientes tiveram alta hospitalar, retornando para a casa com os seus tutores, com as devidas orientações sobre os cuidados no pós-operatório.

1.4.5 Avaliação de dor

Após o procedimento cirúrgico os animais foram submetidos à avaliação de dor pós-operatória pelos mesmos dois avaliadores proficientes com os métodos de avaliação e cegos ao procedimento realizado, com as escalas FGS e EUNESP nos momentos: T1, T2, T3 e T4 (3, 6, 12 e 24 horas após a extubação, respectivamente).

Animais que atingiram acima de 8 pontos (8 de 30) na EUNESP ou maior a 4 pontos (4 de 10) na FGS na avaliação de um dos avaliadores, receberam analgesia resgate com cloridrato de metadona (0,1mg/kg, IM), seguida de reavaliação após 30 minutos até a normalização dos parâmetros, segundo estas escalas.

Todos os animais receberam um pequeno curativo no local dos portais desde o início da ambientação, afim de manter a ferida cirúrgica escondida dos avaliadores. Os curativos e medicações foram realizadas por equipe extra, não inclusas na avaliação analgésica, em momentos em que não havia avaliação de dor para interferir o mínimo possível no comportamento das gatas.

1.4.6 Avaliação de temperatura

A temperatura central foi avaliada através de transdutor de temperatura esofágica e a temperatura periférica através de termômetro infravermelho na região interdigital. O ΔT_{c-p} foi obtido através da fórmula: ΔT_{c-p} = (T° central – T° periférica). Os valores foram avaliados antes e depois da HBOT nos grupos submetidos a terapia - afim de determinar se houve perda de temperatura e quantifica-la, imediatamente após a indução anestésica e ao fim da anestesia, no momento da extubação (em animais submetidos a cirurgia).

A sala foi mantida a uma temperatura de 25 a 27°C e umidade controlada através de termômetro com higrômetro, sendo avaliados de maneira constante por membro da equipe.

1.4.7 Estresse oxidativo

A avaliação da resposta inflamatória e do estresse oxidativo foram realizados por meio da análise de biomarcadores compatíveis com as amostras. Os tempos de coleta foram: Tempo 1 = Imediatamente antes do início da cirurgia, na estabilização anestésica; Tempo 2 = no momento da extubação; Tempo 3 = 24 horas após o fim da cirurgia. As análises foram realizadas da mesma forma, nos três grupos e nos diferentes tempos.

Animais do grupo GHC tiveram a sua primeira amostra coletada (T1) após a sedação dos animais, T2 após a reversão (30 minutos depois) e T3 24 horas após a reversão. As análises foram realizadas da mesma forma, nos três grupos e nos diferentes tempos, conforme segue abaixo:

1.4.7.1 Superóxido dismutase (SOD)

A atividade da SOD foi mensurada seguindo o método de McCord e Fridovich (1969). Uma unidade da enzima SOD é definida como a quantidade de enzima que inibe em 50% a velocidade de oxidação da adrenalina. Isso conduz à formação do produto de cor vermelha, adrenocromo, que é detectada por um espectrofotómetro. A SOD foi determinada medindo a velocidade de formação de adrenocromo, observado a 480 nm, num meio de reação contendo 50 mM de glicina-NaOH, pH 10 e adrenalina a 1 mM. Os resultados foram expressos como unidades de (UI) SOD por miligrama de proteína.

1.4.7.2 Substâncias reativas ao ácido tiobarbitúrico (TBARS)

Os níveis de TBARS foram determinados de acordo com Jentzsch, Bachmann e Bieesalski (1996) através da medição da concentração de MDA como um produto da peroxidação lipídica, por meio de reação com o ácido tiobarbitúrico (TBA). Resumidamente, a mistura de reação, contendo 200µl de soro ou de padrão (0.03mM MDA), 1 mL de ácido ortofosfórico 0,2 M, e 250µL ácido tiobarbitúrico (0,1 M) foi aquecida a 95°C durante 120 min. A absorbância medida a 532 nm. Os níveis séricos de TBARS foram expressos em ηmoles de MDA/mg de proteína.

1.4.7.3 Acetilcolinesterase (AChE)

A atividade da enzima AChE em sangue total foi determinada pelo método de Ellman et al. (1961) e modificado por Worek et al. (1999). O sangue foi recolhido em tubos vaccutainer. As amostras foram hemolisadas com tampão de fosfato, pH 7,4 contendo Triton X-100 e armazenada a -30 ° C durante 1 semana. A atividade específica da AChE de todo o sangue foi calculada a partir do quociente entre a atividade de AChE e conteúdo de hemoglobina e os resultados foram expressos como mU / umol de Hb.

1.4.7.4 Catalase (CAT)

A determinação da atividade da catalase no sangue foi realizada conforme método modificado de Nelson e Kiesow (1972). Este ensaio envolve a alteração da absorbância a 240 nm, durante 2 min, devido à decomposição da catalase dependente de peróxido de hidrogénio

(H_2O_2). A atividade da enzima foi calculada utilizando o coeficiente de extinção molar (0,0436 cm2 / umol) e os resultados foram expressos como ηmoles / mg de proteína.

1.4.7.5 Butirilcolinesterase (BChE) em soro

A atividade da BChE do soro foi determinada pelo método de Ellman et al. (1961). O sistema de tampão de fosfato de potássio 0,1mol com pH7,4, DTNB 0,30mmol e 50µL de soro será incubado durante 2 min a 30°C e a reação será iniciada pela adição do substrato butirilcolina na concentração de 1mmol. A leitura será realizada pelo método de espectrofotometria de 2 min a 412nm. A atividade enzimática foi expressa em µmol de BcSCh/h/mg de proteína.

2 ARTIGO 1 – INTERFERENCE OF HYPERBARIC OXYGEN THERAPY ON POSTOPERATIVE PAIN AND TEMPERATURE IN FEMALE CATS SUBMITTED TO ELECTIVE VIDEO-ASSISTED OVARIOHYSTERECTOMY

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Original Article

Interference of hyperbaric oxygen therapy on postoperative pain and temperature in female cats submitted to elective video-assisted ovariohysterectomy

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Keywords: Pain management, anesthesia, hyperbaric oxygen therapy, feline medicine, hypothermia

ABSTRACT

Objectives: We aimed to evaluate the effects of pretreatment with hyperbaric oxygen therapy (HBOT) on temperature and postoperative pain in cats undergoing elective ovariohysterectomy (OHE).

Methods: Forty-five healthy cats underwent video assisted OHE after being drawn into three groups: the hyperbaric group (pretreated with HBOT and then operated on), the hyperbaric control group (pretreated with HBOT and not operated on) and the SHAM group (operated without pretreatment). Pain was evaluated using the Feline Grimace Scale (FGS) and UNESP-Botucatu Multidimensional Scale (EUNESP) at baseline (pre-surgery), T1, T2, T3 and T4 (3, 6, 12 and 24 h after extubation). Scores above 8 and 4 points (EUNESP and FGS, respectively) received rescue analgesia. The central and peripheral temperatures and variations between them (ΔT_{c-p}) were evaluated before and after HBOT in the groups undergoing therapy, after induction of anesthesia and at the end of anesthesia in animals undergoing surgery.

Results: Five animals received rescue analgesia (four from the SHAM group and one from the hyperbaric group). There was no difference when comparing the same group over time nor between groups at the same time. In terms of temperatures, there was a difference in location (central or peripheral) and time (initial vs final); the final central temperatures of the groups were lower than the initial ones. The ΔT_{c-p} showed an effect of treatment (hyperbaric vs SHAM) and time (initial vs final). The hyperbaric and SHAM

groups showed a difference in initial and final ΔT_{c-p} and no differences were noted between the groups in the final evaluation time.

Conclusion and relevance: This was the first randomized study in cats submitted to HBOT and the results showed no positive influence of HBOT on postoperative analgesia. There was no difference between central temperature, peripheral temperature or ΔT_{c-p} in animals submitted or not to HBOT. Temperature reduction in healthy female cats after HBOT was not clinically significant.

Introduction

Hyperbaric oxygen therapy (HBOT) is a therapeutic modality where the patient is introduced into a chamber with 100% oxygen and subjected to pressures above 1 absolute atmosphere (ATA)[1]. In animals, the first reports of HBOT date back to 1887, in which it was shown to reduce fever in sepsis rabbits[2]. The advancement in HBOT techniques began to provide higher oxygen transport and better patient outcomes[3,4]. In veterinary medicine, elective ovariohysterectomy (OHE) is used as a method of population control and to treat and prevent numerous diseases[5]. Minimally invasive approaches of OHE are now well established and have advantages over laparotomy[6].

Properly managing pain is fundamental to the feline clinic[7]; recognizing it and the ability to diagnose and treat it requires experience and familiarity with clinical tools. Nonetheless, pain is often neglected, evidencing that cats receive fewer analgesics compared with canines[8–11]. Anesthesia with volatile substances can produce hypothermia

by inhibiting tremors and vasodilation and combining these factors with exposure to an algid environment and the ease with which cats lose temperature contribute to postoperative hypothermia[12–14].

Given this scenario, this study aimed to evaluate the effects of preconditioning with hyperbaric oxygen therapy on the temperature and postoperative pain in healthy cats undergoing elective ovariohysterectomy.

Materials and Methods

Forty-five (45) healthy, non-brachycephalic, female cats aged 1.89 ± 1.06 years and weighing 3.05 ± 0.52 kg were referred for OHE and admitted three days before the procedures for adaptation. Pre-surgical baseline values were established by the Feline Grimace Scale (FGS) and the UNESP-Botucatu Multidimensional Scale (EUNESP) by two blinded evaluators. For the procedure, the cats were separated into three groups, which received the following treatments:

1. Hyperbaric group (HG): 15 animals were pretreated with HBOT at 2 ATA pressure for 45 mins and subsequently underwent video-assisted OHE with two portals;
2. Hyperbaric control group (HCG): 15 animals were pretreated with HBOT at a pressure of 2 ATA for 45 mins without interference from the surgical procedure. OHE was performed only at the end of the evaluations;

3. SHAM group: 15 female cats underwent video-assisted OHE with two portals without pretreatment.

All animals underwent the same anesthetic protocol and were pre-medicated with dexmedetomidine hydrochloride (20 µg/kg IM). After 20 mins, they were also trichotomized and the HCG group had its sedation reversed with atipamezole (10 µg/kg IM) and referred to the evaluations.

In the groups undergoing surgery, anesthetic induction was performed with propofol intravenously (IV) to the effect and orotracheal intubation after anesthesia of the glottis with the sprinkling of 1 mg/kg of lidocaine 1%. Anesthetic maintenance was performed with isoflurane at a concentration necessary to maintain the appropriate anesthetic plane. After stabilization, the animals received atipamezole (10 µg/kg IM) to reverse the effects of dexmedetomidine, avoiding interference in the subsequent evaluation. The OHE-submitted groups were maintained in a continuous infusion of remifentanil hydrochloride (10 µg/kg/h IV) for transoperative analgesia. Fluid therapy was promoted by infusing Ringer's lactate at 3 ml/kg/h IV. The animals were continuously monitored and maintained on mechanical ventilation at an inspiratory pressure of 10 cmH_2O and expired CO_2 concentration ($ETCO_2$) between 30 and 45 mmHg. For this, the respiratory rates were adjusted as necessary.

At the end of the surgery, the animals received dipyrone sodium (25 mg/kg SC, b.i.d) for three days. The HCG group received the same volume of 0.9% NaCl solution at the same intervals.

Pain assessment

After OHE, the animals were submitted to postoperative pain assessment by the FGS and EUNESP scales at T1, T2, T3 and T4 (3, 6, 12 and 24 h after extubation, respectively). Animals with scores above 8 points (8/30) for EUNESP or 4 points (4/10) for FGS by one of the evaluators received rescue analgesia with methadone hydrochloride (0.1 mg/kg IM) and reevaluation after 30 mins until the parameters normalized. All animals received a small dressing at the surgical site in order not to reveal the surgical technique to the evaluators.

Temperature evaluation

The central temperature (CT) was evaluated using an esophageal transducer and the peripheral temperature (PT) using an infrared thermometer in the interdigital region. The ΔT_{c-p} was obtained through the formula: ΔT_{c-p} = (CT-PT). The values were evaluated before and after HBOT in the groups undergoing therapy to determine whether there was temperature loss and quantify it immediately after anesthesia induction and at the time of

extubation. The room was kept at a temperature between 25-27°C and the humidity was monitored using a thermometer with a hygrometer.

Statistical analysis

The data were submitted to the Shapiro-Wilk normality test and considered non-parametric when $P < 0.05$. Pain scale data were subjected to Friedman's non-parametric test to detect significant differences between the same group at predetermined intervals. For the intra-group, fixed-time comparisons, the Kruskal-Wallis test followed by Dunn's post-test was used. The data were graphically displayed as the maximum value, 75% percentile, median, 25% percentile and minimum value.

The pre- and post-hyperbaric temperature variations were evaluated by applying a dependent variables Student's t-test. The temperature between the HG and SHAM group in the pre- and postoperative times were evaluated and methods compared (central and peripheral) via a three-way analysis of variance (ANOVA) (treatment versus measurement method versus time) followed by Newman-Keuls post-hoc test. The ΔT_{c-p} was analyzed by two-way ANOVA (time versus treatment) and data were expressed as mean ± standard deviation. Values were considered statistically different when $P < 0.05$.

Results

The surgical time was 48.41 ± 4.75 mins and the anesthetic time was 65.58 ± 6.73 mins from induction of anesthesia to extubation. Five animals received rescue analgesia, four being from the SHAM group, all at the third hour after extubation, two by the EUNESP scale, one by the FGS and one by both scales. The fifth animal was from the HG and had rescue analgesia after extubation by the FGS at the sixth hour.

There was no statistical difference in the analgesic evaluation compared with the same group during the time evaluations (Figure 1). The Kruskal-Wallis test showed values of $P = 0.503$ (baseline), $P = 0.301$ (T1), $P = 0.232$ (T2), $P = 0.060$ (T3) and $P = 0.729$ (T4). Friedmann's test followed by Dunn's post-test showed no differences in the same group over time in the EUNESP evaluation (Figure 1A). In the FGS, there were also no significant differences between groups when analyzed in a fixed time period, $P = 0.619$ (baseline), $P = 0.051301$ (T1), $P = 0.142$ (T2), $P = 0.511$ (T3) and $P = 0.941$ (T4). No difference was observed in this scale concerning the comparison between groups at a fixed time period (Figure 1b).

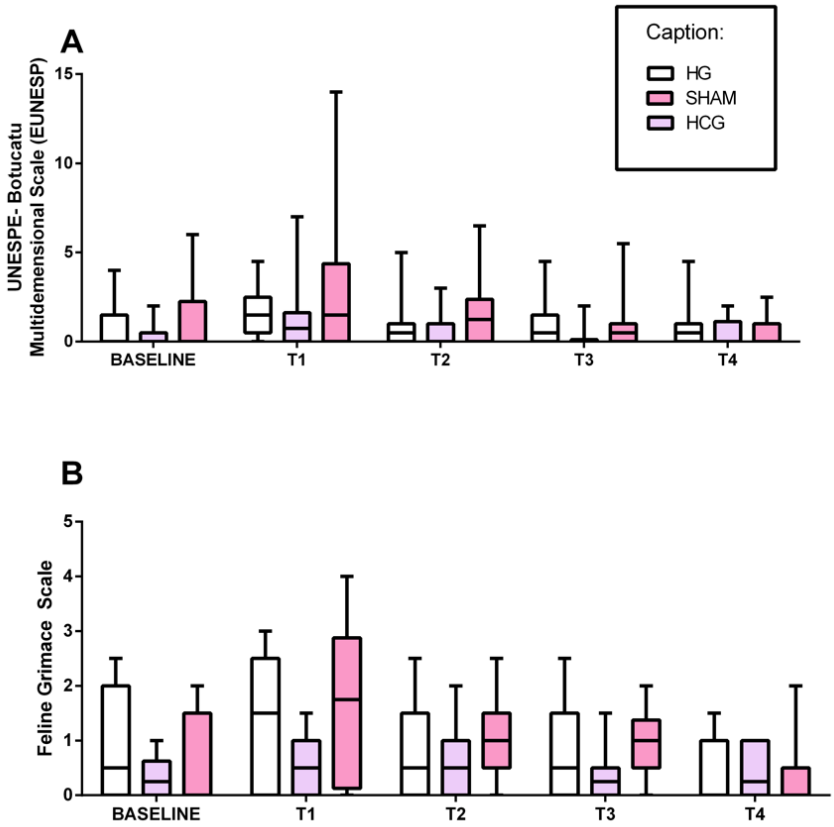

Figure 1: Evaluation of analgesia scores in female cats using the (a) UNESP de Botucatu scale (EUNESP) and (b) Feline Grimace Scale (FGS). Evaluations performed before and after extubation: baseline (before surgery), T1 (3 h), T2 (6 h), T3 (12 h) and T4 (24 h) of the HG, SHAM and HCG, respectively. The data are presented as the maximum value, 75% percentile, median, 25% percentile and minimum value

The Students t-test of dependent variables revealed a statistical difference (P <0.0001) between pre- (38.43 ± 0.32) and post-hyperbaric (38.11 ± 0.35) rectal temperature assessment for HBOT (Figure 2).

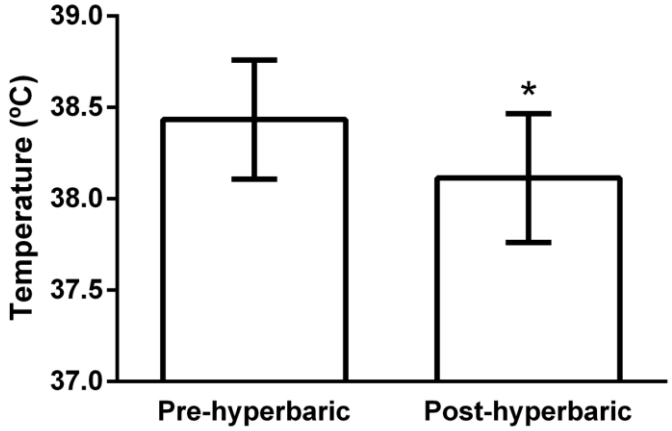

Figure 2 Pre- and post-hyperbaric rectal temperature assessment of female cats subsequently undergoing OHE. Data were presented as mean ± standard deviation. *Indicates statistical difference for the pre-hyperbaric group and values were considered significant when P <0.5.

The three-way ANOVA showed an effect of site of CT and PT measurements: [F = 145.78, *P* <0.0001], time (initial vs final) [F = 59.70, *P* <0.0001] and interaction between time and site [F = 106.46, *P* <0.0001]. The Newman-Keuls post-hoc test showed that the final CTs of the HG and SHAM groups (34.2 ± 0.7 and 34.2 ± 0.9, respectively) were lower than the initial ones (37.2 ± 0.6 and 37.2 ± 0.9, respectively), with *P* >0.001 values. There was no statistical difference between initial and final CTs in the HG and SHAM group (*P* = 0.340 and *P* = 0.107, respectively). Differences between final CTs were also observed in the SHAM group (*P* = 0.088) (Figure 3).

The two-way ANOVA for $\Delta T_{c\text{-}p}$ showed an effect of treatment (HG vs. SHAM) [F = 4.47, *P* <0.038] and time (initial vs final) [F = 110.57, *P* <0.0001]. The HG and

SHAM groups demonstrated a difference in initial and final ΔT_{c-p} with P <0.001 for both groups. While the initial ΔT_{c-p} of the HG and SHAM groups was 5.9 ± 1.0 and 4.7 ± 2.0, the final ΔT_{c-p} was 1.9 ± 1.2 and 1.16 ± 1.5, respectively. There was no difference between HG and SHAM groups at the final evaluation time (P = 0.530) (Figure 3).

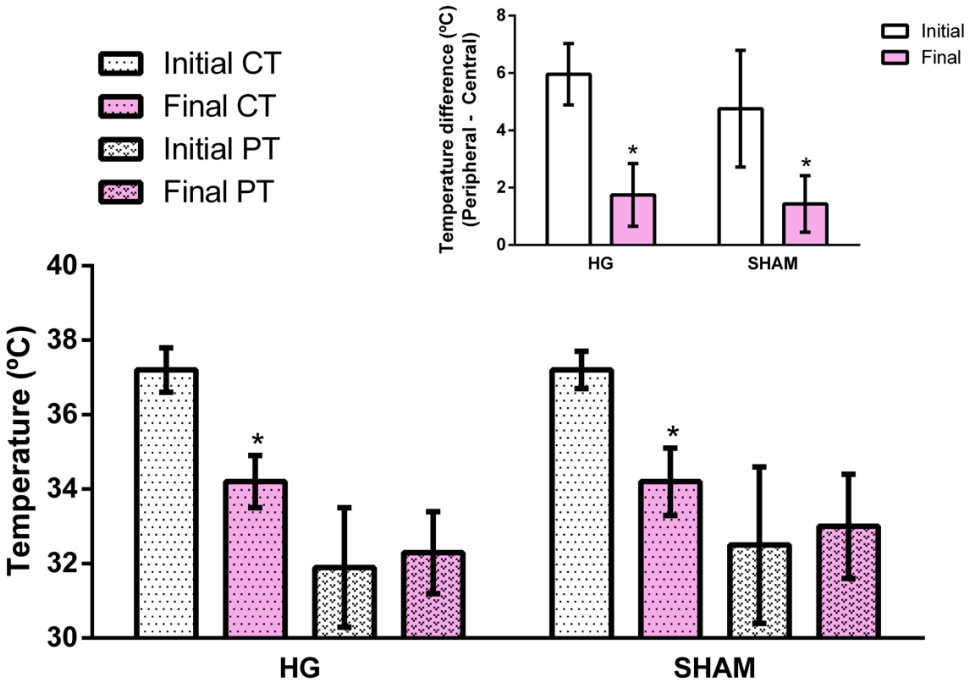

Figure 3 Comparison between initial and final temperatures in the OHE submitted to HBOT and comparison between CT and PT measuring methods between the HG and SHAM group. The ΔT_{c-p} for the time variable was evaluated. Data were expressed as mean ± standard deviation. Statistical differences were considered when P <0.05. *Difference between final and initial temperature; key: CT: initial (pre-surgical) and final (post-surgical) central (esophageal) temperature; PT: initial (pre-surgical) and final (post-surgical) peripheral temperature.

Discussion

Pain is an unpleasant sensation that is difficult to understand and animals are incapable of self-reporting, so we must recognize it. Adequate analgesia prevents suffering, improves healing, decreases morbidity and avoids persistent chronic postoperative pain[15]. Given that HBOT therapy benefits pain management in humans and rats[16–18], we asked whether the same therapy could help control postoperative pain in cats. Therefore, we applied HBOT in female cats that underwent video-assisted OHE and compared them to female cats that did not undergo the surgical procedure or were not submitted to HBOT. Considering that stress is an essential modulator of behavior in cats, we evaluated the behavior of the cats during the HBOT to identify whether the therapy would be a stressful factor. To the authors' knowledge, this is the first work that evaluates the use of HBOT for analgesic purposes and its response to temperature in cats.

During pain assessment (EUNESP and FGS), we observed a higher number of rescues in the SHAM group due to higher pain scores, although there were no significant differences over the four-time evaluations nor between groups when comparing fixed time (Figure 1). This is due to the low pain stimulus caused by the video-assisted[6,19] procedure allied to using dipyrone in OHE, corroborating studies that have demonstrated this[20,21].

We hypothesized that the animals were pretreated with only one session of HBOT in light of research in humans demonstrating that a single session of HBOT attenuates acute and chronic signs in patients with hyperalgesia induced by burn wounds, confirming the central anti-inflammatory and neuroplasticity effects of HBOT[22].

Considering the sample size of 45 animals, in which five required complementary analgesia and four did not undergo HBOT, studies involving a larger number of cats could provide different results. The protocol used for pretreatment with HBOT was based on a prospective study[23] in which 230 HBOTs for dogs and cats were performed at a pressure of 2 ATA for periods ranging from 45 to 60 mins. In the aforementioned studies, HBOT was well tolerated and no major adverse effects were reported during treatment, corroborating our study, in which no noteworthy adverse effects were reported during the 30 sessions performed.

In view of the behavioral and physiological peculiarities that cats may manifest[24] and how stress can be determinant in the quality of life of these animals[25], a differentiated treatment is necessary. During this study, from triage to discharge, we attempted to follow techniques and management that aimed to minimize stress, such as hospitalization in a calm environment, avoiding excessive handling and keeping the animals with blankets and clothes from their guardians, among other measures[24,26].

In mice, the mere presence of the evaluator can decrease the scores because stress can inhibit pain characteristics[27]. However, it is known that this is not necessary for cats

and it has been proven that the pain scores evaluated with the FSG do not change with the presence of the evaluators[28]. Even so, in this study, the animals were housed with the evaluators for three days prior to the evaluations in order to minimize any effects.

It is protocol in some veterinary centers to sedate or tranquilize patients for HBOT, although we noticed this was unnecessary[23]. To evaluate the behavior of the cats during HBOT, the camera operator evaluated them every five mins during the entire session. Our study found that 53.4% (16/30) were classified as calm, remaining serene, lying down, sleeping or grooming for >75% of the sessions. Nonetheless, seven (23.3%) remained curious, exploring or sitting without evident anxiety, while 23.3% (7/30) were considered anxious, manifesting arched posture, fearful expression, attempted escape or scratching the camera and constant vocalization. As only collaborative animals were selected for this study, it is suggested that the use of sedatives be at the veterinarian's discretion, observing the behavior and particularities of each patient.

Since research involving HBOT in cats is scarce, it is essential to associate this therapy with basic parameters of healthy animals to enrich future studies and the development of safe monitoring protocols. During the 30 sessions of HBOT performed, the animals had a loss of 0.32 ± 0.03°C. A study involving dogs submitted to the same time and pressure protocol obtained similar results, which are statistically significant but not physiologically important[29], as the animals had a loss of less than 0.5°C within the physiological parameters for the species.

Studies in humans point out that the association of HBOT with therapeutic hypothermia in patients intoxicated by carbon monoxide is beneficial[30]. In feline medicine, this association has yet to be proven; however, in our study, we demonstrated that it is possible to maintain thermal homeostasis in healthy animals, thereby subsidizing future research.

Cats have a large surface area in relation to their body weight, making them very susceptible to transoperative hypothermia [31]. This susceptibility was seen in this study, considering the difference between the initial and final surgical CT in the SHAM and HG groups (Figure 3). Even with the difference between the initial and final CTs, there was no difference when comparing the groups, showing that preoperative HBOT did not negatively influence the temperature loss of the cats.

Besides the difference in body conformation, several factors cause an anesthetized animal to lose temperature, making thermoregulation difficult[32]. Volatile anesthetics such as isoflurane and other components of the anesthetic protocol can produce hypothermia[12,14], regardless of the use of HBOT, as evidenced by our findings. The drop in CT potentiated by anesthesia may be related to the elimination of behavioral responses, including searching for a warm environment and physiological responses such as inhibiting shivering, reducing metabolic rates and vasodilation[12,14,31,32].

It is known that general anesthesia increases ΔT_{c-p} over time and this condition was not evidenced in our study[32]. On the contrary, we noticed a negligible increase in PT

in both groups, decreasing ΔT_{c-p} as the surgical time increased (Figure 3). We attribute this lower ΔT_{c-p} to the use of preoperative atipamezole to reverse the analgesic effects of dexmedetomidine. With this inversion, there is also the reversion of other effects, such as vasoconstriction and systemic vascular resistance caused by this drug[33–36].

Conclusions

The results obtained in this randomized clinical trial showed no significant positive influence in reducing postoperative pain scores in female cats pretreated with hyperbaric oxygen therapy and submitted to elective ovariohysterectomy. There were no differences between central, peripheral or ΔT_{c-p} temperatures when comparing animals submitted to pretreatment with HBOT with those that were not, showing no interference of this therapy in these physiological parameters. In this novel randomized study, it was possible to conclude that reduced temperatures in healthy female cats after a hyperbaric session has no clinical significance

Conflict of interest

None of the authors of this article has a financial or personal relationship with other people or organizations that may influence the content of the article, declaring any potential conflict of interest.

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Ethical approval

This work involved the use of non-experimental animals (owned) outside of established internationally recognised high standards (‘best practice’) of individual veterinary clinical patient care. The study therefore had Ethics Approval from an established committee as stated in the manuscript.

Informed consent

No animals or people are identifiable within this publication, and therefore additional informed consent for publication was not required.

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3 ARTIGO 2 – HYPERBARIC OXYGEN THERAPY POSITIVELY INTERFERES WITH OXIDATIVE METABOLISM IN FEMALE CATS UNDERGOING VIDEO-ASSISTED ELECTIVE OVARIOHYSTERECTOMY

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Hyperbaric oxygen therapy positively interferes with oxidative metabolism in female cats undergoing vídeo-assisted elective ovariohysterectomy.

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Running head:

PRECONDITIONING WITH HBOT

ABSTRACT

This study aimed to determine whether hyperbaric oxygen therapy (HBOT) alters oxidative biomarkers after elective ovariohysterectomy (OHE). For this purpose, 45 healthy female cats were randomized into three groups: the hyperbaric group (HG): 15 animals pretreated with HBOT and submitted to OHE; the hyperbaric control group (HCG): 15 animals pretreated with HBOT without surgery; and the sham group (SHAM): 15 female cats submitted to OHE without pretreatment. The following biomarkers were evaluated: superoxide dismutase (SOD) and catalase (CAT), reactive oxygen species (ROS), thiobarbituric acid reactive substances (TBARS), acetylcholinesterase (AChE), and butyrylcholinesterase (BChE). The collection times were: T1 = before the surgery for the operated groups and after sedation in the HCG; T2 = 30 min after reversal of sedation (HCG) or at the time of extubation in the other groups; T3 = 24 h after T2. There was no difference regarding serum dosage of ROS, AChE, or BChE. There was an increase in TBARS in the T3 SHAM compared to the T3 HG (p=0.043). Moreover, CAT activity decreased in T2 SHAM and T3 SHAM compared to T1 SHAM (p=0.012 and p<0.001mauric); T2 SHAM had lower CAT activity than T2 HCG (p=0.05). Additionally, the T3 SHAM was lower than the T3 HG (p=0.030) and T3 HCG (p=0.050). There was reduced SOD in the T2 SHAM compared to the T2 HG (p=0.033) and T2 HCG (p=0.027). Similarly, the T3 SHAM decreased compared to the T3 HG (p=0.039) and T3 HCG (p=0.019).

Keywords:

Feline medicine; HBOT; Lipid peroxidation; Oxidative stress markers; Videosurgery .

Introduction

Hyperbaric oxygen therapy (HBOT) is a therapeutic modality in which patients inhale oxygen (O_2) at 100% under a pressure level above 1 absolute atmosphere (absolute technical atmosphere, ATA) in a pressurized chamber, enabling higher oxygen perfusion rates in tissues[15]. Oxygen is necessary to provide energy and enable cellular respiration; hence, deficient oxygen supply can lead to cell death by hypoxia. Sick animals have reduced O_2 transport capacity and their tissues require increasingly higher O_2 levels, leading to system collapse and oxidative stress [32]. Several mechanisms are proposed to account for the physiological benefits of HBOT, including increased plasma oxygen availability, tissue hyperoxygenation, barometric effects, immunomodulation, and reduced oxidative stress [6].

The organism of animals has an oxidative state, which is completely dependent on a balance between oxidant reagents and antioxidant defenses. Situations such as tissue hypoxia generate molecules with unpaired electrons that interact with other molecules, consequently modifying their biochemical structure. The body can produce antioxidant substances that neutralize this effect, although when the production of oxidant agents is high or there is a physiological inability to neutralize them, a biochemical imbalance occurs, called oxidative stress [8, 28]. Oxidative stress can be quantified using different biomarkers by directly measuring free radicals, free radical damage products, or the levels of specific and total antioxidants [1].

Reactive oxygen species (ROS) are generated as natural byproducts of metabolism and encompass a variety of chemical species, including superoxide, hydrogen peroxide, hypochlorous acid, and hydroxyl [28]; these radicals can be generated exogenously or produced by cells from several different sources [17].

Lipid peroxidation consists of a cascade of reactions resulting from the action of free radicals on lipids. The cell membrane is one of the most affected components, leading to

changes in its structure and permeability with consequent release of organelle content, including malonildialdehyde; this marker can be measured by quantifying thiobarbituric acid reactive species (TBARS). To counteract the oxidative imbalance caused by ROS, the organism produces antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT), products of oxygen oxidation in a peroxidase reaction where the two molecules of hydrogen peroxide (H2O2), are catalyzed into water (H2O) and oxygen (O2) [11, 13, 17].

The enzymes acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) are used as local or low-grade systemic inflammation indicators. Acetylcholine (Ach) has an important inflammatory suppressive action, albeit it is rapidly hydrolyzed by AChE and BChE. Therefore, increased AChE and BChE activities may lower Ach levels, reducing its anti-inflammatory effects [14].

In cats, oxidative stress development is well documented in various diseases such as hypertrophic cardiomyopathy, chronic kidney disease, cognitive dysfunction syndrome, feline infectious peritonitis, among others [8, 10, 20, 27]. The interference of HBOT on oxidative stress markers is well documented in human patients, experimental animals, and dogs [2, 7, 19, 31], although data for feline patients is scarce.

Given this lack of research regarding the interference of HBOT on oxidative stress biomarkers in cats and knowing the harmful effects that this disease can promote in the body [17], we aimed to determine whether treatment with pre-surgical hyperbaric oxygen therapy alters oxidative stress biomarkers during video-assisted ovariohysterectomy with two portals. This study was approved by the Ethics Committee on Animal Use (CEUA) of the Federal University of Santa Maria under protocol no. 5134560820.

Material and Méthods

Forty-five healthy female cats aged 1.89+-1.06 years and weighing 3.05+-0.52 kg were used for video-assisted OHE. All animals were acclimated for three days, and on the fourth day, they were randomly divided into three groups that received different treatments, namely:

1. Hyperbaric group (HG): 15 animals were pretreated with 100% oxygen at a pressure of 2 ATA for 45 min and subsequently underwent elective video-assisted OHE with two portals.
2. Control hyperbaric group (CHG): 15 animals were pretreated with HBOT at a pressure of 2 ATA for 45 min without interference from the surgical procedure. Elective OHE was performed at the end of the collections.
3. Sham group (SHAM): 15 cats underwent elective video-assisted OHE with two portals without pretreatment.

Pre-surgical procedures

Patients in the HGs and HCGs were submitted to HBOT with a pressure of 2 ATA for 45 min before the anesthetic-surgical procedure plus 15 min for pressurization and 15 min for depressurization. Preanesthetic medication was performed with dexmedetomidine hydrochloride (20 µg/kg; Dexdomitor, Zoetis, Brazil) applied intramuscularly (IM) and, in the three groups evaluated, the researchers waited 25 min for total sedation to take effect. Anesthetic induction was performed with propofol (Propovan 10 mg/mL; Cristália Prod. Quím. Farm. Ltda., Brazil) administered intravenously (IV). Anesthesia of periglottic tissues was performed with 1 mg/kg lidocaine spray (Xylestesin 2 mg/mL; Cristália Prod. Quím. Farm. Ltda., Brazil) prior to orotracheal intubation. Anesthetic maintenance was performed with isoflurane in 100% oxygen concentration to maintain the animals in an adequate anesthetic plan in a system compatible with the animal's size.

After anesthetic stabilization, prior to surgical antisepsis, atipamezole (10 µg/kg; Antisedan, Zoetis, Brazil) was administered to reverse the sedative, analgesic, and hemodynamic effects of dexmedetomidine, followed by a continuous infusion of remifentanil hydrochloride (10 µg/kg/h; Remifas 5 mg, Cristália Prod. Quím. Farm. Ltda, Brazil) and fluid therapy with a Lactate Ringer solution (3 mL/kg/h). Immediately after the end of the surgery, the animals in the HGs and SHAMs received dipyrone sodium (25 mg/kg; Analgex V, União Química Farmacêutica Nacional S/A, Brazil) subcutaneously every 12 h for 3 days. The animals in the HCG received a proportional volume of 0.9% sodium chloride solution.

Oxidative metabolism

The biomarkers of oxidative stress were evaluated. The collection times were: T1 = immediately before the surgery during anesthetic stabilization; T2 = at the time of extubation; T3 = 24 h after the surgery. The analyses were performed in the same way in the three groups and at different times. The HCG animals had the T1 sample collected after sedation, T2 after anesthesia reversal (30 min after), and T3 24 h after anesthesia reversal; SOD and CAT activity were analyzed using whole blood collected in sodium citrate tubes. Serum samples were collected to measure ROS, TBARS, AChE, and BChE.

Statistical analysis

Data were subjected to one-way repeated measures analysis of variance (ANOVA), followed by Duncan's post-hoc test. Data were expressed as mean ± standard deviation of the mean, and $p<0.05$ values were considered statistically different.

Results

One-way ANOVA with repeated measures showed a significant effect of prior treatment with HBOT (p=0.028) on serum TBARS levels. There were no significant effects of the time variable (p=0.507) or treatment vs. time interaction (p=0.165) on serum TBARS levels. Duncan's post-hoc test showed significantly higher TBARS levels in the T3 group, while the SHAM had more lipoperoxidation (14.07±1.44) than the HG (7.95±1.49), with a p-value=0.043 (Figure 1A). One-way ANOVA showed no effect of treatment (p=0.715), time (p=0.600), nor treatment vs. time interaction (p=0.761) on serum ROS generation (Figure 1B).

One-way ANOVA with repeated measures for CAT activity showed a significant interaction between treatment and time (p<0.001) and an effect of treatment (p=0.021), although no effect of time (p=0.10). The post-hoc test showed reduced CAT activity in the T2 SHAM (1.75±0.09) and T3 SHAM (1.64±0.07) compared to the T1 SHAM (2.00±0.08), with values of p=0.012 and p<0.001, respectively. In addition, the T2 SHAM (1.75±0.09) had lower CAT activity than the T2 HCG (2.18±0.10), with a p-value=0.05. Moreover, the T3 SHAM showed lower CAT activity than the T3 HG (2.11±0.07) and T3 HCG (1.99±0.07), with p-values=0.030 and p=0.05, respectively (Figure 2A). One-way ANOVA with repeated measures showed an increase in the treatment variable (p<0.001), albeit without any effect of time (p=0.729) or interaction between treatment vs. time (p=0.496) for serum SOD activity. Duncan's test showed decreased serum SOD activity in the T2 SHAM (1.66±0.09) compared to the T2 HG (2.06±0.09) and T2 HCG (2.10±0.09), with values of p=0.033 and p=0.027, respectively. Similarly, the T3 SHAM (1.66±0.08) showed decreased enzyme activity compared to the T3 HG (2.07±0.09) and T3 HCG (2.13±0.09), with p-values=0.039 and p=0.019, respectively (Figure 2B).

One-way ANOVA did not show any effect of treatment (p=0.959), time (p=0.768), or treatment vs. time interaction (p=0.259) on AChE activity (Figure 3A). Similarly, one-way

ANOVA showed no effect of treatment (p=0.544), time (p=0.566), or treatment vs. time interaction (p=0.670) on BChE activity (Figure 3B).

Discussion

Reactive oxygen species, such as superoxide and hydrogen peroxide, are produced mainly by mitochondria during cellular respiration. Knowing this, we can affirm that all aerobic organisms are subject to oxidative stress because the DNA of these organisms is continuously exposed to ROS [8, 24, 30]. This topic generates more and more interest; after countless studies over the years, research has shown that biological oxidation, the integrity of the body's antioxidant defenses, plays an important role in the course of various diseases and even in aging, as a change in biological oxidation or a decrease in endogenous antioxidants can lead to DNA damage and the synthesis of essential proteins for the organism [8, 12, 22].

Given this crucial issue and knowing that tissue injuries from surgeries produce oxidative stress [1], we questioned whether the HBOT used preventively could help reduce oxidative stress caused by surgery in cats. Furthermore, evaluating the concentration of oxidative stress biomarkers in healthy cats after a session of HBOT allows us to provide substantial results that will serve as a basis for further research on the subject. This is the first study that evaluates oxidative stress biomarkers after HBOT in cats.

Hyperbaric oxygen therapy is a therapeutic modality in which the patient is subjected to a chamber with pure (100%) oxygen at a pressure typically 1.5 to 3 times higher than standard air pressure at sea level (i.e., absolute technical atmosphere) [4, 15]. This is a low complexity treatment with numerous well-documented biochemical effects in humans and animals, including increased endogenous synthesis of antioxidants, modulation of inflammation, angiogenesis, and antimicrobial activity [4, 16, 31].

Studies have demonstrated safety and few side effects in small animals after HBOT. Recently, prospective clinical trials have been published, showing that cats tolerate HBOT sessions very well [6, 23]. With this in mind, each session's pressure regimen and time were based on these trials using a pressure of 2 ATA and 45-min duration. No adverse effects or complications occurred during the 45 sessions. In most studies in small animals, HBOT is performed after the lesion has been produced [5, 19] although the preventive treatment with hyperbaric oxygen therapy is still little reported and has aroused the interest of many researchers [18] including this trial, in which HBOT was performed preventively.

It is already known that surgical procedures, like any other trauma, produce oxidative stress associated with oxidant production and deplete antioxidant mechanisms due to incisional injuries, visceral manipulation, and inflammatory cell activation [3, 29]. It is also known that less tissue trauma results in less inflammation and that laparoscopic surgeries cause less oxidative stress compared to open procedures [1, 11]. In order to minimize variation in induced inflammatory effects, all surgeries were performed by the same surgeon and camera operator. The room was kept at a stable temperature and humidity control during the procedures, and no anti-inflammatory drugs were administered prior to the last collection time.

Reactive oxygen species are generated as natural byproducts of metabolism and have a dual role in the body; moreover, ROS can be beneficial through their participation in defenses against infectious agents, cell signaling, and induction of mitogenic response, among others [26, 28]. Nevertheless, their excess can be harmful, leading to protein and nucleic acid oxidation. These free radicals may often be increased in many organs in cases of hypoxia [28], however, it was not possible to detect this increase in this study, and no difference in ROS values was evidenced between cats that were and were not submitted to HBOT.

One of the consequences of oxidative stress is lipid peroxidation, which consists of a cascade of reactions resulting from the action of free radicals on lipids [21]. When evaluating

the TBARS values 24 h after surgery (T3), we noted a significant decrease in the values in the animals that underwent the HBOT session (HG) compared to the animals that did not (SHAM). Knowing that TBARS evaluates the levels of malondialdehyde and that this is a secondary product of oxidative stress formed during lipid peroxidation [21, 25], we evidenced that HBOT could reduce this effect. Another piece of data that corroborates this hypothesis was that there was no statistical difference between the two groups submitted to HBOT (HG or HCG), which further strengthens the efficacy of HBOT in reducing lipid peroxidation.

We believe that this is because the surgical technique used induces an insufficient inflammatory reaction for this detection in the immediate postoperative period. We know that installing the oxidative stress process occurs at the expense of an imbalance between oxidant and antioxidant compounds. Superoxide dismutase and catalase are enzymes intimately involved in the antioxidant system and are considered part of the first line of defense of the organism in protecting tissues against oxidative damage caused by ROS [17, 30].

The profile of antioxidant enzymes in this study was similar to TBARS, with no statistical difference between the groups that underwent HBOT (HG and HCG), regardless of the surgical procedure. We believe that the reduced SOD and CAT activities occurred due to the surgical stimuli in the SHAM. Nonetheless, there was an increase in the activity of these enzymes with the HBOT pretreatment, and these observations support our suspicions that HBOT is beneficial in maintaining endogenous antioxidant levels. This is evidenced in the decrease of enzymes in the group without HBOT pretreatment (SHAM) compared to the two groups pretreated with HBOT (HG and HCG).

When we evaluated the first 24 h post-surgery of the cats in the SHAM, we noticed a significant decrease over time, evidencing the consumption of antioxidant enzymes in the body. Similar findings were observed in a study with rats in which bile duct ligation was performed (with and without HBOT), and treatment with HBOT sharply increased the mean SOD and

CAT activity and decreased TBARS levels [2]. These data further support the theory that HBOT can minimize the deleterious effects of surgery on oxidative stress.

Furthermore, AChE and BChE act by regulating acetylcholine, an important enzyme in suppressing inflammation. In surgical procedures of OHE in bitches, one study identified the elevation of these biomarkers in the first three postoperative hours [11]. There was no difference between the groups for either of the two cholinesterases nor an increase or decrease detected with a statistical difference in our study. We believe that there was not enough inflammatory process to detect this increase because we are dealing with minimally invasive procedures.

Another study conducted with bitches after OHE also reported unaffected AChE and BChE activity, both in a conventional surgical approach and in laparoscopic surgery; however, unlike our study, anti-inflammatory drugs were used postoperatively, which may have modulated pro-inflammatory cytokine production [13]. Regarding the use of HBOT, one study evaluated other inflammatory biomarkers (e.g., C-reactive protein, circulatory cytokines, and changes in iron homeostasis) in bitches that underwent OHE and subsequently received or not two sessions of HBOT, and there were no significant differences in these biomarkers [20]. This result rectifies our data and suggests that new studies be performed about the inflammatory profile of animals submitted to HBOT.

The present study achieved its objective of elucidating the effects of preoperative hyperbaric oxygen therapy on the expression of some biomarkers of oxidative damage and evaluating this therapeutic effect on oxidative enzymes in female cats submitted to elective ovariohysterectomies. The HBOT proved to be of substantial value in treating inflammatory response, promoting favorable influence on reducing oxidative stress and serum levels of these biomarkers. This is the first study addressing the significantly positive results of preoperative HBOT for video-assisted elective ovarian hysterectomy in healthy cats to the best of our knowledge. Considering the positive effects of HBOT in cats, associated with the tendency of

using video-laparoscopic techniques in these animals, we believe that the union of these treatment modalities constitutes an advance in feline medicine. The results obtained in this study may also serve as a basis for future investigations covering HBOT in other domestic species.

Conflicts of interest

The authors declare no potential conflicts of interest; no author of this article has a commercial or financial interest or partnership for commercial purposes with this work.

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Figures:

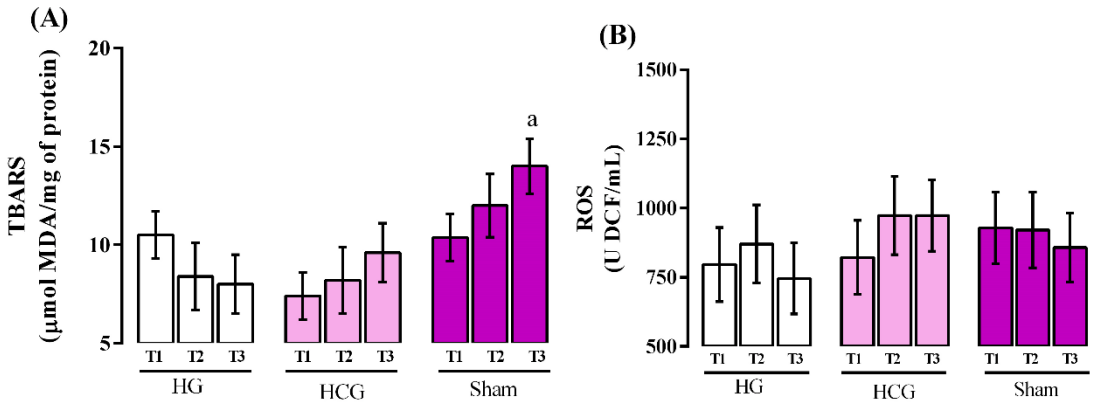

Figure 1: Serum analysis of TBARS levels and ROS generation of I) female cats submitted to hyperbaric therapy with subsequent OHE (HG); II) female cats only submitted to hyperbaric therapy (HCG) and III) female cats only submitted to OHE (Sham). Blood samples were collected before surgery (T1), immediately after surgery (T2), and 24 h after the procedure (T3). Data were expressed as mean ± standard deviation of the mean. Differences were considered significant when $p<0.05$.

[a] Significant difference for the HG at the same time;

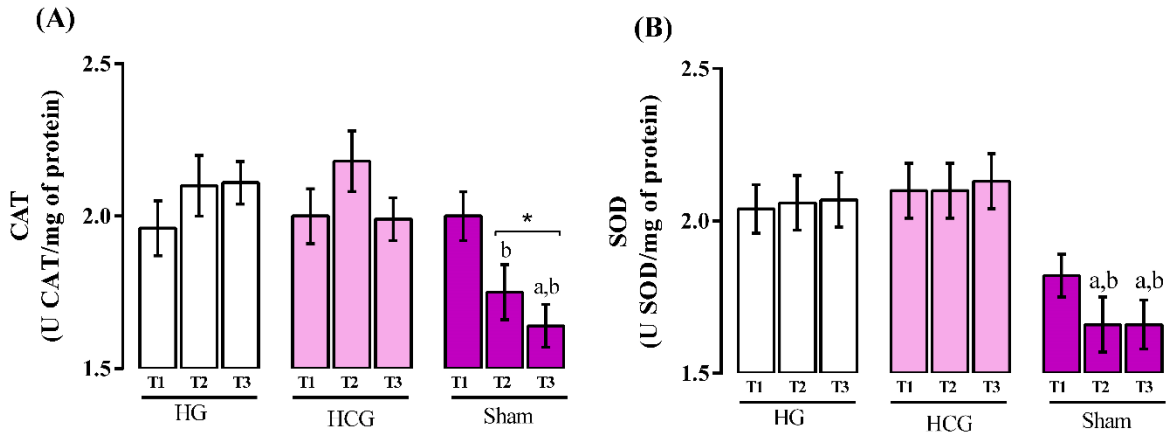

Figure 2: Serum analysis of catalase (CAT) and super dismutase (SOD) activity of I) female cats submitted to hyperbaric therapy with subsequent OHE (HG); II) female cats only submitted to hyperbaric therapy (HCG), and III) female cats only submitted to OHE (Sham). Blood samples were collected before surgery (T1), immediately after surgery (T2), and 24 h after the surgical procedure (T3). Data were expressed as mean ± standard deviation of the mean. Differences were considered significant when $p<0.05$.

* Significant difference for T1 within the same experimental group; [a] Significant difference for the HG at the same time; [b] Significant difference for HCG at the same time

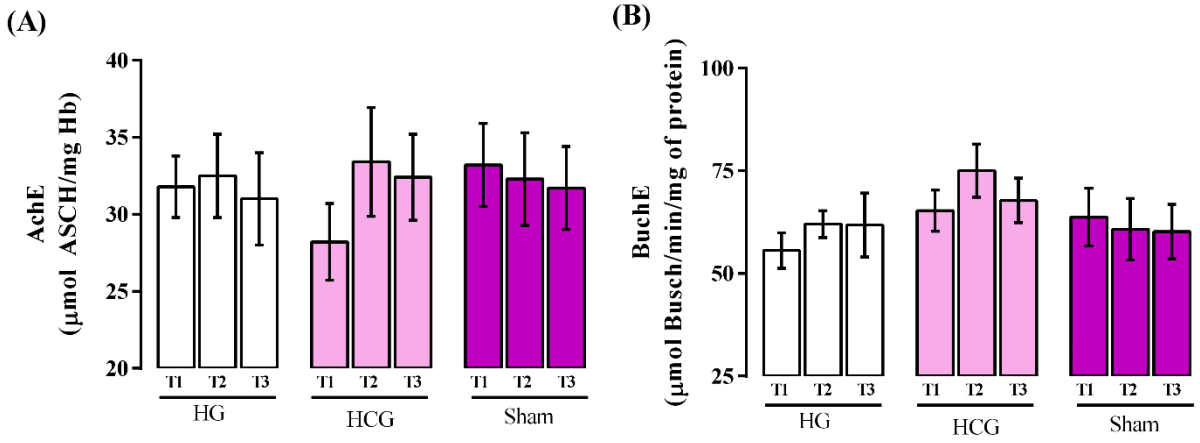

Figure 3: Serum analysis of AChE and BChE activity of I) female cats submitted to hyperbaric therapy with subsequent castration (HG); II) female cats only submitted to hyperbaric therapy (HCG), and III) female cats only submitted to castration (Sham). Blood samples were collected before surgery (T1),

immediately after surgery (T2), and 24 h after the procedure (T3). Data were expressed as mean ± standard deviation of the mean. Differences were considered significant when $p<0.05$.

4 DISCUSSÃO

Durante a revisão bibliográfica realizada, não foi possível encontrar trabalhos que comparem os efeitos da oxigenioterapia hiperbárica sobre a resposta inflamatória local e sistêmica de felinos. Tampouco foram encontrados trabalhos avaliando o grau de alteração que a HBOT causa na temperatura de felinos ou sua interferência na analgesia pós-operatória. Desta forma, este é o primeiro estudo disponível para a avaliação da interferência da HBOT nos padrões fisiológicos em felinos. Além disso, o presente estudo é o primeiro a explorar os parâmetros basais em felinos saudáveis submetidos à HBOT precedendo procedimento eletivo, dados estes que são de suma importância para referenciação futura no uso desta opção terapêutica em animais doentes.

Devido ao aumento da população de gatos como espécie de escolha para estimação, e a frequência da realização da ovariohisterectomia (OVH) videoassistida eletiva na instituição de pesquisa em questão, tem-se que a avaliação dos efeitos da HBOT na expressão de proteínas de fase aguda e estresse oxidativo trará informações inéditas e relevantes.

O estudo discorrido apresentou importantes resultados no que tange à influência positiva da oxigenoterapia hiperbárica como pré-tratamento para procedimentos cirúrgicos videoassistidos de ovariohisterectomia eletiva em fêmeas da espécie felina. É sabido que os processos inflamatórios são inerentes a qualquer procedimento cirúrgico, mesmo quando precedidos de técnicas minimamente invasivas .(ARSALANI-ZADEH et al., 2011; BAYSAL et al., 2009; THOMAS; BALASUBRAMANIAN, 2004).

A lesão tecidual induz a liberação de mediadores inflamatórios como bradicininas, prostraglandinas, histaminas e citocinas que tem importante papel nos processos de reparação tecidual como o recrutamento de células de defesa, agregação plaquetária e ativação de nociceptores, desencadeando os sinais cardiais do processo inflamatório. A dor, a hipertermia, o edema e perda de função nos tecidos lesados, quando não bem controlados, desencadeiam um desequilíbrio de funções fisiológicas entre a produção de substâncias oxidantes e antioxidantes, com predomínio de radicais livres, levando a danos irreversíveis em ácidos nucleicos, proteínas e lipídios. Esse desequilíbrio é denominado de estresse oxidativo e culmina invariavelmente em morte celular (DE WOLDE et al., 2021; FINKEL; HOLBROOK, 2000; HEDLUND, 2008).

Vários biomarcadores estão circulantes na corrente sanguínea das diferentes espécies e sua concentração é passível de ser mensurada para a avaliação da presença de estresse oxidativo já nas primeiras horas que sucedem a lesão tecidual (BASSO et al., 2014; CUI; KONG;

ZHANG, 2012; MCMICHAEL, 2007). Dentre muitos biomarcadores sensíveis a testes de avaliação de estresse oxidativo destacam-se as Espécies Reativas ao Oxigênio (ROS), Substâncias Reativas ao Ácido Tiobarbitúrico (TBARS), enzimas antioxidantes como a Catalase (CAT) e Superóxido Dismutase (SOD) e as proteínas de fasse aguda como a Acetilcolinesterase (AChE) e Butirilcolinesterase (BChE), são excelentes preditores de danos celulares oriundos de oxidação biológica (BASSO et al., 2014; DAS, 2012; FINKEL; HOLBROOK, 2000; THOM, 2009).

O manejo da dor em felinos é uma prática comumente negligenciada, visto que a espécie ainda guarda resquícios comportamentais de sua herança ancestral, por consequência, a exteriorização de suas sensações é mascarada pela pouca vocalização, pouca deambulação e apetite comedido, característicos da espécie (ARMITAGE et al., 2005; EVANGELISTA et al., 2019; HUNT et al., 2015; MILLS; KARAGIANNIS; ZULCH, 2014). É papel fundamental do médico veterinário, ser proficiente na correta identificação dos sinais clínicos de dor nas diferentes espécies, postulando uma condição sine qua non quando se refere aos felinos (EPSTEIN et al., 2015; WATANABE et al., 2020).

Não obstante, é notório que quando submetidos a um procedimento cirúrgico, a lesão tecidual e a manipulação visceral desencadearão processos inflamatórios, porém, com a utilização de abordagens cirúrgicas cada vez menos invasivas e protocolos anestésicos eficientes, o trauma intervencionista impõem-se cada vez menor, possibilitando uma pronta recuperação com a necessidade cada vez menor de metabolização de fármacos pelo organismo e o rápido retorno da homeostase fisiológica (EPSTEIN et al., 2015; ROBERTSON, 2018; ROBERTSON et al., 2018).

Mediante essas afirmações, empreendeu-se avaliar a contribuição da oxigenoterapia hiperbárica como tratamento pré-operatório com vistas a reduzir o processo fisiopatológico do estresse oxidativo, bem como a redução de efeitos álgicos e benefícios na termorregulação de pacientes felinas submetidas a OVH eletiva videoassistida.

As abordagens pré-operatórias por oxigenoterapia hiperbárica e os benefícios advindos dessas duas técnicas são pouco postulados entre os médicos veterinários, o que motivou-nos a realizar esse estudo randomizado, até então inédito, associando o que ambas as técnicas podem trazer de melhor para o sucesso no tratamento de afecções que acometem felinos domésticos, uma especialidade da medicina veterinária que ganha espaço a cada dia no cotidiano das rotinas clínicas do Brasil e do mundo.

5 CONCLUSÃO

Após a avaliação de diversos biomarcadores de estresse oxidativo, alteração na termorregulação e resposta álgica de 45 gatas submetidas a OVH eletiva videoassitida com e sem pré-tratamento com oxigenoterapia hiperbárica, obtivemos os resultados descritos a seguir: A HBOT não exerceu influência significativa na diminuição dos escores de dor nas fêmeas felinas, assim como não se observou alteração relevante na temperatura central, periférica ou na variação entre ambas quando comparadas com os animais que não foram submetidos ao pré-tratamento com HBOT. Desta forma se pode concluir que a técnica de videocirurgia exerceu impacto muito mais positivo no trans e pós-operatório do que a oxigenoterapia hiperbárica, destacando a significância relevante dos procedimentos videoassistidos. Não obstante, a HBOT demonstrou ser de importante valia no tratamento da resposta inflamatória, promovendo uma influência favorável na redução do estresse oxidativo e nos níveis séricos desses biomarcadores. Baseado na singularidade do trabalho proposto e por tratar-se de um estudo inicial que abordou os referidos aspectos, assim como pela inovação trazida pelas técnicas de videolaparoscopia na medicina veterinária, concluímos que os resultados obtidos com essas novas abordagens no tratamento de redução do estresse oxidativo constituem avanços na medicina felina, servindo como referencial para futuros estudos abrangendo outras espécies domésticas e estendendo-se como opção terapêutica para outras alterações fisiopatológicas.

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ANEXOS

ANEXO A: Manuscript Submission Guidelines: Journal of Feline Medicine and Surgery (JFMS)

1, Article types

Journal of Feline Medicine and Surgery (JFMS) considers manuscripts submitted in the following formats.

Original Articles

Papers should be as concise as possible, and generally not exceed 3,000 words (excluding references). Each paper should have a self-contained Abstract (up to 300 words, structured with subheadings as detailed in Preparing your manuscript), followed by Introduction, Materials and methods, Results, Discussion, Conclusions, Acknowledgements, Conflict of Interest, Funding, Ethical Approval and Informed Consent statements, and References. Note that Original Articles on well-recognised diseases that report valuable national or regional data on disease prevalence, or other relevant data, should be submitted to the sister journal, the Journal of Feline Medicine and Surgery Open Reports (jfmsopenreports.com) as a Short Communication. The manuscript submission guidelines for JFMS Open Reports can be found here.

Review Articles

Offers of reviews and topics for consideration should be directed to the Editors, via the editorial office (jfms@icatcare.org), for initial editorial approval. Reviews should provide an update on recent advances in a particular field and the length should not generally exceed 4,000 words. They should include an abstract (up to 300 words), followed by subheadings directed by the content, as well as Conclusions, Acknowledgements, Conflict of Interest, Funding, Ethical Approval and Informed Consent statements, and References.

Short Communications

Short communications reporting relevant research or sufficiently substantial pilot studies are considered for JFMS. They should generally not exceed 1,500 words. They should include a self-contained Abstract (up to 300 words, structured with subheadings as detailed in Preparing your manuscript), followed by Introduction, Materials and methods, Results, Discussion,

Conclusions, Acknowledgements, Conflict of Interest, Funding, Ethical Approval and Informed Consent statements, and References.

Short Communications on well-recognised diseases that report valuable national or regional data on disease prevalence, or other relevant data, should be submitted to the sister journal, the Journal of Feline Medicine and Surgery Open Reports (jfmsopenreports.com) – the manuscript submission guidelines for which can be found here.

Case Series

Large prospective and retrospective case series are considered for JFMS. Depending on the information contained, a Case Series may be up to 2,500 words in length. It should include a brief Abstract (up to 300 words structured with subheadings detailed in Preparing your manuscript) followed by Introduction (optional), Case series description, Discussion, Conclusions, Acknowledgements, Conflict of Interest, Funding, Ethical Approval and Informed Consent statements, and References. Small case series and individual case reports that provide novel information should be submitted to the Journal of Feline Medicine and Surgery Open Reports – the manuscript submission guidelines for which can be found here.

Letters to the Editor

Letters commenting on papers recently published in JFMS will be considered for publication in the journal. Letters should not exceed 1,000 words (including references and one table or figure). The Editors may send the letter to the authors of the original paper for comment so that both letter and reply may be published together.

Manuscripts should be clearly labelled ‘Original Article’, ‘Review Article’, ‘Short Communication’, ‘Case Series’ or ‘Letter to the Editor’.

Journal of Feline Medicine and Surgery Resident Best Paper Award

The award recognises quality and excellence for early career authors who publish in JFMS. Authors who are in a recognised veterinary residency programme (eg, ABVS, EBVS and ANZCVS residency) at the time of submission of their paper will automatically be eligible for consideration for the award, subject to its acceptance for publication. Accepted papers will be

considered for the award in the year in which they are published. Details about this award for interested residents and resident supervisors can be found here.

2. Editorial policies

2.1 Peer review policy

JFMS operates a single-blinded peer review process in which the reviewer's name is withheld from the author. The reviewer may at their own discretion opt to reveal their name to the author in their review but our standard policy is for the reviewer's identity to remain concealed. Each manuscript is reviewed by at least two referees. All manuscripts are reviewed as rapidly as possible, and an editorial decision is generally reached within 6–8 weeks of submission. Generally, JFMS does not accept more than two revisions to a paper.

2.2 Authorship

Papers should only be submitted for consideration once consent is given by all contributing authors. Corresponding authors should carefully check that all those whose work contributed to the paper are acknowledged as contributing authors.
The list of authors should include all those who can legitimately claim authorship. This is all those who:

Made a substantial contribution to the concept and design, acquisition of data or analysis and interpretation of data,
Drafted the article or revised it critically for important intellectual content,
Approved the version to be published.
Have participated sufficiently in the work to take public responsibiity for appropriate portions of the content.
Authors should meet the conditions of all of the points above. Each author should have participated sufficiently in the work to take public responsibility for appropriate portions of the content.

When a large, multicentre group has conducted the work, the group should identify the individuals who accept direct responsibility for the manuscript. These individuals should fully meet the criteria for authorship.

Acquisition of funding, collection of data, or general supervision of the research group alone does not constitute authorship. All contributors who do not meet the criteria for authorship should be listed in the Acknowledgements section (see 2.3 below). Please refer to the International Committee of Medical Journal Editors (ICMJE) authorship guidelines for more information on authorship.

Please note that manuscripts must be submitted with declaration statements in the following order: Acknowledgements (where relevant), Conflict of Interest, Funding, Ethical Approval and Informed Consent. Manuscripts may be returned if these statements are not included.

2.3 Acknowledgements

All contributors who do not meet the criteria for authorship should be listed in an Acknowledgements section. Examples of those who might be acknowledged include a person who provided purely technical help (see also section 2.2).

2.3.1 Third party submissions

Where an individual who is not listed as an author submits a manuscript on behalf of the author(s), a statement must be included in the Acknowledgements section of the manuscript and in the accompanying cover letter. The statements must:

Disclose this type of editorial assistance – including the individual's name, company and level of input
Identify any entities that paid for this assistance
Confirm that the listed authors have authorized the submission of their manuscript via third party and approved any statements or declarations, e.g. conflicting interests, funding, etc.
Where appropriate, SAGE reserves the right to deny consideration to manuscripts submitted by a third party rather than by the authors themselves.

2.3.2 Writing assistance

Individuals who provided writing assistance (eg, from a specialist communications company) do not qualify as authors and so should be included in the Acknowledgements section. Authors must disclose any writing assistance – including the individual's name, company and level of input – and identify the entity that paid for this assistance.

It is not necessary to disclose the use of language polishing services.

Any acknowledgements should appear first at the end of your article prior to your Conflict of Interest statement.

2.3.2 Prior presentation at conferences

Details of any prior presentation of study findings/results at conferences or meetings should be included in an 'Author note' section after the 'Acknowledgements' and before the 'Conflict of Interest' statement.

2.4 Declaration of conflicting interests

It is the policy of JFMS to require a declaration of conflicting interests from all authors enabling a statement to be carried within the paginated pages of all published articles.
Please ensure that a 'Conflict of Interest' statement is included at the end of your manuscript, after any acknowledgements and before the 'Funding' statement. If no conflict exists, please state that 'The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article'.

For guidance on conflict of interest statements, please see the ICMJE recommendations here.

2.5 Funding

JFMS requires all authors to acknowledge their funding in a consistent fashion under a separate ‘Funding’ heading. Please visit the Funding Acknowledgements page on the SAGE Journal Author Gateway to confirm the format of the acknowledgment text in the event of funding, or state that: ‘The authors received no financial support for the research, authorship, and/or publication of this article.’

2.6 Clinical and Research Ethics, and Informed Consent

Prior to undertaking studies and prior to submitting a manuscript to JFMS, authors should read these guidelines to ensure requirements have been adequately met.

Circumstances relating to the use of animals in clinical and experimental studies must meet international standards as set out in:

The International Guiding Principles for Biomedical Research Involving Animals (1985) from the Council for International Organizations of Medical Sciences, available at https://cioms.ch/shop/product/international-guiding-principles-for-biomedical-research-involving-animals-2/ (or from the Executive Secretary CIOMS, % WHO, Via Appia, CH-1211 Geneva 27, Switzerland)
The Consensus Author Guidelines on Animal Ethics and Welfare for Veterinary Journals from the International Association of Veterinary Editors, available at http://www.veteditors.org/consensus-author-guidelines-on-animal-ethics-and-welfare-for-editors/
In addition to the above, for manuscripts submitted to JFMS, the Editors would not normally support publication of:

Any experimental studies directly resulting in euthanasia of the cats.
Studies using non-experimental (eg, client-owned) cats that may cause the cat a level of pain, suffering, distress or harm higher than that induced by inserting a hypodermic needle, and/or where the procedure is not part of ‘Recognised Veterinary Practice’. Recognised Veterinary Practice would include investigations, procedures and therapies that are part of normal clinical practice and that would be of direct benefit for the individual cat (or potentially to the group to which it immediately belongs). Where investigations, procedures or therapies are unproven, or

where there is deliberate exposure of cats to procedures or interventions that might be deleterious to their health without direct clinical benefit to them, it is highly likely that experimental cats should be used with appropriate attention to their health and welfare, with the requisite ethical approval (see below). If authors are in any doubt, they are encouraged to contact the Editors prior to manuscript submission.
The Editors would also expect that for all manuscripts submitted:

Where appropriate, analgesia, sedation and/or anaesthesia must have been used and the authors should have adequately discussed the use of analgesia for the welfare of the cats involved.
Any drugs or therapeutic agents used must have been obtained legally and ethically, following all relevant locally applicable regulations.
Research involving experimental animals must always have received prior approval from an appropriate ethics committee with oversight of the facility in which the studies were conducted, and this may also apply to some studies involving client-owned animals (see 2.6.1 Ethical approval).
The Editors reserve the right to reject manuscripts on ethical or welfare grounds when, in their opinion, studies involve unnecessary pain, distress, suffering, harm, or potential harm to animals; and where the above guidelines have not been followed.

2.6.1 Ethical Approval

All material published in JFMS must adhere to high ethical standards concerning animal welfare and meet with the above guidelines. Irrespective of the nature of the work (eg, prospective, retrospective or experimental studies, case series or review), JFMS requires all authors to make one of the following four ethical approval declarations (using the exact wording) in an ‘Ethical approval’ section at the end of their manuscript, stating:

a) The work described in this manuscript involved the use of non-experimental (owned or unowned) animals. Established internationally recognised high standards (‘best practice’) of veterinary clinical care for the individual patient were always followed and/or this work involved the use of cadavers. Ethical approval from a committee was therefore not specifically required for publication in JFMS. Although not required, where ethical approval was still obtained, it is stated in the manuscript

b) The work described in this manuscript involved the use of non-experimental (owned or unowned) animals and procedures that differed from established internationally recognised high standards (‘best practice’) of veterinary clinical care for the individual patient. The study therefore had prior ethical approval from an established (or ad hoc) committee as stated in the manuscript.

This statement might, for example, apply to randomised and/or controlled trials (including where established interventions are being compared with each other), as well as studies where novel medications, techniques, devices or interventions established as safe but not currently part of ‘Recognised Veterinary Practice’ (see 2.6 Clinical and research ethics, and informed consent) are used.

Authors must state in the Materials and methods the nature of the institutional, national or international ethical review body used, and, if available, the ethical approval number.

If an existing ethical review body was not available, authors should state why in the Materials and methods, and should describe the nature of an ad hoc committee that was used (which must have included at least some individuals independent of the institute[s]/clinic[s] involved in the work).

c) The work described in this manuscript involved the use of experimental animals and the study therefore had prior ethical approval from an established (or ad hoc) committee as stated in the manuscript.

Authors must state in the Materials and methods the nature of the institutional, national or international ethical review body used, and, if available, the ethical approval number.

If an existing ethical review body was not available, authors should state why in the Materials and methods, and should describe the nature of an ad hoc committee that was used (which must have included at least some individuals independent of the institute[s]/clinic[s] involved in the work).

d) This work did not involve the use of animals and therefore ethical approval was not specifically required for publication in JFMS.

Authors may select this option if, for example, the manuscript is solely a clinical review or clinical guidelines using previously published data, or reports on questionnaire or in vitro

findings. This statement is not suitable for manuscripts containing novel animal-specific data (including retrospective studies).
For any queries regarding the best-fit statement, please contact jfms@icatcare.org.

2.6.2 Informed consent and informed consent for publication

JFMS requires all authors to make one of the following two informed consent declarations (using the exact wording below) in an 'Informed consent' section at the end of their manuscript, stating:

a) Informed consent (verbal or written) was obtained from the owner or legal custodian of all animal(s) described in this work (experimental or non-experimental animals, including cadavers) for all procedure(s) undertaken (prospective or retrospective studies).

b) This work did not involve the use of animals (including cadavers) and therefore informed consent was not required.

Authors may select this option if, for example, the manuscript is solely a clinical review or clinical guidelines using previously published data, or reports on questionnaire or in vitro findings, and does not involve the publication of any novel animal-specific data.
In addition to informed consent for use of animals within a study, informed consent for publication is required where any animal or person may be identifiable as a result of the publication (eg, a recognisable photograph, description or unique identifiable features, etc). Authors are therefore required to also state within the 'Informed consent' section either:

a) For any animals or people individually identifiable within this publication, informed consent (verbal or written) for their use in the publication was obtained from the people involved.

b) No animals or people are identifiable within this publication, and therefore additional informed consent for publication was not required.

2.7 Reporting guidelines

Authors and researchers are encouraged to consult the relevant EQUATOR Network reporting guidelines for different studies, including, for example, the Consolidated Standards of Reporting Trials (CONSORT) for randomized controlled trials. Other resources can be found at NLM's Research Reporting Guidelines and Initiatives

2.8 Data

JFMS requests that any primary data used by authors in their research article is published as Supplementary material, or that detailed information is provided in the article on how the data can be obtained. This information should include links to third-party data repositories or detailed contact information for third-party data sources. Data available only on an author-maintained website will need to be loaded onto either the journal's platform or a third-party platform to ensure continuing accessibility. Examples of data types include (but are not limited to) statistical data files, replication code, text files, audio files, images, videos, appendices, and additional charts and graphs necessary to understand the original research. The Editors may consider limited embargoes on proprietary data. The Editors can also grant exceptions for data that cannot legally or ethically be released. All data submitted should comply with Institutional or Ethical Review Board requirements and applicable government regulations. For further information, please contact Jennie Atkinson (Jennie.atkinson@sagepub.co.uk), Publishing Editor at SAGE Publications.

3. Publishing policies

3.1 Publication ethics

SAGE is committed to upholding the integrity of the academic record. We encourage authors to refer to the Committee on Publication Ethics' International Standards for Authors and view the Publication Ethics page on the SAGE Author Gateway.

3.1.1 Plagiarism

JFMS and SAGE take issues of copyright infringement, plagiarism or other breaches of best practice in publication very seriously. We seek to protect the rights of our authors and we always investigate claims of plagiarism or misuse of published articles. Equally, we seek to protect the reputation of the journal against malpractice. Submitted articles may be checked with duplication-checking software. Where an article, for example, is found to have plagiarised other work or included third-party copyright material without permission or with insufficient acknowledgement, or where the authorship of the article is contested, we reserve the right to take action including, but not limited to: publishing an erratum or corrigendum (correction); retracting the article; taking up the matter with the head of department or dean of the author's institution and/or relevant academic bodies or societies; or taking appropriate legal action.

3.2 Contributor's publishing agreement

Before publication, SAGE requires the author as the rights holder to sign a Journal Contributor’s Publishing Agreement. SAGE’s Journal Contributor’s Publishing Agreement is an exclusive licence agreement, which means that the author retains copyright in the work but grants SAGE the sole and exclusive right and licence to publish for the full legal term of copyright. Exceptions may exist where an assignment of copyright is required or preferred by a proprietor other than SAGE. In this case copyright in the work will be assigned from the author to the societies. For more information please visit our Frequently Asked Questions.

3.3 Open Access and author archiving

Journal of Feline Medicine and Surgery offers optional open access publishing via the SAGE Choice programme. For more information on Open Access publishing options at SAGE please visit SAGE Open Access. For information on funding body compliance, and depositing your article in repositories, please visit SAGE’s Author Archiving and Re-Use Guidelines and Publishing Policies.

3.4 Permissions

Authors are responsible for obtaining permission from copyright holders for reproducing any illustrations, tables, figures or lengthy quotations previously published elsewhere. For further

information including guidance on fair dealing for criticism and review, please visit our Frequently Asked Questions.

4. Preparing your manuscript for submission

This section explains how to format, style and reference your paper for JFMS. The title, keywords and abstract are key to ensuring readers find your article online through online search engines such as Google. Please refer to the information and guidance on how best to title your article, write your Abstract and select your keywords by visiting the SAGE Journal Author Gateway for guidelines on How to Help Readers Find Your Article Online.

4.1 Formatting

The preferred format for your manuscript is Word. LaTeX files are also accepted. Word and LaTeX templates are available on the Manuscript Submission Guidelines page of our Author Gateway.

The text should be double-spaced throughout and with a minimum of 3 cm for left and right hand margins and 5 cm at head and foot. Text should be standard 10 or 12 point. All lines should be numbered on manuscripts using continuous line numbering. Figures, tables and Supplementary material should all be cited in the text in numerical order.

Title page

The title should be concise (20 words maximum) with no abbreviations

Abstract

The second page of the manuscript must contain only the abstract, which should be of no more than 300 words and must be clearly written and comprehensible to readers before they come to read the paper.

For Original Articles and Short Communications, the Abstract should be structured with the following four subheadings: ‘Objectives’, ‘Methods’, ‘Results’, and ‘Conclusions and

relevance'. For Case Series, the abstract should be structured with the following two subheadings: 'Case series summary', and 'Relevance and novel information'. For Reviews, the abstract can either have no subheadings or subheadings of the author's choice.

Abbreviations should be avoided and reference citations are not permitted.

Any manuscripts submitted without a structured abstract will be returned to the author prior to peer review, thus delaying the evaluation process of the manuscript.

4.2 Artwork, figures, other graphics and tables

For guidance on the preparation of illustrations, pictures and graphs in electronic format, please visit SAGE's Manuscript Submission Guidelines. Figures supplied in colour will appear in colour online and in print.Tables should be provided in an editable format (eg, drawn in Microsoft Word or Microsoft Excel). The minimum image quality required is 300dpi at 1000 x 1000 pixels.

4.3 Style Guide

JFMS has its own style guide: JFMS Style Guide 2020

4.4 Abbreviations, symbols and drug names

Each scientific abbreviation must be explained at its first occurrence in the paper; for example:

• complement fixation test (CFT).

Do not use propriety symbols (eg, ® or ™) or ltd, etc, in medications or company names.

Medications should be referred to by their recommended International Nonproprietary Name (rINN). A list of these generic names is coordinated by the World Health Organization at http://www.who.int/medicines/services/inn. Where appropriate, the proprietary name and the manufacturer should be given in parentheses when first mentioned; for example:

• carprofen (Rimadyl; Zoetis).

4.5 Supplementary material

This journal is able to host additional materials online (eg, datasets, podcasts, videos, images, etc) alongside the full-text of the article. These will still be subjected to peer review. For more information please refer to our guidelines on submitting supplementary files.

4.6 Reference style

JFMS adheres to the SAGE Vancouver reference style. View the SAGE Vancouver guidelines to ensure your manuscript conforms to this reference style.

If you use EndNote to manage references, you can download the SAGE Vancouver EndNote output file.

In general only primary sources of information should be cited – citing reviews or book chapters where primary sources are referred to is generally not acceptable. Where relevant, authors should make note of [Abstract] and [Letter] in their references.

4.7 English language editing services

Authors seeking assistance with English language editing, translation, or figure and manuscript formatting to fit the journal's specifications may consider using SAGE Language Services. Visit SAGE Language Services for further information.

4.8 Disclaimer

The following disclaimer appears in print and online.
Journal of Feline Medicine and Surgery is an international journal. Advertising material may promote and/or authors may discuss products and formulations that are not available or licensed in the individual reader's own country.

Furthermore, drugs may be mentioned that are licensed for human use, and not for veterinary use. Readers need to bear this in mind and be aware of the prescribing laws pertaining to their own country. It is the responsibility of the reader to check that a product is authorised for use in their own country. The authors, Editors, owners and publishers do not accept any responsibility for loss or damage arising from actions or decisions based on information contained in this publication; ultimate responsibility for the treatment of animals and interpretation of published materials lies with the veterinary practitioner. The opinions expressed are those of the authors and/or companies placing advertisements, and the inclusion in this publication of material relating to a particular product, method or technique does not amount to an endorsement of its value or quality, or the claims made by its manufacturer.

5. Submitting your manuscript

JFMS is hosted on SAGE Track, a web-based online submission and peer review system powered by ScholarOne™ Manuscripts. Visit https://mc.manuscriptcentral.com/jfms to login and submit your article online.

IMPORTANT: Please check whether you already have an account in the system before trying to create a new one. If you have reviewed or authored for the journal since 2011 it is likely that you will have had an account created. For further guidance on submitting your manuscript online please visit ScholarOne Online Help.

5.1 ORCID

ORCID applies only to papers published in the Classic editions of JFMS, due to the required verification expected by ORCID.

As part of our commitment to ensuring an ethical, transparent and fair peer review process SAGE is a supporting member of ORCID, the Open Researcher and Contributor ID. ORCID provides a unique and persistent digital identifier that distinguishes researchers from every other researcher, even those who share the same name, and, through integration in key research workflows such as manuscript and grant submission, supports automated linkages between researchers and their professional activities, ensuring that their work is recognized.

The collection of ORCID iDs from corresponding authors is now part of the submission process of this journal. If you already have an ORCID iD you will be asked to associate that to your submission during the online submission process. We also strongly encourage all co-authors to link their ORCID ID iD to their accounts in our online peer review platforms. It takes seconds to do: click the link when prompted, sign into your ORCID account and our systems are automatically updated. Your ORCID iD will become part of your accepted publication's metadata, making your work attributable to you and only you. Your ORCID iD is published with your article so that fellow researchers reading your work can link to your ORCID profile and from there link to your other publications.

If you do not already have an ORCID iD please follow this link to create one or visit our ORCID homepage to learn more.

Please note that only ORCID iDs validated prior to article acceptance will be authorised for publication, and we are unable to add or amend ORCID iDs at later stages (eg, at proof stage).

Once an ORCID account is set up you are able to add papers manually to your account to ensure all your work is accounted for. We would recommend this for all papers published in the Clinical Practice editions of JFMS.

5.2 Information required for completing your submission

You will be asked to provide contact details and academic affiliations for all co-authors via the submission system and identify who is to be the corresponding author. These details must match what appears on your manuscript. At this stage please ensure you have included all the required statements and declarations and uploaded any additional supplementary files (including reporting guidelines where relevant). Manuscripts must be submitted with declaration statements in the following order: Acknowledgements (where relevant), Conflict of Interest, Funding, Ethical Approval and Informed Consent.

5.2.1 Social Media - Twitter @CatVets and @ISFMCats

JFMS uses Twitter (through both the ISFM channel @ISFMCats and the AAFP channel @CatVets) to engage with debate on Social Media. Authors and readers are encouraged to join the ongoing discussion around the twitter account on issues related to the Journal. JFMS authors are offered the option of providing their Twitter handle to be published alongside their name and email address within their article. Providing a Twitter handle for publication is entirely optional, if you are not comfortable with JFMS promoting your article along with your personal Twitter handle then please do not supply it.

By providing your personal Twitter handle you agree to let JFMS and SAGE Publications to use it in any posts related to your Journal article. To include your Twitter handle within your article please provide this within the ScholarOne submission form when prompted and on the separate title page in the format outline below (please refrain from adding it to the manuscript itself to facilitate anonymous peer review).

As an example of how to supply this information please see the example below:

Joe Bloggs, Department of Veterinary Science, University Hospital, Town, Zip code, USA
Email: JoeBloggs@email.com
Twitter: @drjoebloggs

6. On acceptance and publication

6.1 SAGE Production

Your SAGE Production Editor will keep you informed as to your article's progress throughout the production process. Proofs will be sent by PDF to the corresponding author and should be returned promptly.

6.2 Access to your published article

SAGE provides authors with online access to their final article.

6.3 OnlineFirst publication

OnlineFirst allows final revision articles (completed articles in queue for assignment to an upcoming issue) to be published online prior to their inclusion in a final journal issue which significantly reduces the lead time between submission and publication. For more information please visit our OnlineFirst Fact Sheet

ANEXO B: Instructions for Authors The Journal of Veterinary Medical Science (JVMS)

1. General information

Revised on May 27, 2021

The Journal of Veterinary Medical Science (JVMS) is an open access, peer-reviewed journal that covers fields in basic, applied and clinical research in veterinary science.

JVMS is the official journal of the Japanese Society of Veterinary Science and releases 12 issues per year. The journal publishes work on the following fields: Anatomy, Physiology, Biochemistry, Pharmacology, Ethology, Wildlife Science, Pathology, Toxicology, Laboratory Animal Science, Parasitology, Bacteriology, Virology, Public Health, Immunology, Epidemiology, Avian Pathology, Internal Medicine, Clinical Pathology, Surgery, and Theriogenology.

2. Types of papers

Full papers, Notes, and Review articles that are principally related to veterinary science and are unpublished and not under consideration for publication elsewhere will be accepted for publication. The length of papers, including Tables, Figures, and any other appendices, should not exceed 8 PDF pages for a Full paper or Review article, and 5 for a Note. (One PDF page corresponds to about 850 words, excluding the title, figures, and tables.)

All submitted manuscripts are examined by editors and multiple reviewers, and the Editor-in-Chief or Vice Editor-in-Chief determines their acceptance/rejection.

3. Compliance with ethical standards

Before submitting manuscripts, authors should carefully read the following ethical standards to ensure compliance with the same. Manuscripts judged by the editors and Editorial Office as not compliant with these standards may be rejected without review. Similarly, such manuscripts may be retracted even after being accepted or published.

3-1. Duplicate submission

Manuscripts submitted to JVMS should not have been previously published, are not currently in press, or under consideration for publication in other journals (even in part/any language).

However, reports by national, public, or private research institutions and research grant reports are exempted from this rule.

If any related manuscripts are under consideration for submission to other journals, currently in press, or were previously published, authors should inform the Editor-in-Chief at the time of submission. However, manuscripts that are deposited to publicly accessible preprint servers are not deemed to have previously been published (see [3-3. Preprints]).

If authors choose to submit their manuscripts to other journals prior to JVMS's final decision to accept/reject these manuscripts, they should retract the submission to JVMS first.

3-2. Originality

Submitting a manuscript to JVMS assures that the manuscript is original. JVMS screens for unoriginal content included in manuscripts using software. Authors submitting manuscripts to JVMS are considered to consent to this screening. Manuscripts lacking originality to an unacceptable level are rejected based on the editors' judgment.

3-3. Preprints

To widely disseminate research, JVMS permits authors to register their manuscripts on preprint servers officially approved by relevant research communities before or at the time of submitting these manuscripts to JVMS. This applies only to original-version manuscripts describing primary studies. Manuscripts revised based on reviewers' comments, accepted for publication, or already published in JVMS cannot be registered on preprint servers. However, there is no restriction on the registration of links to published papers on preprint servers.

3-4. Authorship

Submitting a manuscript to JVMS signifies that all co-authors have confirmed and approved the author list and final version of the manuscript. Any alteration to the author list after submission, such as adding or deleting author names, or changing the order of authors, is basically unacceptable.

3-5. Identity of images

When authors digitally manipulate or process images, revisions should be minimized and processing should be applied to entire image within the standard ranges specified by relevant research communities. Furthermore, when major revisions have been implemented, authors

should clearly explain such revisions in their manuscripts. All images in each manuscript should accurately reflect original data. Authors should neither move, delete, add, nor emphasize individual parts of images. Editors have the right to request authors to submit original, unprocessed images. If authors have difficulty in submitting requested images, their manuscripts may be rejected.

3-6. Reprinting of copyrighted materials

When manuscripts contain materials to which authors themselves do not hold the copyrights, authors must obtain the relevant permission(s) from copyright holders to reprint these materials. When manuscripts contain materials that were previously published as papers, authors must obtain permission(s) from copyright holders and publishers of original articles to reprint these materials. Additionally, authors should refer to the original articles in their manuscripts.

A copy of the permission(s) for reprinting should be attached when authors submit their manuscripts for the first time.

3-7. Data and materials availability

When necessary, authors should describe acceptance numbers and detailed corporate information in their manuscript to specify sources of publicly available data and materials, such as open repositories and private manufacturers.

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• Hori, M., Yazama, F., Matsuura, Y., Yoshimoto, R., Kaneda, T., Yasumoto, T., Ozaki, H. and Karaki, H. 2018. Inhibition of actin polymerization by marine toxin pectenotoxin-2. J. Vet.

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• Sasaki, H., Sasaki, N., Nishino, T., Nagasaki, K., Kitamura, H., Torigoe, D. and Agui, T. 2014. Quantitative trait loci for resistance to the congenital nephropathy in tensin 2-deficient mice. PLoS One 9: e99602. doi:10.1371/journal.pone.0099602.

• Hamm, L. L., Alpern, R. J. and Preisig, P. A. 2013. Cellular mechanisms of renal tubular acidification. pp. 1917–1978. In: Seidin and Giebisch's The Kidney, 5th ed. (Alperm, R. J., Caplan, M. J. and Moe, O. W. eds.), Elsevier, Amsterdam.

• Wild, D. G. 2013. The Immunoassay Handbook, 4th ed., Elsevier Science & Technology, Oxford.

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