OVARIOHISTERECTOMIA POR CELIOTOMIA OU VIDEOCIRURGIA CONSIDERANDO O ESTRESSE OXIDATIVO E O ESTUDO CITOGENÉTICO EM GATAS.

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JUNHO - 2015

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Tese apresentada ao Centro de Ciências e Tecnologias Agropecuárias da Universidade Estadual do Norte Fluminense Darcy Ribeiro, com requisito parcial para a obtenção do grau de Doutora em Ciência Animal na área de concentração em Sanidade Animal.

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“Dedico este trabalho a minha mãe

Rosemary Azeredo Andrade (em memória).

Você virou uma estrela de primeira grandeza

e brilhará para sempre no meu coração.”

“Não se pode ter a cabeça erguida diante dos homens, sem antes tê-la baixado sobre os livros.” **Ruy Barbosa** (1849-1923).

RESUMO

Com o advento da técnica cirúrgica minimamente invasiva, a castração por cirurgia laparoscópica vem ganhando popularidade por apresentar vantagens, como menor trauma tecidual e manipulação visceral objetiva, em comparação com procedimento cirúrgico tradicional por celiotomia. Esse estudo analisou os dados disponíveis sobre o impacto da cirurgia minimamente invasiva em relação à OVH laparotômica considerando o estresse oxidativo e resultados de um estudo citogenético em linfócitos do sangue periférico no cromossomo de gatas. Foram utilizados nesse experimento quarenta gatas sem raça definida, separadas em quatro grupos, sendo os animais de dois grupos apenas anestesiados (um grupo de dez foi anestesiado durante treze minutos e o outro grupo durante quarenta e três minutos). Nos outros dois grupos, os animais foram submetidos a duas técnicas distintas de ovariohisterectomia por laparoscopia e por celiotomia (técnica tradicional). Para os dois outros grupos que foram submetidos à cirurgia, 3 mL de sangue foram recolhidos a partir da veia cefálica, em três momentos diferentes, por punção venosa em tubos de vácuo com heparina de lítio, de cada paciente. A primeira colheita, de 1ml antes da anestesia, a segunda, de 1ml imediatamente após a cirurgia, e a outra, vinte e quatro horas após a cirurgia. Os resultados obtidos foram produto da técnica operatória e não do anestésico utilizado. Houve significância estatística no grupo da laparotomia em relação ao grupo da laparotomia no tempo três em relação ao estresse oxidativo, e no tempo dois no grupo da laparoscopia em relação ao grupo da laparotomia. Em conclusão, o presente estudo mostrou que a laparoscopia gerou significativamente menos estresse oxidativo do que a cirurgia aberta, e apesar de ter provocado maior dano ao DNA (como quebras e aberrações), pôde se observar no grupo da laparoscopia uma tendência à normalidade dos danos no tempo três.Essa situação não foi observada no grupo da laparotomia, sugerindo um maior risco de danos nesse grupo ao longo do tempo.

Palavras-chave. videocirurgia, estresse oxidativo, radicais livres, alterações cromossômicas.

ABSTRACT

With the advent of minimally invasive surgical technique, castration by laparoscopic surgery is gaining popularity for its advantages as less tissue trauma and objective visceral manipulation compared to traditional laparotomy surgery. This study analyzed data on the impact of minimally invasive surgery compared to the laparotomy OVH through oxidative stress and a cytogenetic study in peripheral blood lymphocytes in cats' chromosomes. In the experiment were used 40 mongrel cats, separated into 4 groups, two groups of anesthesia (one group of 10 animals was anesthetized for 13 minutes and the other group for 43 minutes). The two other groups were subjected to the two different ovariohysterectomy techniques, laparoscopy through celiotomy (traditional technique) and laparoscopy. For each patient were collected 3 ml blood samples from the cephalic vein at three different times by venipuncture into vacuum tubes with lithium heparin. The collect times were: 1ml before anesthesia, 1 ml immediately after surgery and 1ml 24 h after surgery. The results showed that the abnormalities presented were not product of the anesthetic technique used. There was statistical significance for the oxidative stress between the laparotomy group and the laparotomy group at times 3, and 2. In conclusion this study showed that laparoscopy generated significantly less oxidative stress than open surgery, and although it caused major damage to DNA, could be observed in the laparoscopy group a tendency to normality of the damage in time 3, this was not observed in the laparotomy group, suggesting a greater risk of damage in this group over time.

Keywords. videosurgery, oxidative stress, free radicals, chromosomal alterations, pain.

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1. INTRODUÇÃO

A ovariohistectomia (OVH) é a operação mais executada na medicina veterinária segundo o senso comum e dados da literatura (ATALLAH et al., 2013; DAVIDSON, 2004), entretanto, sua execução apresenta uma diversidade de formas diferentes, sem que se saiba qual a técnica que apresenta um menor trauma operatório e, consequentemente, capaz de mitigar os casos de morbi-mortalidade (HOWEO, 2006).

Em gatas pré-púberes e naquelas que já atingiram a maturidade sexual, a OVH representa o método contraceptivo mais utilizado pelos médicos veterinários para evitar a reprodução e ocorrências de doenças do trato reprodutivo nessa espécie (BLOOMBERG, 1996; CONCANNON & MEYERS-WALLEN, 1991).

Novas técnicas cirúrgicas minimamente invasivas vêm sendo estudadas para tentar substituir as OVH realizadas por celiotomia. O interesse pela laparoscopia deve-se também aos benefícios que esta modalidade apresenta, tais como o acesso reduzido, e por possibilitar o diagnóstico e o tratamento de determinadas enfermidades em um único ato anestésico. Além disso, essas operações vêm demonstrando benefícios, quando comparadas às técnicas convencionais amplamente difundidas, trazendo menor trauma aos tecidos, menos desconforto pós-operatório, redução do período de hospitalização e melhor resultado cicatricial (DAVIDSON, 2004).

No entanto, diferentes autores revelaram algumas preocupações no que se refere aos efeitos do pneumoperitônio. Qualquer forma de trauma, incluindo a cirurgia, procedimentos cirúrgicos laparoscópicos e abertos podem resultar em estresse oxidativo e um dos principais alvos dessas espécies reativas de oxigénio é o DNA (ácido desoxiribonucléico).

Uma vez que, a molécula é responsável por toda a informação genética de todas as células de um organismo vivo, e ainda pelo fato de que não há estudos sobre o estresse oxidativo e seu impacto no DNA em gatas ovariohisterectomizadas, torna-se necessário realizar estudo comparativo dos efeitos de ambas as técnicas cirúrgicas.

Apesar das vantagens da técnica laparoscópica já serem bem estabelecidas e parecerem convincentes, para a real substituição da técnica da laparotomia, torna-se necessário primeiro, a completa compreensão dos eventos ocorridos durante o pneumoperitônio na espécie felina, e compará-los com os gerados durante a laparo-

tomia. Tais eventos podem provocar alterações no DNA, e tais alterações poderíam ser carcinogênicas para a espécie.

O objetivo geral dessa investigação é analisar o impacto da cirurgia minimamente invasiva em relação à laparotomia em gatas, inferindo sob os aspectos analisados quanto à melhor opção.

O objetivo especifico foi avaliar a produção de estresse oxidativo durante as técnicas operatórias , compará-las, e ainda avaliar a produção de quebras e aberrações cromossômicas em estudo citogenético em linfócitos do sangue periférico de gatas.

Sob a hipótese de que a técnica videocirúrgica testada para OVH em gatas não apresenta diferenças em relação à convencional por celiotomia em relação ao estresse oxidativo e produção de danos cromossomais.

2 - FUNDAMENTAÇÃO TEÓRICA

2.1- Ovariohisterectomia

O termo ovário-histerectomia (OVH) se refere à remoção cirúrgica do útero e ovários, conhecida popularmente como castração (MiILLER et al., 2015; COISMAN et al., 2013; SPARKERS, 2011; DORN & SWIST, 1977). É a prática cirúrgica mais comumente realizada na Medicina Veterinária, servindo de base para o controle populacional através da prevenção do estro, impedindo a reprodução da espécie, sendo esse seu principal objetivo (ATALLAH et al., 2013; COISMAN et al., 2013; DUPRÉ et al, 2009; HOWEO, 2006). A aplicação de hormônios sintéticos infere alterações significativas à saúde do animal e apenas limita sua capacidade reprodutiva (ATALLAH et al., 2013; EHRHARDT, 2012; DUPRÉ et al., 2009; FINGER et al., 2009). Outras razões para a realização da OVH incluem: diminuição do risco de desenvolvimento de neoplasias mamárias, infecções uterinas, pseudocieses, prolapsos vaginal e uterino, neoplasias (uterinas, ovarianas e vaginais), correções de distocias, torção uterina e até mesmo para controle de algumas doenças endócrinas, como diabetes, epilepsia e dermatoses (demodicose), (ATALLAH et al., 2013; SPARKERS, 2011; DORN & SWIST, 1977).

2.1.1- Técnicas para realização de OVH em gatas

Várias técnicas desenvolvidas para eliminar a capacidade do felino de se reproduzir são descritas na literatura, incluindo ovariectomia (OVE), a ovário-histerectomia (OVH) - (REECE et al., 2012; ROMAGNOLI, 2010; FINGER et al., 2009; HOWEO, 2006). A OVH realizada por meio da técnica das três pinças por uma celiotomia mediana pré-retroumbilical é considerada a mais tradicional, sendo indicada por autores clássicos (ROMAGNOLI, 2010). Entretanto, com intuito de reduzir o trauma cirúrgico, uma técnica de mini-laparotomia Key-role (buraco da fechadura) é frequentemente utilizada em gatas, para sua esterilização. Nesse procedimento, um gancho snoock é usado de forma cega para expor os ovários, os quais são ligados por via extra-abdominal. Porém, quando existe uma exposição limitada devido a uma pequena incisão abdominal e o gancho é usado de forma cega, o risco inadver-

tido de trauma de tecido abdominal e hemorragia em um pedículo friável pode ser potencializado, embora tais complicações ainda não tenham sido relatadas (VAN NIMWEGEN & KIRPENSTEIJN, 2007). A OVH tradicional tem como principal variação o acesso pelo flanco, mais realizado em gatas (REECE et al., 2012; JANSSENS & JANSSENS, 1991) e através da laparoscopia (EHRHARDT, 2012; REECE et al., 2012; THOMAS et al., 2011).

Apesar da OVH por laparotomia ser a técnica mais realizada em clínicas particulares e hospitais universitários (EHRHARDT, 2012; RODRIGUES et al., 2012), técnicas laparoscópicas têm sido descritas para a realização da esterilização na medicina veterinária desde 1985, demonstrando variação com relação aos acessos, número de trocárteres e à localização desses (COISMAN et al., 2013; BUOTE et al., 2011; DUPRÉ et al., 2009). Em gatas, os relatos apontam que, principalmente, na Europa, o procedimento laparoscópico para castração mais realizado é a ovariectomia (OVE) enquanto que no Brasil, a ovariohisterectomia é a mais empregada através da cirurgia híbrida (COISMAN et al., 2013).

2.2 - Técnica operatória - Cirurgia híbrida

Esta se caracteriza pelo emprego combinado da cirurgia laparoscópica, a cirurgia aberta e vem ganhando muitos adeptos, por possibilitar a otimização do tempo cirúrgico (BUOTE et al., 2011). Matyjasik et al., em 2011 ainda descreveram sobre restrições na realização de cirurgias laparoscópicas em alguns felinos que não se enquadrariam nessa técnica, que mesmo estando em condições desfavoráveis poderiam não resistir, já que na cirurgia laparoscópica o tempo cirúrgico pode ser maior. Felinos obesos que apresentem líquidos abdominais e ainda que apresentem muitas aderências, as indicações de laparoscopia também se tornam restritas.

Robertson et al., em 2014, relataram que embora o felino seja um paciente pequeno no tamanho, seu abdômen distensível permite mais espaço para a manipulação cirúrgica do que o oferecido por um paciente canino de tamanho similar. As características favoráveis, tais como rápida recuperação pós-operatória, reforço diagnóstico, precisão, ampliação e iluminação das áreas restritas, escuras e pequenas, e campo de visão em um pequeno espaço de trabalho, elevaram a conscientização e a captação da laparoscopia em pacientes felinos. Em resumo, conclui-se

que uma abordagem laparoscópica oferece excelente visualização dos órgãos abdominais do gato (Robertson et al., 2014b).

• Figura 1. Cirurgia híbrida - ovariohisterectomia em gata. Dinâmica no centro cirúrgico

2.2.1- Equipamentos

De acordo com vários autores, a cirurgia laparoscópica do aparelho reprodutor de gatas requer tais equipamentos para sua execução (SCHIOCHET, 2015; BRUN, 2015; RUNGE et al., 2014b; BRUN, 2000).

- Microcâmera Com Processador De Imagem
- Insuflador Eletrônico -Fonte De Luz
- Cabo de luz De Fibra Óptica

13. Monitor De Vídeo

23. Placa De Captura De Imagem

1. Endoscópio Rígido de 5mm ou 2,7mm De Diâmetro E Ângulo De Visão De 0 grau.

Também há necessidade de instrumentos básicos laparoscópicos, tais como:

1. Mangueira de gás com conectores nas pontas pata o insuflado de CO_2
1. Cabo para eletrocirurgia monopolar e bipolar
1. Trocarte curto com rosca de 5mm ou 3mm de diâmetro
1. Pinça Kelly, uma pinça Roddick-olsen, pinça de Maryland e uma tesoura de Metezenbaum, além do material para cirurgia aberta, que deve ser delicado e é utilizado especialmente na pele e nos casos de conversão cirúrgica.
1. Em relação à hemostasia podem ser utilizados bisturi elétrico (mono ou bipolar) ultra-sônico, ligasure, clipes de titânio (médio ou pequeno) com seu respectivo aplicador.
1. Utilizar em felinos instrumentais pediátricos ou neonatais (5 ou 3 mm de diâmetro).

2.2.2- Pré-operatório e anestesia

As recomendações que se fazem necessárias no pré-operatório incluem tricotomia desde a região central do apêndice xifóide até o púbis e, aproximadamente, 5 cm lateral às cadeias mamárias. Após assepsia, sondagem uretral com auxílio de um espéculo nasal humano pediátrico, utilizando uma sonda uretral número quatro. Outra recomendação que se faz necessária é a utilização de colchão térmico no transoperatório, para evitar a perda de calor que se faz presente, em consequência do dióxido de carbono (BRUN, 2015; KIM et al, 2010; NIMWEGEN & KIRPENSTEINJN, 2007).

Campos e Roll, em 2003, relataram que a anestesia dos pacientes submetidos à videocirurgia conta com a presença de complicações durante esses procedimentos, relacionadas com o pneumoperitônio, com a diminuição do débito cardíaco e o aumento da pressão arterial como resultado da resistência vascular sistêmica e pulmonar, tendo como consequência alterações hemodinâmicas e respiratórias. Consequentemente, a anestesia para esses procedimentos tornou-se mais complexa nos últimos anos (OLIVEIRA, 2005).

Em virtude disso, o anestesista deve ser cauteloso em relação à utilização do fármaco no transoperatório, usando menor quantidade possível. O protocolo anestésico deve conter anestésicos de curta duração, obtendo-se maior estabilização hemodinâmica e respiratória, maior relaxamento muscular, despertar mais rápido com

o mínimo de desconforto ou efeitos colaterais no pós-operatório (MIGLIARI & De VUONO, 2000).

Oliveira (2005) e Nunes (2007) publicaram um protocolo anestésico indicado para cirurgias laparoscópicas baseado nas observações hemodinâmicas a partir de experimentos, o qual se sustenta para os felinos. Em relação à medicação pré anestésica (MPA), o midazolam recebeu destaque, o propofol como indutor anestésico e também para ser usado em infusão contínua. O midazolam e o etomidato também podem ser utilizados como agentes indutores e os opióides devem ser indicados de acordo com a duração estimada da cirurgia e intensidade da dor. Em relação à manutenção anestésica, os mesmos autores concordaram que o isofluorano e o sevofluorano são os mais indicados devido ao fato de não sensibilizarem o miocárdio, as catecolaminas e determinarem redução na resistência vascular periférica. Em resumo, o protocolo para procedimentos laparoscópicos deve levar em consideração dados obtidos durante a avaliação pré-anestésica, além de proporcionar ao paciente amnésia, diminuição da intensidade da dor e, sobretudo, analgesia (OLIVEIRA, 2005).

Nunes (2007) ainda acrescentou que a ventilação controlada é indispensável, bem como a análise do CO_2 expirado pela capnografia (EtCO2). Ainda no transoperatório, indica-se monitorar a frequência e o traçado cardíacos, a pressão arterial e a gasometria (SCHIOCHET, 2015).

2.3- Comparação entre as técnicas de OVH por celiotomia e laparoscopia em gatas.

A técnica tradicional para a realização da OVH utilizando o método das “três pinças” é considerada segura e confiável, recomendada pela maioria dos autores, tanto para gatas jovens quanto para idosas (EHRHARDT, 2012; DEVITT et al., 2005; DAVIDSON et al., 2004). Porém, complicações pós-cirúrgicas podem ocorrer, tais como: hemorragias, infecção, edema e deiscência de sutura, peritonite, evisceração, formação de fístulas e granulomas inflamatórios, ligação dos ureteres e hidronefrose, aderências, piometra de coto e síndrome do ovário remanescente (JOYCE & YATES, 2011).

Na medicina veterinária, também há um grande arsenal literário relatando as vantagens da OVH laparoscópica em gatas sobre a técnica tradicional. Sendo elas:

menor trauma cirúrgico, baixo potencial hemorrágico, menor formação de aderências, melhor preservação da imunidade celular, menor resposta inflamatória peritoneal, menor resposta endócrino-metabólica, menor dor no pós-operatório com recuperação mais rápida, menor risco de deiscência de sutura e período de convalescência menor (SMITH & DEVINE, 2013; BUOTE et al., 2011; KIM et al., 2011; FREEMAN et al., 2010; SCHIOCHET et al., 2009; CAMBRIDGE, 2000).

Em relação à ovariohisterectomia por laparoscopia, (OVHL) uma das vantagens é que permite uma melhor visualização do limite do ovário, permitindo sua ressecção completa, evitando a síndrome do ovário remanescente (FURNEAUX, 2011; NIMWEGEN & KIRPENSTEINJN, 2007). Entretanto, essa vantagem pode ser maior em cães, haja vista, que o campo visual nos gatos, mesmo com a laparoscopia, é menor, podendo ocorrer uma maior incidência de casos de hemorragia também nas gatas (JOYCE & YATES, 2011). As cadeias técnicas laparoscópicas para OVH estão bem documentadas, portanto Van Nimwegen & Kirpensteijn em 2007 relataram que a ovariectomia laparoscópica (LapOVE) também era possível em gatas e era um processo relativamente simples, apesar do tamanho reduzido do abdômen. Em seguida, estudos clínicos foram publicados sobre o potencial para acesso de portal único LapOVE em gatas (KIM et al., 2011; THOMAS et al., 2011). O LapOVE-portal único poderia proporcionar uma melhor visualização do ovário e mesovário associado com manipulação mínima de órgãos adjacentes e permitir um exame rápido do abdômen para disfunções subclínicas (CASE & ELLISON 2013; GRINT et al., 2006).

Em contrapartida, há autores que descrevem sobre as dificuldades que os médicos veterinários encontram em executar tal técnica, pois requer uma grande e processual curva de aprendizado, haja vista que as habilidades psicomotoras são fundamentais para conduzir de forma adequada e segura um procedimento vídeocirúrgico, dificultando o domínio da técnica (RUNGE et al., 2014a; RUNGE et al., 2015b; BAKHTIARI, 2012). Os cirurgiões têm necessidade de conhecer, treinar e praticar esse tipo de procedimento (NÁCUL et al., 2015; ROBERTSON et al., 2014).

A complexidade de adquirir equipamentos, devido ao seu alto custo e a potencial maior duração de execução da técnica, quando comparada à técnica tradicional, ainda são barreiras a serem ultrapassadas (COOPER et al., 1996). Hancock e Batista afirmam que a laparoscopia ainda é pouco estudada entre os médicos veterinários, pois tal fator está relacionado à complexidade na aquisição dos equipamentos, atribuída, principalmente, ao seu alto custo e a uma mais longa execução da

técnica, isso comparada à técnica tradicional. Segundo os autores, para atenuar tal processo, faz-se necessário o estudo por aperfeiçoamento de modelos de treinamento exequíveis para a formação de cirurgiões laparoscópicos capazes de simular adequadamente as situações vivenciadas na sala de cirurgia (HANCOCK et al., 2005; BATISTA et al., 1998).

Além disso, as operações videolaparoscópicas provocam alterações fisiopatológicas que devem ser conhecidas e estudadas (COHEN et al., 2003). A necessidade de adquirir coordenação motora em prol da utilização de um novo tipo de instrumental cirúrgico e o fato de que o cirurgião deve conduzir suas manobras em um ambiente tridimensional, através de imagem bidimensional, em que é privado da sensação tátil direta com os tecidos caracterizam a cirurgia laparoscópica e, portanto, diferem-na da cirurgia tradicional (ROBERTSON, et al., 2014; BAKHTIARI, 2012; HANCOCK et al., 2005).

2.4 - Pneumoperitônio

A videocirurgia vem sendo considerada a técnica mais segura para realização de OVH por ser menos invasiva, podendo estar associada com uma menor inflamação sistêmica pós-operatória e ainda fomentar a preservação da função imune (SMITH & DEVINE, 2013; KIM et al., 2011; FREEMAN et al., 2010; SCHIOCHET et al., 2009; BECKER, 2004). A resposta ao estresse cirúrgico parece ser atenuada e antecipada. E assim, esperam-se respostas fisiológicas menos pronunciadas. Contudo, este procedimento minimamente invasivo requer pneumoperitôneo para visualização intra-abdominal adequada e manipulação operatória (SAMMOUR et al., 2009; DORWEILER et al., 2007; ADAMS et al., 1999).

O pneumoperitôneo, que é a introdução de um gás na cavidade abdominal para a criação de um espaço em seu interior e que permite a inserção dos instrumentos e a manipulação sobre os órgãos internos abdominais e pélvicos (KHOURY et al., 2009; KUCUKAKIN et al., 2009; YILMAZ et al., 2004), pode, entretanto, criar alterações adversas que têm sido observadas em muitos órgãos. Tais alterações têm sido associadas com o aumento na carga de pressão de CO2 sistêmica e intra-abdominal que ocorre durante a insuflação (CAGIDO, 2011; KHOURY et al., 2009; TSUCHIHA et al., 2008). O gás ideal para o estabelecimento de pneumoperitônio durante a laparoscopia deve ser inerte e não deve sustentar a combustão

(TSUCHIHA et al., 2008; KOIVUSALO & LINDGREN, 2000). Na cirurgia laparoscópica, o CO_2 é o gás mais utilizado na rotina cirúrgica, porque é rapidamente absorvido e excretado, facilmente disponível, barato e não inflamável (CHIAO-FEN et al., 2011; FARIAS et al., 2011; YILMAZ et al., 2004).

Entretanto, a insuflação de dióxido de carbono (CO_2) e sua deflação consiste, no pneumoperitôneo, de um modelo de lesão de isquemia-reperfusão (SZCZUBIAL et al., 2015). Haja vista que o gás introduzido exerce efeitos prejudiciais por meio do aumento da pressão intra-abdominal, que pressiona vasos esplênicos, circulações hepática, renal e mesentérica e os fluxos sanguíneos da mucosa intestinal, (SAMMOUR et al., 2009). Os distúrbios metabólicos e enzimáticos existem durante isquemia e são muito bem estabelecidos, porém evidências clínicas e experimentais demonstram que os principais eventos que levam a disfunções celulares relacionam-se com a subsequente reperfusão, ou seja, durante a reintrodução do oxigênio no meio isquêmico, e isso se dá durante a deflação de CO_2 (CHIAO-FEN et al., 2011; MALM et al., 2005). Pode resultar em um número de efeitos deletérios, principalmente, necrose de células irreversivelmente lesadas. Todas essas lesões, alterações metabólicas e mortes celulares causadas pela lesão isquemia-perfusão, provocam o aparecimento de radicais livres e, consequentemente, estresse oxidativo (CHIAO-FEN et al., 2011; SAMMOUR et al., 2009).

2.5 - Técnica Cirúrgica e o Estresse Oxidativo

Qualquer forma de trauma, incluindo procedimentos cirúrgicos, pode resultar em estresse oxidativo (YIANNAKOPOULOU et al., 2013). Dependendo da técnica cirúrgica, esta pode causar ou não no organismo animal estresse cirúrgico, que se define como o impacto exercido sobre o corpo durante e após procedimentos cirúrgicos, que leva a um estresse fisiológico de resposta complexa, envolve a ativação dos mediadores oxidativos, inflamatórios, endócrinos, metabólicos e imunológicos (ANDRADE, 2005; ESTÉCIO & SILVA, 2002). E todo esse sistema pode culminar com produção de radicais livres, levando a um estresse oxidativo que pode causar ou não morte celular através da genotocixidade (ESTÉCIO & SILVA, 2002; SUN & OBERLEY, 1996).

Os radicais livres são moléculas reativas com eletróns desemparelhados, que são continuamente produzidas no organismo por mitocôndrias e leucócitos, porém

podem ser produzidos através de fatores exógenos durante o metabolismo celular através de exposição ao álcool, tabaco e ainda intervenções cirúrgicas (ANDRADE, 2005). Também podem ser gerados por ação das células fagocitárias, como macrófagos, neutrófilos e monócitos (CIMEN, 2008; TSUCHIHA et al., 2008). Exerce importantes funções biológicas, tal como defesa antibacteriana, mas também pode-causar danos (GRANGER et al., 2007). Em sistemas biológicos, a membrana celular constitui um dos focos de ação dos radicais livres. Além das membranas celulares, as membranas das organelas intracelulares, tais como a mitocôndria, o retículo endoplasmático e o núcleo também são afetados (VALKO et al., 2007). Normalmente, a morte celular ocorre por vias distintas, como necrose ou apoptose, porém, quando na presença do estresse oxidativo, essa morte ocorre pela perda da integridade da membrana e perda da homeostase celular (CIMEN, 2008).

Os organismos aeróbicos desenvolveram defesas que agem como agentes antioxidantes para superar os efeitos nefastos dos radicais livres (ANDRADE, 2005; CERHAN, 2003). Os radicais livres formados são convertidos em elementos não danosos por esses agentes. Já em 1989, Starke-Reed e colaboradores publicaram que diversos fatores poderiam levar a um aumento do desequilíbrio entre a produção de radicais livres e a proteção antioxidante, conduzindo a um estresse oxidativo com subsequente lesão aos tecidos. Estudos experimentais com ratos, têm demonstrado correlação causal entre maior produção de radicais livres de oxigênio e maior lesão pancreática e colônica, assim como redução da lesão tecidual com o emprego de antioxidantes (SCOLA et al., 2013; KAGEYAMA et al., 2008; JONES, 2006). No entanto, a patogênese do estresse oxidativo e as medidas para diminuí-la na cirurgia laparoscópica e na cirurgia abdominal estão além do escopo dessa pesquisa e estão sendo revistos em outros artigos.

A produção descontrolada dos radicais livres pode provocar inúmeras afecções nos gatos como: inflamações crônicas, doenças degenerativas e câncer (KEEGAN & WEBB, 2010).

Mas uma célula é normalmente capaz de superar o estresse oxidativo, se as perturbações no equilíbrio redox forem pequenas, restabelecendo o equilíbrio normal intracelular (KAGEYAMA et al., 2008; CERHAN et al., 2003; STARKED-REED, 1989).

A formação de radicais livres não é observada apenas nas cirurgias laparoscópicas, mas também aumenta durante cirurgias abdominais (principalmente com

manipulação intestinal) como resultado de produção de isquemia originada durante a manipulação do órgão e da ativação de leucócitos em processos inflamatórios agudos e crônicos (CASE & ELLISON, 2013; BULBULOGLU et al., 2011; ANDRADE, 2005; RAMACHANDRAN et al., 2001).

O impacto do estresse oxidativo na cirurgia minimamente invasiva não está totalmente investigado. A gravidade do pneumoperitônio induzindo o estresse oxidativo sugere ser tempo e pressão utilizados dependentes (SZCZUBIAL et al., 2015; BUKAN et al., 2004; RAMACHANDRAN et al., 2001). No entanto, os dados publicados relacionando o pneumoperitônio de longo prazo são muito restritos e estes são controversos, por enquanto.

Além disso, a medição do estresse oxidativo nos animais é problemática, haja vista que valores de referência não existem. Assim, questões metodológicas surgem, que não permitem a extração de conclusões válidas (SCOLA et al., 2013; YIANNAKOPOULOU, et al., 2013).

2.6- Estresse Oxidativo e Genotoxidade

A genotoxicidade ou toxicologia genética é uma especialidade que se ocupa da identificação e do estudo da ação de qualquer agente físico, químico ou biológico que produz efeitos tóxicos e genotóxicos, sobre o material genético. As substâncias genotóxicas são todas as que têm afinidade para interagir com o DNA, e cada alteração na sua estrutura poderá modificar sua função (JONES, 2006).

A genotoxicidade pode ter origem através dos radicais livres, que podem ter como alvos, por exemplo, as proteínas, os lipídeos e os ácidos nucléicos. O radical hidroxila, por exemplo, é extremamente reativo e com alta capacidade de gerar danos celulares, já que o organismo não apresenta sistema enzimático de defesa para esse radical, podendo ocasionar modificações em bases purínicas e pirimidínicas, gerando mutações de DNA, (CHIHUAILAF et al.; 2002 KRUMAN et al., 1997).

Os primeiros alvos citados acima, ou seja, as proteínas e os lipídeos, podem se desnaturar ou simplesmente perder a sua função (KAGEYAMA et al., 2008). Em relação aos danos causados ao DNA (genotoxicidade) pode-se observar um aumento na incidência de mutação de ponto, quebras cromossômicas. Tais quebras podem afetar apenas um filamento do cromossomo (cromátide), originando uma quebra

cromatídica, ou ambos os filamentos, produzindo uma quebra cromossômica, algumas aberrações como alterações de tamanho ou forma dos cromossomos (aberrações estruturais) que resultam de quebras simultâneas na mesma célula, envolvendo um ou mais cromossomos, podendo originar cromossomos rearranjados. (JONES, 2006; ESTÉCIO & SILVA, 2002).

Como consequência de todos esses danos, o DNA pode produzir falhas na transcrição por alterações cromossômicas, a segregação e a expressão do gene (PRADO et al., 2010), provocando uma mutação ou, conforme a intensidade da agressão, inviabilizar a vida da célula, o que finalizaria em morte por apoptose ou por necrose (PRADO, 2003).

Um dos indicadores biológicos para avaliar danos no material genético é o estudo citogenético de aberrações cromossômicas em linfócitos de sangue periférico.

A genotoxidade em linfócitos tem sido relatada em vitro em animais expostos a anestesias. Além disso, alguns autores acreditam que a genotoxidade anestésica pode ser devido ao seu poder de redução e oxidação metabólica que dão origem a reações metabólicas e a espécies reativas de oxigênio. Sabe-se que danos oxidativos ao DNA podem ser reparados e retirados pelo sistema de reparação, o qual inclui genes anomalos, porém não se sabe a interferência dos anestésicos na expressão de cada gene.

Há numerosos estudos relatando os efeitos da anestesia por inalação, em células de indivíduos expostos, mas não se sabe muito sobre a capacidade de procedimentos cirúrgicos para induzir lesões oxidativas do DNA. No entanto, a cirurgia é frequentemente associada com alteração imunológica perioperatória temporária, e alguns anestésicos voláteis parecem contribuir para um linfocitopenia transiente após a cirurgia.

3. CONCLUSÃO

A análise dos resultados do presente estudo permitiu concluir que a técnica videocirúrgica testada para OVH em gatas apresentou diferenças em relação à convencional por celiotomia em relação ao estresse oxidativo e produção de danos cromossomais. Foi atribuída a manipulação intestinal transoperatória durante a técnica da laparotomia e o tempo cirúrgico da laparoscopia, como possíveis causas dessas diferenças

4. LIMITAÇÃO DO ESTUDO

Seria ideal ter dosado componentes de uma equipe de enzimas antioxidantes como a glutationa peroxidase (GSH-Px), superóxido dismutase (SOD) e catalase (CAT).

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6. CAPÍTULO 1

(Feline Surgery)

Original Article

ASSESSMENT OF THE OXIDATIVE STRESS RESPONSE IN MINIMALLY INVASIVE SURGERY AND LAPAROTOMY ON FELINES FOLLOWING OVARIOHYSTERECTOMY

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Keywords:	Feline, Reactive Oxygen Species, Surgical Manipulation, Intestine, Laparoscopic, Lipid Peroxidation, Protein Carbonyl
Abstract:	Objectives: The purpose of this study was to examine the alterations in lipid peroxidation and protein carbonylation, two reliable biomarkers associated with oxidative damage, after laparoscopic or tradicional ovariohysterectomy (OVH) in female cats. Methods: The study included forty healthy female cats, from which similar amounts of blood samples were collected under anesthesia before surgery, immediately after and 24 hours after surgery. Serum oxidative stress (OS) was evaluated by assessing protein carbonyl groups and 4-hydroxynonenal plasma levels, using immunoblotting techniques. Results: A loss of control over reactive oxygen species (ROS) production occurred in female cats after OVH, which lead to increased oxidative damage, particularly in the late post-operative period, suggesting that, traditional OVH is associated with an immediate increase of OS, while OVH by laparotomy is related with the risk of OS in the late period after surgery. Conclusion: Given that OS contributes to the pathogenesis of various diseases, OVH female cats may present an increased risk of OS-associated disorders. Relevance: OS is an integral part of the surgical stress response. Minimally invasive surgery causes fewer traumas, and thus attenuated stress response is anticipated. However, the occurrence of pneumoperitoneum is involved in the production of free radicals. A strategy to counteract the deleterious effects of OS after OVH may be advisable, although further studies are necessary before a definitive recommendation.

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ASSESSMENT OF THE OXIDATIVE STRESS RESPONSE IN MINIMALLY INVASIVE SURGERY AND LAPAROTOMY ON FELINES FOLLOWING OVARIOHYSTERECTOMY

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ABSTRACT

Objectives: The purpose of this study was to examine the alterations in lipid peroxidation and protein carbonylation, two reliable biomarkers associated with oxidative damage, after laparoscopic or tradicional ovariohysterectomy (OVH) in female cats. **Methods:** The study included forty healthy female cats, from which similar amounts of blood samples were collected under anesthesia before surgery, immediately after and 24 hours after surgery. Serum oxidative stress (OS) was evaluated by assessing protein carbonyl groups and 4-hydroxynonenal plasma levels, using immunoblotting techniques. **Results:** A loss of control over reactive oxygen species (ROS) production occurred in female cats after OVH, which lead to increased oxidative damage, particularly in the late post-operative period, suggesting that, traditional OVH is associated with an immediate increase of OS, while OVH by laparotomy is related with the risk of OS in the late period after surgery. **Conclusion:** Given that OS contributes to the pathogenesis of various diseases, OVH female cats may present an increased risk of OS-associated disorders. **Relevance:** OS is an integral part of the surgical stress response. Minimally invasive surgery causes fewer traumas, and thus attenuated stress response is anticipated. However, the occurrence of pneumoperitoneum is involved in the production of free radicals. A strategy to counteract the deleterious effects of OS after OVH may be advisable, although further studies are necessary before a definitive recommendation.

Keywords. Feline, Reactive Oxygen Species, Surgical Manipulation, Intestine, Laparoscopic, Lipid Peroxidation, Protein Carbonyl.

Introduction

Laparoscopy is widely used because it offers many advantages to patients including reduced postoperative pain, shorter hospital stay (shortened convalescence)[1,2], reduction of intraoperative bleeding, fewer blood product transfusions and shorter care in the post anesthesia unit[3]. Laparoscopic surgery through smaller abdominal incisions and less tissue manipulation causes fewer traumas and thus is expected be followed by a less pronounced stress response[4,5]. However, this procedure requires the completion of a pneumoperitoneum for adequate visualization of the operative manipulation[6,7]. Depending on the pressure level of the gas used, this technique may cause severe inflammatory and metabolic responses, which induce hemodynamic alterations in patients[8,9], can affect several homeostatic systems leading to changes in acid-base balance, blood gases, cardiovascular systems and respiratory physiology[9,10]. The insufflation and deflation of the pneumoperitoneum is like a model of ischemia-reperfusion injury, and thus the induction of oxidative stress and OS response is anticipated[6,7].

Numerous studies reported an increase of OS in both women and female laboratory animals after ovariectomy[8, 9, 10, 11]. A higher risk of many diseases associated with OS, such as cardiovascular diseases, renal diseases, osteoporosis and Parkinsonsm, has been observed in women after ovariectomy[11,12,13]. In cats there have been reports of renal and liver disease, as well diabetes[10]. Even so the impact of minimally invasive surgery on OS is not fully understood[11] and the data are not ideal and are often controversial[12]. In fact, there is little information about the evaluation of antioxidative/oxidative status in ovariohysterectomized cats.

Material e Methods

Experimental design

This study includes 40 felines (domestic cats), American Society of Anesthesiology (ASA) physical status 1, ranging in age from 6 months to 3 years, in weight from 2 kg to 4 kg, that were referred to the Veterinary Medical Teaching Hospital for elective ovariohysterectomy, were included in the study after reading and signing a consent form carried out by the owners. Cats were selected as healthy after performing clinical examination, basic profile (complete blood count, urinalysis) and biochemical analysis study.

The exclusion criteria were: pregnancy, oestrus or lactation, and aggressive cats. Cats were randomly selected to four groups, one for ovariohysterectomy by laparotomy (n=10), another for ovariohysterectomy laparoscopic group (n=10), anesthetized group for 13 minutes (n=10) and anesthetized group for 43 minutes (n=10). None of the cats in either group were purebred. The study was performed according to the national guidelines after approval by the National Ethical Committee for Laboratory Animals (2007-07-27; document no. 018 939) and conducted in accordance with international standards on animal welfare as defined by the European Communities Council Directive of 2 November 1986 (86/609/EEC).

Sample collection

For each group, 3 mL of blood were collected by venipuncture from the cephalic vein, in three different times, into vacuum tubes with lithium heparin, from each individual. The first collection of 1 mL was made immediately pre anesthesia (T1); the second collection of 1 mL was made immediately after the end of surgery (T2); and a third collection was made 24 hours after surgery (T3). The laparo-

scopic surgery took on average 43 minutes to perform while the laparotomy took on average 13 minutes. Blood samples were immediately frozen and kept at -80ºC until use.

Lipid peroxidation levels

The 4-hydroxy-2-nonenal (4-hydroxynonenal; 4-HNE) is a highly reactive aldehyde generated by the exposure of polyunsaturated fatty acids to peroxides and ROS. 4-HNE levels were quantified using a specific goat anti-4-hydroxynonenal polyclonnal antibody according to the method described by Kruman and collaborators[15]. Firstly, samples were lysed by sonication. Protein concentration of samples was then determined by using the BCA Protein Assay Kit (Pierce, Thermo Fisher) following the manufacturer's instructions. Samples were then diluted to a concentration of 0.001 μg/μL using phosphate buffer saline (PBS). Activated polyvinylidenedifluoride (PVDF) membranes were used in the slot-blot technique, which was performed using a Hybri-slot manifold system (Biometra, Göttingen, Germany). The membranes were then blocked by incubating during 90 min with a 5% non-fat milk Tris-buffered saline solution (TBS) with 0.05%. Tween-20 containing 5% skimmed dried milk. Afterwards, the blocked membranes were incubated overnight with rabbit anti-4-HNE antibody (1:5000; Ref. AB5605; Merck Millipore). Samples were visualized using rabbit anti-goat IgG-AP (1:15000; Ref. A4187; Sigma-Aldrich). Membranes were then reacted with ECF (GE Healthcare) and read using a BioRad FX-Pro-plus (Bio-Rad). Densities from each band were quantified using the BIO-PROFIL Bio-1D Software from Quantity One (Vilber Lourmat). The results are expressed as fold variation to control situation (immediately before surgical intervention).

Protein oxidation levels

Protein oxidation levels were evaluated by assessing protein carbonyl content in the samples, which is commonly used as a marker for OS. To evaluate protein carbonyl content a specific rabbit anti-dinitrophenol (DNP) polyclonal antibody (Sigma-Aldrich) was used. Firstly, samples were lysed by sonication. Protein concentration of samples was then determined by using the BCA Protein Assay Kit (Pierce, Thermo Fisher) following the manufacturer's instructions. Lysed samples were derivatized using 2,4-dinitrophenylhydrazine (DNPH) according to the method described by Dias and collaborators 16. In brief, a volume of lysed sample containing 10 μg of total protein was mixed with the same volume of sodium dodecyl sulfate (SDS) 12% and centrifuged to minimize nucleic acid interference in the assay (5000xg, 5 minutes). The samples were then mixed with two volumes of DNPH 20 mM diluted in trifluoroacetic acid (TFA) 10% and incubated for 30 minutes in a dark environment. The reaction was then stopped using 1.5 volumes of Tris 2 M with 18% β-mercaptoethanol. Samples were then diluted to a concentration of 0.001 μg/μL using PBS. Activated PVDF membranes were used in the slot-blot technique, which was performed using a Hybri-slot manifold system (Biometra). The membranes were then blocked by incubating during 90 min with a 5% non-fat milk TBS with 0.05% Tween-20 containing 5% skimmed dried milk. Afterwards, the blocked membranes were incubated overnight with rabbit anti-DNP antibody (1:5000; Ref. D9656; Sigma Aldrich). Samples were visualized using goat anti-rabbit IgG-AP (1:5000; Ref. Sc2007; Santa Cruz Biotechnology). Membranes were then reacted with ECF (GE Healthcare) and read using a BioRad FX-Pro-plus (Bio-Rad). Densities from each band were quantified using the BIO-PROFIL Bio-1D Software from Quantity One (VilberLourmat). The results are expressed as fold variation to control situation (immediately before surgical intervention).

Statistical analysis

The variables were tested to see if they followed a normal distribution using the Kolmogorov-Smirnov test with Lilliefors correction. To test if there were differences within times per group the Friedman test was used. The Wilcoxon signed-rank test was used to test the differences between pairs of times (before versus immediately after, before versus 24h, immediately after versus 24h). To test for differences between groups (laparotomy versus laparoscopy) the Mann-Whitney U test was used. This test was also used to test if there were differences between the animals that undergone surgery (laparotomy and laparoscopy) and the ones that were only under the effect of the anesthesia (control groups).

Results

Table 1. Descriptive results of the protein carbonyl and the lipid peroxidation for each group.

ROS	Group	Time	Descriptive of the results			
			Minimum	Median	Mean	Maximum
Protein carbonyl	Laparotomy	T2	0.97	1.03	1.13	1.54
		T3	0.91	1.19	1.34	1.98
	Laparoscopy	T2	1.02	1.02	1.04	1.14
		T3	1.27	1.27	1.33	1.64
Lipid peroxidation	Laparotomy	T2	0.88	0.97	0.98	1.07
		T3	0.90	1.02	1.02	1.10
	Laparoscopy	T2	0.81	0.96	0.94	0.96
		T3	0.86	0.97	0.96	1.00
	Control for laparotomy	T2	1.11	1.58	1.55	1.89
		T3	0.70	0.71	0.73	0.80

	Control for laparoscopy	T2	1.25	1.77	1.71	1.96
		T3	0.78	0.87	0.87	0.99

The effect in ROS due to the surgery can be observed when we compare the animals that undergone surgery with the ones that suffered only the effect of the anesthesia (Figure 1). The animals that were only anesthetized had a higher variation of lipid peroxidation at T2 when compared to the ones that suffer surgery, while the opposite was observed for T3. These differences were statistically significant ($p<0.05$) for each time period between the control groups and the surgery groups.

[Insert Figure 1].

Within group comparison for the laparoscopy group showed statistically significant differences ($p<0.0001$) between times for protein carbonylation. The same was observed for lipid peroxidation ($p<0.0001$). For the laparotomy group no statistically differences were observed between times for protein carbonylation ($p=0.058$) and lipid peroxidation ($p=0.209$).

When comparing groups (laparotomy versus laparoscopy) there was a statistically significant difference between groups for T3 (24h after surgery) for the lipid peroxidation ($p=0.038$). The group that undergone laparotomy had higher values of lipid peroxidation compared to the group that undergone laparoscopy (Figure 2).

[Insert Figure 2]

Discussion

The degree of OS was evaluated by measuring the final products of the generated injury using different biomarkers[4,14]. The OS can be measured by the quantity of the ROS, the damage caused by ROS or by the levels of antioxidant response generated[15,16].

Previous studies have shown that there is not a single biomarker that can really represent OS and the result of each can vary immensely[14], taking this into account in the present study we used two different biomarkers: The 4-HNE chosen as a measure of lipid peroxidation due to form a highly stable adduct with protein side specific chains[15,16] and protein oxidation levels assessed by the protein carbonyl content since this is an extremely reliable and stable biomarker[15].

On the comparison between the two surgery groups and the groups that were only anesthetized, there was a statistically significant difference. There was a tendency to normalization of the biomarkers values in T3 at the anesthetized groups, suggesting that the results obtained in this study are due to surgery and not only the anesthesia.

Each biomarker had different results. Comparing the laparoscopy and laparotomy groups there was significant difference for Lipid Peroxidation in T3 were laparotomy group had higher values. The possible reason for such an event is found in the fact that the plasma membrane is one of the most affected by ROS damage, inducing changes in the structure and permeability of cell membranes.

These results are concordant with studies that suggests a lower OS by the laparoscopic technique in wich pneumoperitoneum triggers ischemic and reperfusion sindrome[7,8], even representing a minimally invasive technique. It is believed that these findings may be related to the use of a moderate pneumoperitoneum pressure. That would explain how the laparoscopy group having longer surgical time (average 43 minutes), with a 10 mmHg pressure did not provide during this period higer OS damage when compared

with laparotomy group. Suggesting that the laparoscopic procedure did not become an ischemia-reperfusion model as cited by several authors[17, 18, 19, 20].

Guven et al. in 2010 supports, that the hemodynamic changes determined by pneumoperitoneum may vary with time and pressure used during surgery. According to these authors the pneumoperitonium induction for laparoscopic surgery with pressure levels between 10 to 15 mmHg bring higher pressure levels in the portal system than levels between 7 and 10 mmHg [18, 21].

Sammour et al. in 2009, in a retrospective study for laparoscopy in relation with OS, demonstrated that time and the intra-abdominal pressure during pneumoperitoneum, can significantly interfere with the hemodynamic changes and production of OS[22].

In an experimental study with 30 Wistar rats, a group was submitted to pneumoperitoneum for 30 and another for 60 minutes, both with a 10 mmHg pressure. The first group showed no hemodynamic changes, which no longer occurred with the second[23]. In that study the time which the animal was subjected to pneumoperitoneum seems to be relevant, which was not observed in the present study, since despite the time of surgical laparoscopy group was higher, the Lipid Peroxidation remained lower than in the group laparotomy in T3.

The protein carbonyl increase related to the laparotomy group matches with the report described by ANUP et al., 1994, which concluded through an experiment with mice that smooth handling of the intestine is able to induce OS. During the experiment, the animals underwent laparotomy and as a result suffered light manipulation in every segment of the small intestine (ID) for one minute without causing actual harm to the ID, only with the intestinal manipulation that occurs during abdominal surgery. This study indicated that the ID can be highly susceptible to injury during abdominal surgery and gentle handling of the intestines is able to induce OS.

Simmy Kunissery A. Thomas and Balasubramanian[27] reported in 2004 that the every abdominal surgery involves intestinal manipulation to reach the organs below. They further argued that during sur-

gery there might be a decrease in blood flow in the intestine due to a decrease of blood pressure triggering an ischemia and reperfusion injury as in the pneumoperitoneum. Despite the short time of surgery in the laparotomy group, the higher production of ROS may be justified by a growing body of experimental data[25,26,27] claiming that the gastrointestinal tract is extremely sensitive to surgical stress, even in remote locations. It justifies the increased OS in the laparotmy compared with the stress caused by inflation of the laparoscopy.

However, the same study cited above noted that the greatest surgical stress damage was 1 hour after surgery and changes recovered within 24 hours after surgery. They concluded that it is likely that, in large abdominal surgery, the recuperation time can be much higher for the changes to reverse and sometimes they could be irreversible.

Into the present study, the greatest response of OS was at lipid peroxidation in 24 hours after surgery for the laparotomy group and when compared with the laparoscopy group there were statistically significant differences between groups. Other significant differences were observed within the laparoscopy group when comparing T2 and T3 time. The statistical significance may be due both to a reduction in the ability of antioxidant defenses to clean them in the related postoperative with its redistribution and increased consumption, as well as a delayed release of ROS[9,10,15,16,28].

According to Kehlet and several authors, the debate on laparoscopic surgery decreasing the response to surgical stress is on an upward trend studies[30,31,32]. We all know that any form of trauma, including surgery, results in OS, and that both laparoscopic and open surgical procedures can produce changes in several endocrine metabolic responses and changes in immune function, being more pronounced during open surgery[30]

Conclusion

In conclusion, our study showed that both laparotomy and laparoscopy caused an increase on OS. However, laparoscopic caused significantly less OS than laparotomy. Our findings suggest that laparotomy OVH is related with the risk of OS in the late period after surgery. Given that OS contributes to the pathogenesis of several chronic diseases, this may suggest an increased risk of disorders in ovariohysterectomized female cats; however, long-term future studies are required to confirm this hypothesis.

Figure and Images

Figure 1. Boxplot of the lipid peroxidation results per group for the two time periods (T2 – immediately after surgery, T3 – 24h after surgery) and the four groups.

Figure 2. Histogram of the results of lipid peroxidation for time T3 (after 24h of the surgery) for the laparotomy and laparoscopy groups.

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Conflict of interest

The authors do not have any potential conflicts of interest to declare.

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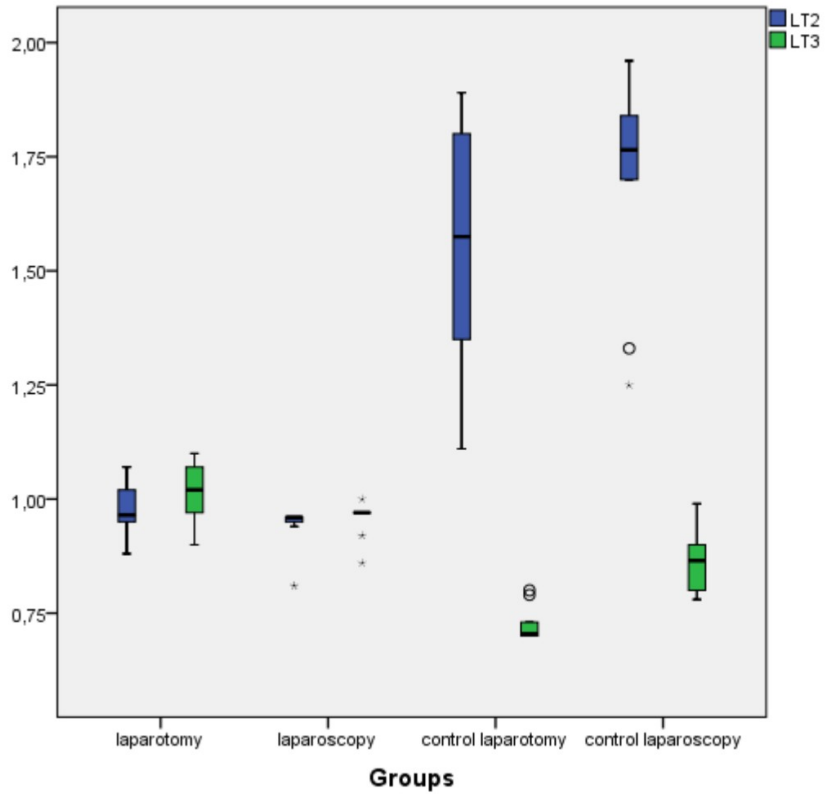

Figure 1. Boxplot of the lipid peroxidation results per group for the two time periods (T2 – immediately after surgery, T3 – 24h after surgery) and the four groups.

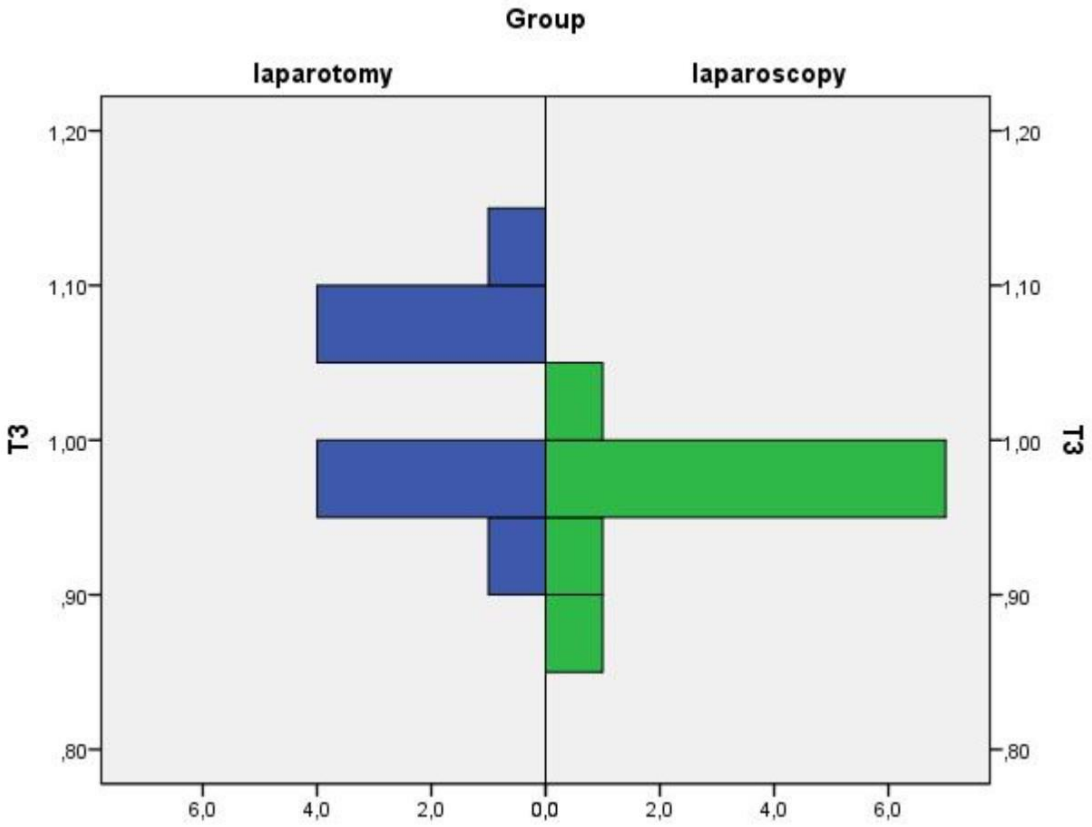

Figure 2. Histogram of the results of lipid peroxidation for time T3 (after 24h of the surgery) for the laparotomy and laparoscopy groups.

7. CAPÍTULO 2

(Feline Surgery)

Original Article

THE IMPACT OF MINIMALLY INVASIVE SURGERY IN THE TRADITIONAL RELATIONSHIP OVH LAPAROTOMY THOUGH A CYTOGENETIC STUDY IN PERIPHERAL BLOOD LYMPHOCYTES.

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ABSTRACT

Objectives: Assesing the production of breaks and chromosomal aberrations in cytogenetic study in minimally invasive surgery compared to laparotomy in cats. **Methods:** The study included forty healthy female cats. Blood samples Were collected before surgery, immediately after and 24 hours after surgery. Colllect similar were made in two groups of animals wich were only anesthetized and evaluated for the production of lipid peroxidation. While the groups that underwent surgery were evaluated for production breaks and aberrations. **Results:** The results showed significant differences in the values of breaks and aberrations in the laparoscopy group at T2 relative to laparotomy group, suggesting that the time which the animal was subjected to pneumoperitoneum seems to be relevant. **Conclusion:** It concluded that the study in peripheral blood lymphocytes in cats submitted the operations indicated a greater genotoxic effect on T2 in the laparoscopy group. It also demonstrated that this increase of genotoxic effects had decreased 24 hours after surgery due to possible DNA repair. These partially reversible genotoxic effects were observed in the laparotomy group compared aberrations. **Relevance:** Since the molecule is responsible for all the genetic information of all cells of a living organism, and also by the fact that there are not studies on DNA damage at cats undergoing ovariohysterectomy. It becomes necessary a comparative study from the effects of both surgical techniques.

Key-words: damage, breaks, aberrations, repair, laparoscopy, surgery

INTRODUCTION

Ovariohysterectomy (OVH), the most common surgical procedure in veterinary practice, prevents or reduces the risk of developing breast cancer and pyometra, and male attraction during estrus[1,2,3,4]. However laparoscopic technique has became standard for ovariohysterectomy (OVH) with results comparable to those of open surgery[5]. Laparoscopic Ovariohysterectomy (LOVH) has potential advantages, including decreased pain and shortened hospitalization and convalescence[7]. Laparoscopic surgery through smaller abdominal incisions and less tissue manipulation causes fewer traumas and thus is expected be followed by a less pronounced stress response[1,7,8].

However any form of trauma, including surgery, both laparoscopic and open surgical procedures can result in oxidative stress and which is one of main targets of these reactive oxygen species include DNA, as well as sugars, lipids and proteins, although not degrade the which does not occur with the DNA, since the molecule is responsible for all genetic information of all cells of a living organism[9].

Numerous studies reported an increase of oxidative stress increases in both women and female laboratory animals after ovariectomy. However the impact of oxidative stress impact of oxidative stress on the ADN on minimally invasive surgery is not fully understood[3,7]. There is anything information about the evaluation of oxidative status and its impact sobre o DNA in ovariohisterectomized cats.

The genotoxicity and genetic toxicology is a specialty that deals with the identification and study of the action of any physical, chemical or biological agent that produces toxic and genotoxic effects on genetic material [10]. The genotoxic substances are all those which have an affinity to interact with the ADN[11]. It is well known that ADN can undergo oxidative damage, such as produced during surgical procedures.

This study analyzed the available data on the impact of minimally invasive surgery in the traditional relationship OVH laparotomy through the oxidative stress production and a cytogenetic study in peripheral blood lymphocytes, in cats chromosome.

Material e Methods

Experimental design

This study includes 40 cats ranging in age from 6 months to 3 years, ranging in weight from 2 kg to 4 kg hat were referred to the University veterinary Hospital for elective ovariohysterectomy were included in the trial after agreement and signed consent were obtained from the owners and after the cats were determined to be healthy by means of physical examination including, heart and respiratory rates, rectal temperature, abdominal palpation, arterial pressure and basic blood analysis and biochemical analysis (urea, creatinine, ALT and AST). The exclusion criteria were: I. pregnancy, II. oestrus or lactation, III. Animals with less that 6 months and bigger that 3 years, IV. ASA bigger 1, V. Dehydration animals, and aggressive cats. The cats were then randomly allocated to a ovariohysterectomy by laparotomy group (n=10) and ovariohysterectomy by laparoscopy group (n=10), a third only anesthetized group for 13 minutes (n=10) and another anesthetized group for 43 minutes (n=10), these two last groups simulating the surgical times of the first two groups, in sequence. The study was submitted and approved by the ethical committee of Up. All owners provided written informed consent.

HNE levels (highly reactive aldehyde produced by exposure of polyunsaturated fatty acids to peroxides and oxygen reactive species) were quantified using a specific antibody goat anti- 4-hydroxynonenal polyclonnal according to the method described by Kruman reviewers[12].

Sample collection

For each experiment, 3 ml of blood was collected, in three different times, by venipuncture into vacuum tubes with lithium heparin, from each patient.

First time: 1ml before anesthesia, second time: 1ml Immediately after surgery, third time: 1ml 24h after surgery. Then, the groups submitted to surgery added 1 ml peripheral blood sample to a 15 ml sterile tube containing 7 ml of RPMI 1640 (Sigma) complete medium supplemented with 15 % fetal calf serum (GIBCO), antibiotics (10,000 units/mL of penicillin and 10,000 μg/mL of streptomycin) - (GIBCO) and 29 mg/mL of L-glutamine (Sigma). Concanavalin A (15 μg/mL, final concentration), and one drop of sterile sodium heparin (this prevents coagulation problems). Store cell cultures placed in an incubator at 37°C with 5% CO_2 atmosphere.

After 96 h days of culture (tubes, were maintained with the highest inclination to improve cell growth, gently agitated cell cultures once a day) cells were harvested after a 1,5 h incubation with 50 μL colcemid® (GIBCO) followed by hypotonic treatment with 75 mM KCl and fixation in 1:3 solution of acetic acid:methanol.

Top spin at 1200g for 8 min, removed the supernatant, and added KCl 0.75 M (0.56 g %) drop by drop to arrive at 2 mL by shaking the tube gently. Mixed cells thoroughly by using Pasteur pipet, and then added more solution to arrive at 14 mL. Mixed cells with a Pasteur pipet and stored the cell suspension at 37°C for 20 min. Then, add 1 mL of fixed solution (FS) (acetic acid/methanol 1:3) and mixed.

- Top spin at 2.700g for 10 min, removed the supernatant, and added (drop by drop) 2 mL FS. Then, mixed thoroughly with a Pasteur pipet and added more fix solution to arrive at 10 mL. Mixed with a Pasteur pipet and stored at room temperature for 20 min.

- Top spin at 2.700rpm and removed the supernatant. Added 5 mL of FS, mixed with a Pasteur pipet, and stored at room temperature for 10 min.
- Repeated as in step 7 and store at 4°C overnight.
- The following day, repeated as in step 7.
- Repeated as in step 7 by adding 0.5-1.0 mL fresh FS (the quantity varied on pellet size).
- Spreaded two drops of cell suspension on slides previously cleaned with ethanol and immersed in cold distilled water.
- Air-dry the slides and checked cell density with a microscope by using phase-contrast. Chromosome preparations were made by the standard air drying method.
- Cytogenetic analysis was performed on coded slides and an average of 50 Giemsa-stained metaphases (mode = 100, range = 22-100) was observed. To avoid bias in cell selection, consecutive metaphases, which appeared intact with sufficient well-defined chromosome morphology, were selected for the study. Each cell was scored for chromosome number and structural abnormalities. Achromatic areas less than a chromatid in width were scored as gaps; achromatic areas more than a chromatid in width were scored as breaks. Tri-radial and quadri-radial configurations and dicentric and ring chromosomes were scored as rearrangements. Gaps were excluded in the selection of chromosome aberrations and rearrangements were scored as two breaks. As CI parameters, percentage of aberrant cell and number of breaks per cell were used.

Results

All animals

	N	Mean	Std. Deviation	Minimum	Maximum

abT1	20	,250	,6387	0,0	2,0
abT2	20	3,7590	4,26678	0,00	14,00
abT3	20	3,300	2,9218	0,0	10,0

Table 1: There is a statistical difference between groups ($p < 0.0001$) for the ab%. As can be seen by the mean difference due to the time T1 (before surgery) compared to after surgery (T2 and T3). When comparing between every two days, no statistically significant difference between T1 and T2 ($p = 0.003$) and T1 and T3 ($p = 0.001$) but not between T2 and T3 ($p = 0.936$).

DescriptiveStatistics

	N	Mean	Std. Deviation	Minimum	Maximum
qT1	20	,0025	,00639	0,00	,02
qT2	20	,05075	,056781	0,000	,170
qT3	20	,0350	,03171	0,00	,10

Table 2: There is a statistical difference between the groups (p = 0.001) to aq / cel. Can be seen from the average difference is due, once again, at time T1 (before surgery) compared to after surgery (T2 and T3) .When compares between every two days, no statistically significant difference between T1 and T2 (p = 0.003) and T1 and T3 (p = 0.001) but not between T2 and T3 (p = 0 524).

Comparison by group

Laparoscopy

	N	Mean	Std. Deviation	Minimum	Maximum
T1	10	,0030	,00675	0,00	,02
T2	10	,08580	,055083	0,000	,170
T3	10	,0400	,04110	0,00	,10

Table 3: There is a statistical difference between groups (p = 0.016) to the% ab. As can be seen by the mean difference due to the time T1 (before surgery) compared to after surgery (T2 and T3). When comparing between every two days, no statistically significant difference between T1 and T2 (p = 0.007)

and T1 and T3 (p = 0.028) but not between T2 and T3 (p = 0.102).

DescriptiveStatisticsa

	N	Mean	Std. Deviation	Minimum	Maximum
abT1	10	,300	,6749	0,0	2,0
abT2	10	6,5500	4,28306	0,00	14,00
abT3	10	3,800	3,8239	0,0	10,0

Table 4: There is a statistical difference between groups (p = 0.020) for aq / cel. As can be seen from the average difference is due, once again, the time T1 (before surgery) compared to after surgery (T2 and T3). But between the time T2 and compares T3.Quando time between every two days, no statistically significant difference between T1 and T2 (p = 0.007) and T1 and T3 (p = 0.028) but not between T2 and T3 (p = 0239)

For both parameters, which is observed is an increase of the time T1 to the time T2 and later a decrease time T2 to time T3.

Laparotomy

DescriptiveStatistics[a]

	N	Mean	Std. Deviation	Minimum	Maximum
qT1	10	,0020	,00632	0,00	,02
qT2	10	,01570	,032284	0,000	,100
qT3	10	,0300	,01944	0,00	,06

Table 5: There is a statistical difference between groups (p = 0.004) to the% ab. As can be seen by the mean difference due to the time T1 (before surgery) when compared to T3. When comparing between every two days, no statistically significant difference between T1 and T3 (p = 0.006) but not between T2 and T3 (p = 0.198) and T1 and T2 (P = 0273).

DescriptiveStatistics[a]

	N	Mean	Std. Deviation	Minimum	Maximum
abT1	10	,200	,6325	0,0	2,0
abT2	10	,9680	1,66694	0,00	4,00
abT3	10	2,800	1,6865	0,0	6,0

Table 6: There is a statistical difference between groups (p = 0.002) for aq / cel. As can be seen from the average difference is due, once again, the time T1 (before surgery) when compared to T3. When comparing between every two days, no statistically significant difference between T1 and T3 (p = 0.006) but not between T2 and T3 (p = 0.048) and T1 and T2 (P = 0273).

In this type of surgery there is an increased time T1 to the time T3. Comparison between the two types of surgery time and for each parameter:

At laparotomy the results for both the aberrations (p = 0.003) and the chromosomal breaks (p = 0.004) in T2 were higher when compared to laparoscopy.

[Insert Figure 1].

For the time T2% ab values are higher for animals undergoing laparoscopy than undergoing laparotomy. The same is observed for values of q / cel: laparoscopy with higher values than laparotomy.

For the total time of surgery were no statistically significant differences (p <0.0001) between laparotomy and laparoscopy group. The average of the laparoscopic group (mean = 43.1) was higher than that of the laparotomy group (mean = 13.9).

Variable	Laparoscopy		Laparotomy	
	Mean	SD	Mean	SD
Total time	43,1	9,8	13,9	2,1

Partial time	26,7	8,4	6,1	1,3

Table 7: Values of partial and full times of the two procedures.

ROS	Group	Time	Descriptive of the results			
			Minimum	Median	Mean	Maximum
Lipid peroxi-dation	Laparotomy	T2	0.88	0.97	0.98	1.07
		T3	0.90	1.02	1.02	1.10
	Laparoscopy	T2	0.81	0.96	0.94	0.96
		T3	0.86	0.97	0.96	1.00
	Control for laparotomy	T2	1.11	1.58	1.55	1.89
		T3	0.70	0.71	0.73	0.80
	Control for laparoscopy	T2	1.25	1.77	1.71	1.96
		T3	0.78	0.87	0.87	0.99

Table 8: Descriptive results of the protein carbonyl and the lipid peroxidation for each of the groups.

Discussion

The most cytogenetic data is published in humans. However, there are no reports that relate to the human surgery, just to anesthetic gases. In veterinary medicine, the studies found were only reported from cats with cancer.

This papper war realized with the purpose of determining if pneumoperitoneum caused during the OVH laparoscopic procedure is able to generate greater genotoxicity in relation to the traditional technique by laparotomy, under the assumption that the pneumoperitoneum induces the production of oxidative stress leading to DNA damage.

Free radicals cause damage to cellular components, and the most important effect refers to DNA damage such as strand breaks in the DNA polymer and aberrations in the composition of their bases or, yeat, an increased level of repair in addition direct formation of adducts can confirm changes in DNA. Thus, these events are molecular markers that may be used as tools in assessing the genotoxicity caused by the action of free radicals produced during operations[12,13]. In the absence of compensation for damage caused to such a molecule, changes can lead to mutations promoting carcinogenesis[14].

In the present study the data obtained showed significant cytogenetic difference in T2 among individuals exposed to laparoscopy compared to laparotomy group. In that group there was an increase of about greater 5 times for breaks and 6 times for the aberrations in relation to the group undergoing laparotomy. In this case, the surgical time seems to have significant influence on the results, allowing greater exposure of action of free radicals in cells, providing training breaks and aberrations.

These results are in agreement with reports of Yiannakopoulou et al. (2014) who reported that minimally invasive surgery causes less trauma and thus the response to surgical stress and oxidative be attenuated. But other segments suggest that changes related laparoscopic surgery would be linked to the surgical time and the intra-abdominal pressure used[8].

This hypothesis is further supported by studies of Wiesenthal and colleagues (2011) who performed an experimental study with 30 rats Wister, a group was submitted to pneumoperitoneum for 30 minutes and another for 60, both with a 10 mmHg pressure[24]. The first group showed no hemodynamic

changes, which no longer occurred with the second. In this case, as in this study, the time which the animal was subjected to pneumoperitoneum seems to be relevant.

Regarding the pressure of 10mmHg used during pneumoperitoneum, it is assumed that there would have contributed to a reduction in damage to the T3 laparoscopy, since the pressure used was similar to normal pressure levels in the portal system (7-10 mmHg)[15].

Also in 2009 Sammour T. et al. in a retrospective study of essays related to laparoscopy with oxidative stress, indicating that the time and the intra-abdominal pressure (IAP) that the animal is subjected during pneumoperitoneum, can significantly interfere with the hemodynamic changes and production ROS[16].

In laparoscopy group showed a tendency to return to homeostatic balance 24 hours after the procedure for both breaks to as aberrations, such data in accordance with the following Karabıyık reports in 2001, in which the author states that the genotoxic effect starts to decrease postoperative period and reaches normal values on the third postoperative day due to DNA repair[14].

The same was observed in the laparotomy group than in the breaks. Such events may be indicating normal activity of repair system, which corroborates with what was described by Maria V. et al, 2002, which stated that the damage can be removed by different repair mechanisms to ensure the stability of genetic information.

In the laparotomy group there was a progressive increase in aberrations in the course of time, the explanation for this experimental observation is based on the fact that this technique requires greater intra-abdominal manipulation. This event may also be justified by a growing body of experimental data that claim that the gastrointestinal tract is extremely sensitive to surgical stress, even in remote locations. Simmy Kunissery, A. Thomas and Balasubramanian reported in 2004 that the entire abdominal surgery involving intestinal manipulation to reach the target organs. They further argued that during surgery there may be a decrease in blood flow in the intestine due to a decrease of blood pressure and also increased

intestinal manipulation during intraoperative. And so, as in the pneumoperitoneum, triggering an ischemia and reperfusion injury[17,13,22].

In 1999 ANUP et al., Concluded through an experiment with mice that smooth handling of the intestine alone is able to induce oxidative stress. During the experiment, the animals underwent laparotomy and as a result suffered light manipulation in every segment of the small intestine (ID) for 1 minute without causing actual harm to the ID, only simulating the intestinal manipulation that occurs during abdominal surgery. This study indicated that rat small intestine is highly susceptible to damage during abdominal surgery, and the simple gentle handling of the intestine alone is able to induce oxidative stress[18].

However it is assumed that the short time allowed surgery lowest significance of these aberrations in relation to LC group, also allowing the repair system act on breakages. However aberrations have shown persistent 24 hours after surgery. This may be due both to a depletion of the antioxidant system in the related postoperative with its redistribution and increased consumption, as well as a delayed release of free radicals [8, 9, 10].

Attack by free radicals results in DNA damage[10,14]. In the absence of repair, these damages may lead to mutations due to the propensity of the DNA polymerase has to enter certain residues in opposition to damage leading to the appearance of transversions[19]. Because of this, and also because on average there are only two copies of each gene, it is likely that damage to the nuclear DNA are particularly relevant to cell function when compared to multiple copies of any protein, lipid or mitochondrial DNA, and the importance of nuclear DNA to carcinogenesis[21]. The detection of DNA damage is an indication of genotoxicity which can lead to mutations, which may subsequently lead to the development of neoplasms[14].

Conclusion

In conclusion, the study of peripheral blood lymphocytes in cats submitted the operations indicated a greater genotoxic effect on T2 in the laparoscopy group. It also demonstrated that this increase of genotoxic effects had decreased 24 hours after surgery due to possible DNA repair. These partially reversible genotoxic effects were observed in the laparotomy group compared aberrations. However, other genotoxicity studies are required to have knowledge of the time that such techniques provide a range of normal values of the genotoxic effects, since DNA repair is a central mechanism that protects cells from genotoxic insults. The reduction of DNA repair capacity may start a cascade of events leading to severe illness in cats. As the origin of cancer is related to the accumulation of changes in the genetic material, the data showing the genotoxicity at laparotomy can strengthen the association between it and increased risk of cancer. But to confirm this hypothesis, future studies with longer-term evaluations are needed.

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Conflict of interest

The authors do not have any potential conflicts of interest to declare.

Figure and Images

Figure 1. The laparoscopy group at time 2 - note aberrations of chromosomes in cats.

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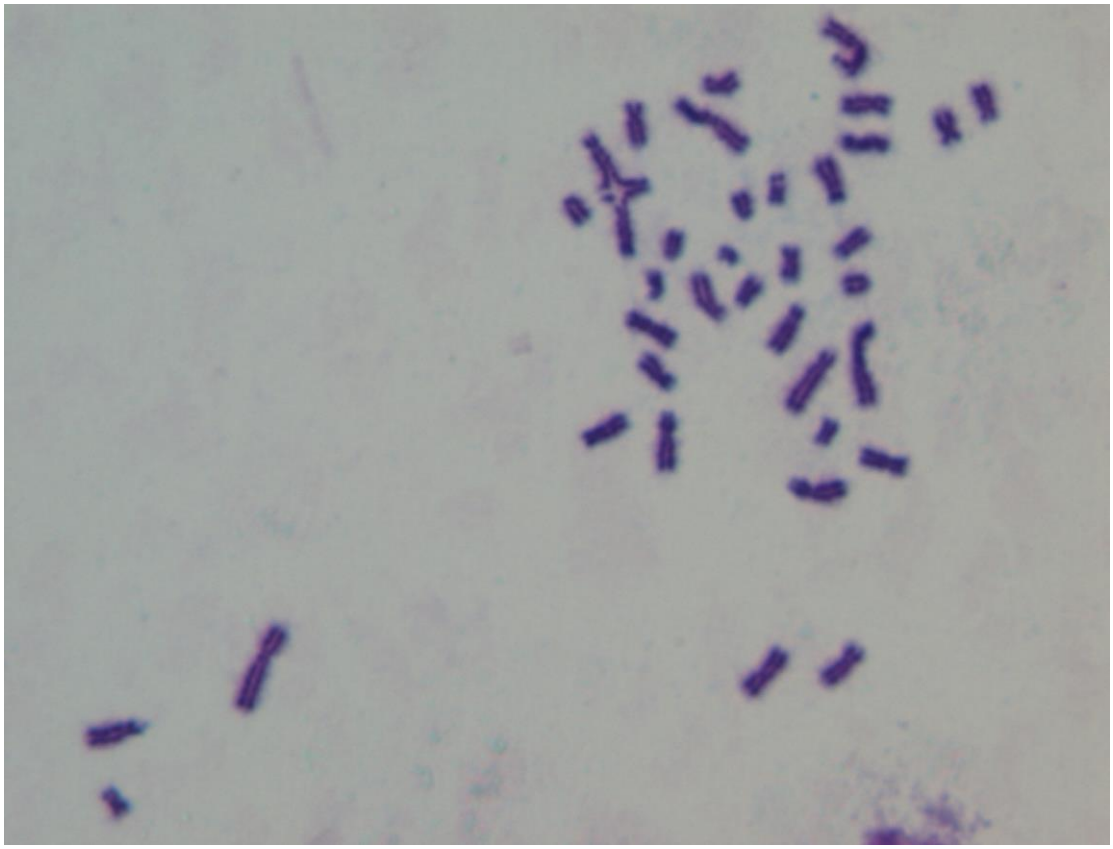

Figure 1. The laparoscopy group at time 2 - note aberrations of chromosomes in cats.