UNIVERSIDADE FEDERAL DE UBERLÂNDIA
FACULDADE DE MEDICINA VETERINÁRIA

LESÕES NÃO NEOPLÁSICAS DA GLÂNDULA MAMÁRIA DE CADELAS E SUA RELAÇÃO COM NEOPLASIAS ADJACENTES

Tais Meziara Wilson
Médica Veterinária

UBERLÂNDIA
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Dissertação apresentada à Faculdade de Medicina Veterinária - UFU, como parte das exigências para a obtenção do título de Mestre em Ciências Veterinárias.

Área de concentração:Saúde Animal

Orientadora: Profª. Drª. Alessandra Aparecida Medeiros-Ronchi

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Título da Dissertação: **LESÕES NÃO NEOPLÁSICAS DA GLÂNDULA MAMÁRIA DE CADELAS E SUA RELAÇÃO COM NEOPLASIAS ADJACENTES**

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Projeto de Pesquisa de vinculação: PATOLOGIA ONCOLÓGICA E EPIDEMIOLOGIA DAS NEOPLASIAS DOS ANIMAIS DOMÉSTICOS

No dia 20 de Janeiro do ano de 2017, às 09 horas, no Anfiteatro do Bloco 4K– Campus Umuarama da Universidade Federal de Uberlândia, reuniu-se a Comissão Julgadora, designada pelo Colegiado do Programa de Pós-Graduação em Ciências Veterinárias, composta pelos Professores(as)/Doutores(as). **Márcio de Barros Bandarra** – UNIVERSIDADE FEDERAL DE UBERLÂNDIA; **Alessandra Estrela Silva Lima** – UNIVERSIDADE FEDERAL DA BAHIA e **Alessandra Aparecida Medeiros-Ronchi,** orientador(a) do(a) candidato(a).

Iniciando os trabalhos o(a) presidente da comissão Dra. Alessandra Aparecida Medeiros-Ronchi concedeu a palavra ao(a) candidato(a) para uma exposição do seu trabalho, contando com o tempo máximo de 50 minutos. A seguir o(a) senhor(a) presidente concedeu a palavra, pela ordem sucessivamente, aos examinadores, que passaram a argüir o(a) candidato(a), durante o prazo máximo de (30) minutos, assegurando-se ao mesmo igual prazo para resposta. Ultimada a argüição, que se desenvolveu dentro dos termos regimentais, a comissão, em sessão secreta, atribuiu os respectivos conceitos. Em face do resultado obtido, a Comissão Julgadora considerou o(a) candidato(a) APROVADA.

Esta defesa de dissertação de mestrado é parte dos requisitos necessários à obtenção do título de mestre. O competente diploma será expedido após cumprimento dos demais requisitos, conforme Regulamento do Programa, Legislação e a Regulamentação Interna da UFU.

Nada mais havendo a tratar o(a) Presidente encerrou os trabalhos às 11 horas e 50 minutos, lavrou esta ata que será assinada por todos os membros da Comissão Examinadora. Uberlândia, 20 de janeiro de 2017.

Prof. Dr. Márcio de Barros Bandarra
UNIVERSIDADE FEDERAL DE UBERLÂNDIA

Profa. Alessandra Estrela Silva Lima
UNIVERSIDADE FEDERAL DA BAHIA

Profa. Dra. Alessandra Aparecida Medeiros-Ronchi
ORIENTADORA

DADOS CURRICULARES DO AUTOR

Tais Meziara Wilson – Nascida em 24 de março de 1991, na cidade de Uberlândia, Estado de Minas Gerais. Graduada em Medicina Veterinária pela Faculdade de Medicina Veterinária da Universidade Federal de Uberlândia com término no ano de 2013. No período da graduação, foi bolsista de Iniciação Científica pelo CNPq, desenvolvendo pesquisa com Leishmaniose Visceral Canina no Laboratório de Patologia Animal da Universidade Federal de Uberlândia. Realizou Residência em Medicina Veterinária nível R1, com término em 2014, na área de Clínica Médica de Pequenos Animais no Hospital Veterinário de Uberaba pelo Instituto de Estudos Avançados em Veterinária José Caetano Borges. Concluiu em 2015 a Pós-Graduação*Lato Sensu* em Acupuntura Veterinária. Atualmente é mestranda em Ciências Veterinárias na área de Saúde Animal, subárea Clínica Médica e Investigação Etiológica, com foco em Patologia Animal, na Universidade Federal de Uberlândia.

Aos meus pais,

Por todo apoio, incentivo, amor e carinho.

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Revisão de literatura

Artigo - Prevalência De Lesões Mamárias Não Neoplásicas Em Cadelas Portadoras De Neoplasia Mamária e Correlação Com Tipo e Grau Histológico

LISTA DE ABREVIATURAS

LNN - Lesão não neoplásica;

ED - Ectasia ductal;

HL - Hiperplasia lobular regular;

HLA - Hiperplasia lobular com atipia;

HLS - Hiperplasia regular com atividade secretória;

HLF - Hiperplasia lobular com fibrose;

EP - Epiteliose;

PI – Papilomatose intraductal.

Ki67 - Índice de proliferação celular

HER2 – Cerb-B2

PCNA – Antígeno nuclear de proliferação celular

LESÕES NÃO NEOPLÁSICAS DA GLÂNDULA MAMÁRIA DE CADELAS E SUA RELAÇÃO COM CARCINOMAS ADJACENTES

RESUMO –São escassos estudos de lesões não neoplásicas (LNN) em cães, assim como a relação entre a presença destas alterações e a ocorrência simultânea de neoplasias mamárias,seja na mama adjacente ou nas demais mamas da cadeia mamária onde ocorreu o tumor. O objetivo desse estudo foi determinara ocorrência de lesões não neoplásicas no parênquima mamário adjacente a tumores e em mamas sem nódulos palpáveis localizadas em cadeias mamárias de cadelas com neoplasias e correlacionar com tipo e grau histológico de carcinoma.Para tanto, foi feita análise histopatológica de 314 amostras de mama com ou sem nódulo palpável de 68 cadelas submetidas à mastectomia. As alterações neoplásicas e não neoplásicas localizadas adjacentes aos tumores e também nas mamas sem nódulos palpáveis na cadeia mamária foram identificadas esua ocorrência foi associada com neoplasias malignas e benignas por análise de contingência(Teste exato de Fischer). A idade mínima das cadelas nesse estudo (3 anos) coincide com a ocorrência do primeiro ou segundo ciclo estral. Em mamas sem nódulos palpáveis associadas a neoplasias na cadeia mamária há ocorrência de LNN e neoplásicas, demonstrando o risco de desenvolvimento de uma nova neoplasia mamária em outras glândulas de cadelas diagnosticadas com carcinoma mamário. Este dado reforça a recomendação de se realizar mastectomia total, mesmo em cadelas que apresentem apenas um nódulo na cadeia. As LNN da glândula adjacente a neoplasias e nas demais mamas sem nódulos estavam mais frequentemente associadas a neoplasias malignas quando comparadas com as neoplasias benignas. As mamas sem nódulos nas cadeias mamárias com mais de um tipo de neoplasia maligna tiveram mais LNN do que aquelas com apenas um tipo de neoplasia. HLA, que é considerada precursora de carcinomas invasivos de mama em cadelas, ocorreu com maior freqüência associada aos carcinomas do que os demais tipos de hiperplasia.

Palavras-chave: Canina; Glândula mamária; Hiperplasia; Displasia; Prevalência.

NON-NEOPLASIC LESIONS OF CANINE MAMMARY GLANDS AND THE RELATIONSHIP WITH ADJACENT CARCINOMAS

SUMMARY – Few studies analyzing non-neoplastic lesions (NNL) in mammary gland of female dogs, as well as the relation between the presence of these alterations and the simultaneous occurrence of mammary neoplasias, either in the mammary tissue adjacent of mammary neoplasm or in the other breasts of the mammary chain with tumor. The objective of this study was to determine the occurrence of non-neoplastic lesions in the mammary tissue adjacent to tumors in breasts with no palpable nodules located in the mammary chains of female dogs with neoplasms and to correlate with histological type and grade of carcinoma. For this, a histological analysis of 314 breast samples with or without a palpable nodule of 68 bitches submitted to mastectomy was performed. The neoplastic and non neoplastic alterations located adjacent to the tumors and also in the breasts without palpable nodules in the mammary chain were identified. Their occurrence was associated with malignant neoplasms and benign by a contingency analysis (Fischer's exact test). The minimum age of bitches in this study (3 years) coincides with the occurrence of the first or second estrous cycle. In breasts with no palpable nodules associated with breast neoplasms there is an occurrence of NNL and neoplastic lesions, demonstrating the risk of developing a new mammary neoplasia in other glands of dogs diagnosed with breast carcinoma. This data reinforces the recommendation to perform total mastectomy, even in female dogs with only one node in the chain. NNL of the gland adjacent to neoplasias and in the other breasts without nodules were more frequently associated with malignant neoplasias when compared with benign neoplasias. Mammary glands without nodules in mammary chains with more than one type of malignant neoplasm had more NNL than those with only one type of neoplasia. ALH, which is considered the precursor of invasive breast carcinomas in bitches, occurred more frequently associated with carcinomas than other types of hyperplasia.

Keywords: Canine; mammary gland; hyperplasia; dysplasia; prevalence.

Sumário

CAPÍTULO 1 – CONSIDERAÇÕES GERAIS

1. INTRODUÇÃO

Lesões não neoplásicas da glândula mamária, tais como hiperplasia ductal, com ou sem atipia são lesões proliferativas não invasivas do parênquima mamário (MOUSER et al., 2010) e possivelmente estão envolvidas no processo de progressão tumoral, principalmente de neoplasias epiteliais (WITHROW; VAIL, 2007).

Modificações no componente epitelial mamário podem estar relacionados com o maior risco de desenvolvimento do câncer de mama. No processo de carcinogênese, a transformação celular é um processo gradativo que se iniciaria com proliferação epitelial típica (hiperplasia típica), com posterior transformação atípica (hiperplasia atípica), para então, ocorrer a transformação em carcinoma não invasivo, que poderia resultar em progressão para invasão tecidual e formação de metástases em órgãos distantes (LAKHANI, 1999).

Esta hipótese tem sido reforçada por vários autores ao verificarem que as alterações hiperplásicas da mama canina representam etapas evolutivas no processo de carcinogênese, por meio da avaliação da expressão de marcadores moleculares como receptores de estrógeno, progesterona, Ki67 e HER2(ANTUOFERMO et al., 2007), expressão de genes envolvidos na proliferação celular (RAO et al., 2009)e proteínas de adesão de células epiteliais (JAKAB et al., 2008).

Similaridades morfológicas e fenotípicas são vizualizadas entre as lesões mamárias encontradas nos humanos e caninos, fazendo deste último um modelo adequado para estudos sobre o câncer em humanos. Além disso, devido a características epidemiológicas, clínicas e antigênicas compartilhadas entre tumores mamários da espécie humana e canina possibilitam que tumores mamários caninos sejam utilizados como modelos comparativos de câncer de mama na mulher (STRANDBERG; GOODMAN, 1974; WARNER, 1976; LINDBLAD-TOH et al., 2005).

Estudos sobre a prevalência de lesões mamárias não neoplásicas em cães são escassos, assim como a relação entre a presença destas alterações e a ocorrência simultânea de neoplasias mamárias (FERREIRA et al., 2010), seja na mama adjacente ou nas demais mamas da cadeia mamária onde ocorreu o tumor.

Recentemente demonstrou-se que há maior expressão de Ki-67 e PCNA na mama não tumoral adjacente a tumores fenotipicamente mais agressivos e esses

fatores estão associados há menor sobrevida. Os autores sugeriram que os fatores de crescimento produzidos pelo tumor podem agir na glândula mamária adjacente não-neoplásica de forma parácrina, contribuindo para um microambiente tumoral altamente proliferativo, o que demonstra que essas lesões devem ser mais bem exploradas (CARVALHO et al., 2016).

2. REVISÃO DE LITERATURA

2.1. Desenvolvimento, anatomia, fisiologia e histologia da glândula mamária.

O parênquima mamário se origina embriologicamente a partir de aparecimento de dois espessamentos ectodérmicos ventrais lineares para o interior de regiões especializadas do mesoderma subjacente. Estes espessamentos são denominados de crista mamária e se desenvolvem em cinco pares de mamas estendendo-se da região axilar até a região inguinal, evidenciando-se por volta do 25º dia de gestação quando o embrião tem 14mm de comprimento (SORENMO et al., 2011; EVANS; DE LAHUNTA, 2013).

As papilas mamárias se originam das células epiteliais do espessamento que, no 30º dia (19 mm), formam um cordão contínuo de células que crescem no mesênquima subjacente, posteriormente se ramificam para formar um broto mamário. No interior de cada broto um lúmen se forma através de um processo de cavitação que se comunica externamente via uma região do epitélio especializado e que se torna o teto na superfície corpórea. Cada broto irá formar o ducto papilarna glândula mamária no adulto (Figura 1) (DYCE; SACK; WENSING, 2004; SORENMO et al., 2011; EVANS; DELAHUNTA, 2013).

No momento do parto a glândula mamária consiste em uma complexa estrutura ductal-lobular-alveolar associada com secreção alveolar. Posteriormente, as glândulas continuam crescendo em proporção com o crescimento corporal e seu desenvolvimento começa na fêmea adulta com a puberdade, com a liberação de estrógenos pelo ovário e se completa durante a gravidez com o aumento dos níveis de progesterona quando o epitélio ductal se prolifera e há uma diferenciação túbulo-alveolar (SILVER, 1966; SORENMO et al., 2011).

Figura 1: Formação do sistema de ductos papilares da glândula mamária. 1 – Broto primário quedaráorigem ao seio lactífero.
Fonte: Modificado de Dyceet al., 2004.

Geralmente os cães desenvolvem cinco pares de mama, porém alguns animais podem apresentar quatro ou seis pares. No sentido crânio-caudal estão dispostas em pares sendo quatro torácicas (M1 e M2 direita e esquerda), quatro abdominais (M3 e M4) e duas inguinais (M5). Cada mama é caracterizada por um corpo mamário (constituído de 8-12 lóbulos, tecido conjuntivo e pele) e uma papila mamária (mamilo) (Figura 2) (SILVER, 1966; EVANS; DELAHUNTA, 2013).

A glândula mamária é uma glândula sudorípara modificada presente no tecido subcutâneo (ALLISSONR; MADDUX, 2009; SORENMO et al., 2011). Caracterizada histologicamente, quando ativa, por uma estrutura túbulo-alveolar composta que secreta os nutrientes do leite de forma apócrina e mesócrina pelos adenômeros túbulo-alveolares, que tem número variável de lobos separados por tecido conjuntivo e adiposo. As unidades secretoras da glândula mamária são constituídas por túbulos e alvéolos secretores formados por células epiteliais cúbicas rodeadas por células mioepiteliais, que contraem sob influência da ocitocina.Uma cápsula fibroelástica de tecido conjuntivo frouxo reveste o corpo da glândula e sua porção secretora é mantida por um estroma de tecido conjuntivo frouxo (SAMUELSON, 2007).

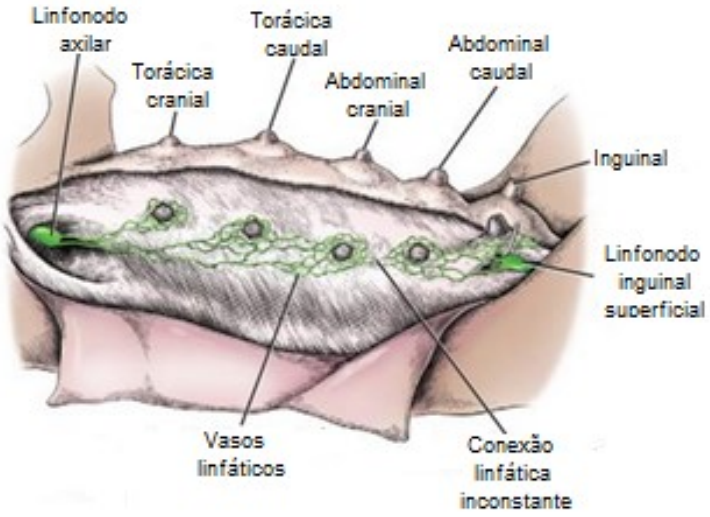

Figura 2: Glândula mamária, topografia e estrutura.
Fonte: Modificado de Evans e DeLahunta, 2013.

Cada lobo é drenado por seu próprio ducto túbulo-lactífero, que antes de se abrir no mamilo, é revestido por epitélio estratificado pavimentoso queratinizado. Cada ducto se dilata formando um seio lactífero, este e o ducto lactífero (maiores) que se dirige a ele são revestidos por epitélio cubóide estratificado, enquanto os ductos menores que sãorevestidos por epitélio colunar simples, ambos são rodeados por células fusiformes mioepiteliais, situadas entre o epitélio e lâmina basal. (GARTNER, G. P.; HIATT, J. L., 2007). As células do alvéolo secretório variam de cubóides a colunares com número variável de gotículas de gordura que se acumulam em seu lúmen, já os alvéolos não secretórios têm células similares com as dos ductos menores. Rodeando os alvéolos encontram-se células mioepiteliais em formato estrelado e em formato de raquete de tênis (Figura 3) (LARSON, 1985; SORENMO et al., 2011).

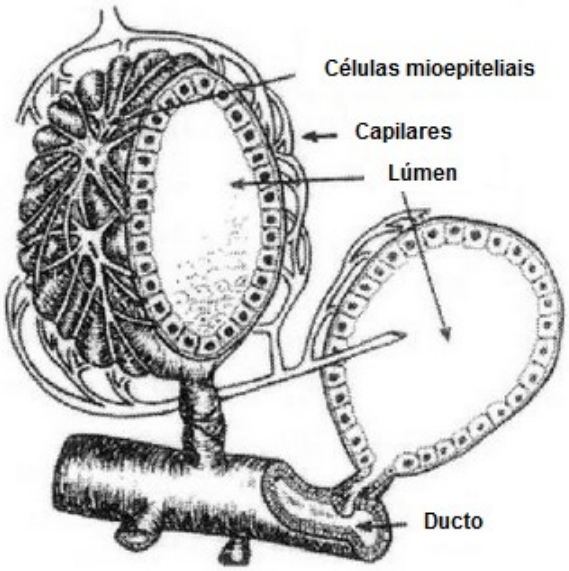

Figura 3: Estrutura do alvéolo e ducto associado.
Fonte: Modificada deLarson, 1985.

O tecido mesenquimal, quesustenta o componente epitelial da glândula mamária,se desenvolveu do mesoderma embriológico e é composto por tecido conjuntivo fibroso, que pode ser dividido em dois componentes: componente intralobular envolvendo os ductos intralobulares, composto por finas fibras colágenas envolvidas por ampla matriz extracelular, enquanto que o componente interlobularsepara os lóbulos e é composto por fibras colágenas mais densas e menos matriz extracelular. Além de tecido conjuntivo fibroso, o mesênquima contêm tecido adiposo, vasos sanguíneos, nervos, e vasos linfáticos, também pode-se encontrar histiócitos, mastócitos e linfócitos no estroma(SORENMO et al., 2011).

A inervação da glândula mamária canina está associada principalmente com a vascularização e consiste de nervos peptidérgicos, que podem estar envolvidos na regulação do fluxo sanguíneo local. A presença de neuropeptídeos sensoriais nos nervos que abastecem o mamilo sugere que estes peptídeos podem também desempenhar um papel na via aferente do reflexo de ejeção de leite(PINHO; GULBENKIAN, 2007).

A glândula mamária dos cães é um órgão hormônio-dependente que tem atividade cíclica associada a fases de desenvolvimento e regressão consecutivas que, altera a histologia mamária gerando características diferentes entre as glândulas ao longo da vida reprodutiva (Figura 4) (REHM; STANISLAUS; WILLIAMS, 2007;

SANTOS; MARCOS; FAUSTINO, 2010). A descrição e o conhecimento da histologia da glândula mamária durante o ciclo estral são importantes para avaliar alterações patológicas que ocorrem no órgão (SORENMO et al., 2011; PEÑA et al., 2014).

No proestro de cadelas adultas é observado tecido glandular atrófico (glândula inativa) composto por ductos interlobulares com pouca e pequenas estruturas lobulares de lúmen vazio e dupla camada de epitélio cúbico rodeado com mioepitélio alongado e contínuo, alguns botões finais e quantidade extensiva de tecido conjuntivo interlobular e intralobular. Nos lóbulos menores, proveniente do ciclo estral anterior, pode haver ocasionalmente estruturas alveolares revestidas por células epiteliais achatadas ou cúbicas vacuolizadas com uma camada descontínua de mioepitélio. No lúmen desses alvéolos e solto no tecido conjuntivo interlobular existem macrófagos com lipofuscina (REHM; STANISLAUS; WILLIAMS, 2007; SANTOS; MARCOS; FAUSTINO, 2010).

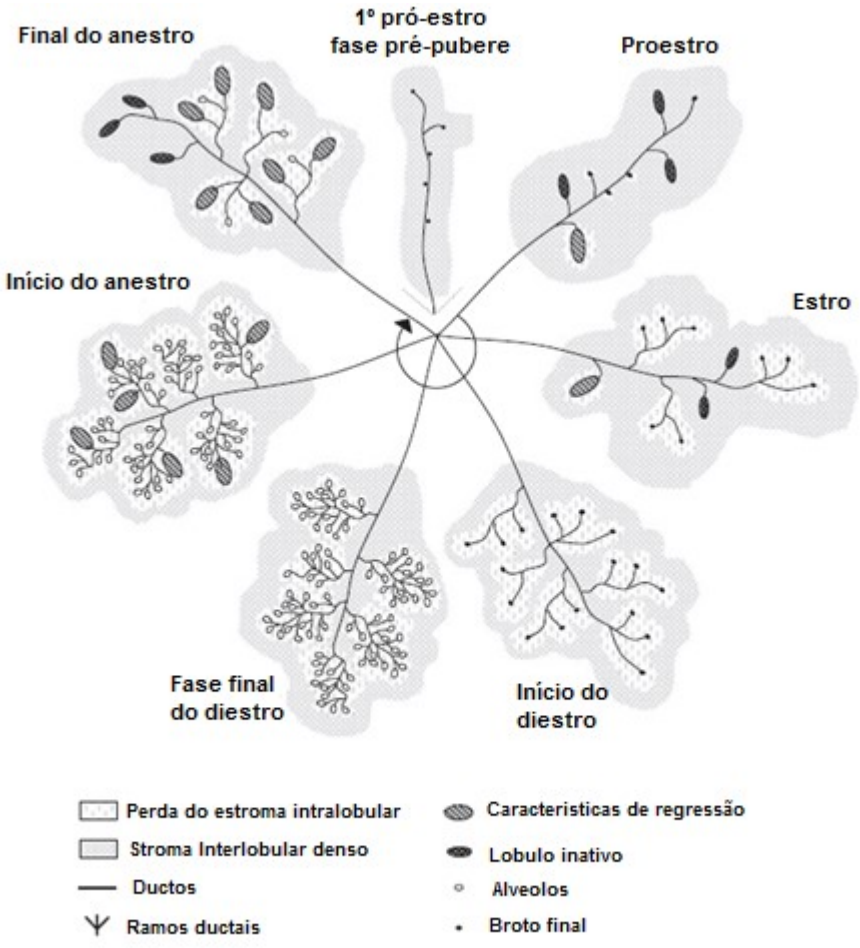

Figura 4: Representação das características histológicas da glândula mamária durante o ciclo estral, incluindo a fase pré-púbere e o 1º proestro.

Fonte: Santos et al., 2010.

No início do estro o epitélio dos ductos interlobulares se tronam proliferados com formação de múltiplos ductos menores revestidos por uma ou mais camadas epiteliais similares às células dos botões finais. Com o aumento dos níveis de progesterona na fase inicial do dietro há maior desenvolvimento dos ductos, com a formação de lóbulos. O epitélio que reveste esses ductos apresentam várias camadas, e as células são pouco aderidas com núcleos eucromáticos arredondados à ovalados com 1-2 nucléolos proeminentes e numerosas figuras de mitose. Os fibroblastos dentro do estroma interlobular têm núcleos mais proeminente e pode apresentar aumento da atividade mitótica. Na fase final do diestro há formação de alvéolos secretores na extremidade terminal dos ductos intralobulares, preenchidos com uma secreção protéica e revestidas por células que variam de cubóide para um epitélio mais fino. No início do anestro os alvéolos contêm menos secreção e são revestidos por células epiteliais sustentadas por uma membrana basal mais proeminente. Alguns lóbulos mostram mudanças associadas com a regressão da glândula mamária com o aumento da quantidade de tecido conjuntivo intralobular e um infiltrado composto por linfócitos e plasmócitos. No final de anestro o lúmen do ducto é preenchido com material eosinofílico, tem menor diâmetro e lóbulos de menor tamanho. As células com núcleos picnóticos formam ductos e alvéolos. O interstício é mais abundante, as fibras de colágenas são compactas, e pode haver um infiltrado por linfócitos, plasmócitos e macrófagos contendo lipofuscina. O estroma interlobular é mais abundante e compacto (SANTOS; MARCOS; FAUSTINO, 2010).

2.2. Frequência das neoplasias mamárias

As neoplasias estão entre as afecções mais comuns em cães, sendo os tumores mamários espontâneos os mais frequentes em cadelas adultas não castradas. Mais de 50% dos tumores são malignos e representam um grupo de grande heterogeneidade em termos de morfologia e comportamento biológico (NERURKAR et al., 1989; RUTTEMAN; WITHROW; MACEWAN, 2001; SORENMO, 2003; SLEECKX et al., 2011).

Os tumores mamários caninos serem o tipo mais comum de neoplasias em cadelas (SLEECKX et al., 2011), os dados sobre sua incidência na população canina são baseados em dados obtidos de hospitais veterinários onde a população é

representativa somente de casos atendidos em clínicas veterinárias ou a partir de biópsias e necropsias realizadas nesses cães (VASCELLARI et al., 2016).

A taxa de incidência anual foi estimada em 198/100.000, sendo três vezes maior que na espécie humana (MISDORP, 2002). Em um estudo realizado na Califórnia, (SCHNEIDER, 1970) foi reportada a incidência anual de 145/100.000 cadelas afetadas. Estudos na Itália mostraram uma taxa anual de aproximadamente 200/100.000 (DOBSON et al., 2002; MERLO et al., 2008; VASCELLARI et al., 2009, VASCELLARI et al., 2016).

Considerando que a maioria dos cães no Brasil são mestiços, torna-se difícil definir se alguma raça específica é mais afetada(KAMIGUCHI et al., 2016). No entanto, uma maior freqüência tem sido reportada em cães das raças Dachshunds, Cocker Spaniels, Poodles toy, GermanShepherds, Pointer, Fox Terrier, Boxer e Beagleque (RUTTEMAN, 1990; MISDORP, 2002).

As glândulas mamárias abdominais caudais e inguinais são as mais afetadas. Múltiplos tumores são encontrados entre aproximadamente 50% a 70% das cadelas com neoplasias mamárias (MISDORP, 2002; SORENMO, 2003; SORENMO et al., 2009). Nos casos de múltiplos tumores, diferentes tipos podem estar presentes em uma mesma mama (RUTTEMAN; WITHROW; MACEWAN, 2001).

Em média 70% das neoplasias mamárias apresentarem características de malignidade (OLIVEIRA FILHO et al., 2010). Dentre os carcinomas o subtipo mais frequentemente descrito na literatura é o carcinoma em tumor misto (CASSALI, 2000; CAVACANTI & CASSALI, 2006; RIBEIRO et al., 2009; TORÍBIO et al., 2012). As neoplasias malignas tem potencial de metastização e a distribuição dessas metástases é determinada de acordo com a drenagem sanguínea e linfática do local do tumor primário, os locais de eleição para metástases são os linfonodos regionais e principalmente os pulmões (DE NARDI et al., 2013).

Fatores prognósticos

Fatores prognósticos podem ser definidos como uma ou mais características clínicas, patológicas ou biológicas específicas de indivíduos e de seus tumores que permitem a previsão da evolução clínica e sobrevida do paciente sem ser submetido a terapias adjuvantes adicionais após a cirurgia inicial (CAVALCANTI; CASSALI, 2006).

De outro modo, a avaliação de marcadores preditivos permite a seleção de pacientes para tratamentos específicos e individualizados (MARINHO et al., 2008).

Diferentes fatores podem influenciar o desenvolvimento de tumores mamários em cães. Exposição à fatores de crescimento e hormônios, principalmente estrógeno e progesterona, aumentam o risco de formação desses tumores, o que pode ser reduzido significantemente pela condução de ovarioesterectomia precoce (SCHNEIDER; DORN; TAYLOR, 1969; QUEIROGA et al., 2005).

A incidência de neoplasias mamárias em cadelas é extremamente alta em regiões onde a ovarioesterectomia precoce (antes dos dois anos de idade) não é uma prática na rotina clínica (DORN et al., 1968). O componente etiológico hormonal é o principal elemento estudado, sendo que o risco de aparecimento desse tumor nas cadelas castradas antes do primeiro cio é de 0,05%, após o primeiro cio 8% e após o segundo, o risco aumenta para 26% (OLIVEIRA et al., 2003; SORENMO, 2003; PETROV et al., 2014). Os hormônios são responsáveis por grande parte do controle no metabolismo das células. Quando há descontrole da secreção hormonal ocorre a proliferação celular com consequentes mutações genéticas, levando a várias alterações, dentre elas o câncer (MISDORP, 2002; SILVA; SERAKIDES; CASSALI, 2004; CASSALI et al., 2014).

A administração de hormônios pode levar a maior probabilidade de ocorrência de neoplasias mamárias (PETROV et al., 2014). A utilização de progestágenos exógenos como método contraceptivo gera uma estimulação da síntese de hormônio do crescimento na glândula mamária, proliferação lóbulo-alveolar e consequente hiperplasia de elementos mioepiteliais e secretórios, induzindo a formação de nódulos benignos em animais (RUTTEMAN; WITHROW; MACEWAN, 2001).

As cadelas de meia idade são mais afetadas (em média 09 a 11 anos) e um aumento da incidência começa a ocorrer aproximadamente aos 06 anos de idade. O desenvolvimento de tumores mamários malignos antes dos cinco anos de idade é pouco frequente, e quando ocorre, normalmente é menos agressivo. Cadelas mais jovens geralmente apresentam displasia ou hiperplasia (ALENZA et al., 2000).

Além de maior chance de serem malignos, os tumores em animais mais velhos também são de maior tamanho (PETROV et al., 2015; KAMIGUCHI et al., 2016). Com o aumento da preocupação com a sanidade dos animais devido a interação homem-animal, a longevidade da população canina tem aumentado. Assim como a possibilidade de encontrar uma maior freqüência de tumores mamários e maior

incidência de tumores malignos em relação aos benignos (BRODEY; GOLDSCHMIDT; ROSZEL, 1983).

Outros fatores como predisposição genética e a dieta podem estar relacionadas com o aparecimento de tumores mamários. Animais obesos e com dietas ricas em gordura, geralmente apresentam maior risco de desenvolver essas neoplasias (BRANDÃO et al., 2013; LIM et al., 2015). Sobrepeso ou obesidade em cadelas pode estar associado com o aparecimento de tumores mamários em cadelas mais jovens e com maior invasão linfática (LIM et al., 2015).

A determinação do estadiamento clínico permite a definição da extensão do tumor, a partir de indicações precisas relativa ao material enviado para análise para o patologista e para a comparação de observações clínicas de diferentes fontes. Isso permite um prognóstico a ser estabelecido e o tratamento a ser planejado (HERMANEK et al., 1997).

O estadiamento clínico é determinado de acordo com o sistema TNM estabelecido pela Organização Mundial de Saúde (OMS) para tumores mamários caninos. Com base neste sistema, o tamanho da lesão primária (T), a extensão da sua expansão para os nódulos linfáticos regionais (N) e a presença ou ausência de metástases distantes (M) deve ser avaliado (OWEN, 1980) (Tabela 1).

Tabela 1: Estadiamento clínico (TNM) de tumores mamários caninos (modificado de OWEN, 1980).

T – Tamanho do tumor primário

T1 <3cm
T2 3-5cm
T3 >5cm

N – Linfonodo regional
N0 Histológico ou citológico – sem metástase
N1 Histológico ou citológico – metástase presente

M – Metástase à distancia
M0 sem metástase à distância
M1 com metástase à distância

	T	**N**	**M**
Estádiol	T1	N0	M0
Estádio II	T2	N0	M0
Estádio III	T3	N0	M0
Estádio IV	Qualquer T	N1	M0
Estádio V	Qualquer T	Qualquer N	M1

O tamanho do tumor é considerado um fator prognóstico independente para tumores mamários em cadelas, os tumores de tamanho 3,0 centímetros ou menores são significativamente correlacionadas com melhor prognóstico em comparação com tumores maiores. Este parâmetro pode ser facilmente obtido e deve ser considerado na tomada de decisões a respeito de terapia complementar (SORENMO, 2003; CAVALCANTI, 2006).

Durante um estudo sobre tumores mamários, relatou-se que a maioria das lesões maiores que 5,0 cm (T3) foram malignas, com uma taxa de proliferação maior e menor positividade para receptores de progesterona (RP), quando comparado com a tumores menores (T1, T2) (FERREIRA et al., 2009).

Com relação ao tipo histológico, neoplasias mamárias são classificadas de acordo com a origem celular. Frequentemente são de origem epitelial, mas podem também ser de origem mesenquimal ou mista. Neoplasias mistas apresentam proliferação de tecido epitelial e mesenquimal, mais tarde podem sofrer metaplasia cartilaginosa ou óssea (CASSALI et al., 2012).

As características macroscópicas como o tamanho, tempo de crescimento e evolução, ulceração, aderência à pele e tecidos subjacentes, são critérios que podem sugerir malignidade ou benignidade da neoplasia (MISDORP, 2002).

2.3. Lesões não neoplásicas da glândula mamária em cadelas

A glândula mamária de cães pode ser acometida por uma variedade de patologias proliferativas epiteliais. Essas lesões podem ser divididas em não-neoplásicas e neoplásicas (SCHNEIDER, 1970). Lesões epiteliais não neoplásicas são frequentemente diagnosticadas em cadelas (MISDORP et al., 1999).

A Organização Mundial de Saúde (MISDORP et al., 1999)propôs uma classificação para as lesões neoplásicas e não neoplásicas de mamas de cadelas, sendo esta classificação amplamente utilizada. Porém, no decorrer dos anos, vários novos subtipos histológicos de neoplasias mamárias em cães tem sido descritos.

Mais recentemente foram propostos outros consensos que estabeleceram critérios de diagnóstico e descrição morfológica dessas lesões, com o intuito de

padronizar a classificação das lesões mamárias e facilitar a comparação entre estudos sobre patologia e prognóstico de lesões mamárias em cadelas (GOLDSCHMIDT et al., 2011; CASSALI et al., 2014). Porém, infelizmente, ainda não há uma padronização quanto à classificação das neoplasias e hiperplasias/displasias mamárias utilizadas em trabalhos científicos na área veterinária, o que dificulta a comparação entre estudos (LIPSCOMB, 2012; MATOS et al., 2012).A Tabela 2mostra a comparação entre as classificações propostas para lesões mamárias não neoplásicas em cadelas.

Tabela 2: Classificação histológica de lesões mamárias não neoplásicas em cadelas.

Misdorpet al.(1999)	**Goldschmidtet al.(2011)**	**Cassaliet al. (2014)**
1. Hiperplasia Ductal	1. Epiteliose	1. Hiperplasia Epitelial
2. Hiperplasia Lobular	2. Hiperplasia Lobular	*1.1. Hiperplasia ductal*
2.1. Hiperplasia epitelial	*2.1. Regular*	*1.2.Hiperplasia lobular*
2.2. Adenose	*2.2. Com atividade Secretória*	*1.3.Adenose*
3. Cisto	*2.3. Com fibrose*	2. Lesão de células colunares
4. EctasiaDuctal	*2.4. Com atipia*	*2.1. Alteração de células colunares*
5. Fibrose Focal (fibroesclerose)	3. EctasiaDuctal	*2.2. Hiperplasia de células colunares*
6. Ginecomastia	4. Papilomatose	*2.3.Lesão de células colunares com atipia*
	5. Alteração Fibroadenomatosa	
	6. Ginecomastia	

Goldschmidt e colaboradores (2011) classificam as lesões não neoplásicas na cadela em: ectasia ductal, hiperplasia lobular (regular, com atividade secretória, com fibrose e com atipia), epiteliose, papilomatose, alteração fibroadenomatosa e ginecomastia.

Histologicamente, a Ectasia Ductal (ED) apresenta dilatação cística dos ductos maiores, no lúmen dos ductos há acúmulo de debris necróticos e variável número de macrófagos espumosos em meio a material lipídico e cristais de colesterol (GOLDSCHMIDT et al., 2011). O padrão de marcação imunohistoquímica da ED é similar aos padrões encontrados na glândula mamária normal(VOS et al., 1993). Afeta cadelas castradas e não castradas de várias idades e não existe associação com o aumento do risco de acontecimento de neoplasia mamária em cadelas com essa alteração (MILLER et al., 2001).

A hiperplasia lobular (HL) é caracterizada pela proliferação não neoplásica dos ductos intralobulares, com aumento do número de ductos e ácinos por lóbulo. Essas lesões podem ser encontradas no parênquima mamário adjacente a neoplasias benignas ou malignas. Podem exibir células epiteliais sem alterações atípicas, sendo denominada hiperplasia lobular regular (HLR). Quando apresentam células cuboidais com atividade secretora e acúmulo de secreção no lúmen dos ácinos, caracterizam hiperplasia lobular com atividade secretória (HLS). No caso da hiperplasia apresentar grande quantidade de tecido conjuntivo fibroso interlobular é denominada hiperplasia lobular com fibrose (HLF). A hiperplasia será denominada como hiperplasia Lobular com atipia (HLA) quando exibir atipia celular com núcleos hipercromáticos, anisocariose e anisocitose e até mesmo variável número de figuras de mitose (GOLDSCHMIDT et al., 2011). A HLA com um alto grau de atipia pode ser difícil de ser diferenciada do carcinoma *in situ* (MISDORP et al., 1999).

A epiteliose (EP), também chamada de Hiperplasia Intraductal, caracteriza-se pela proliferação regular de células epiteliais para dentro do lúmen dos ductos, com agregados celulares preenchendo o lúmen dos mesmos. As células exibem nucléolo hipercromático com núcleo pequeno. A proliferação epitelial papilar intraductal focal a multifocal, não sustentada por tecido fibrovascular, é denominada de papilomatose intraductal (PI) (GOLDSCHMIDT et al., 2011).

Hiperplasia ductal ou lobular pode progredir para displasia e depois para uma neoplasia e a partir de um adenoma benigno se transformar em carcinoma não invasivo e, no estágio final, em formas invasivas(FOSTER, 2013). Estudos demonstram que cadelas que apresentam lesões histológicas pré-cancerosas (hiperplasia atípica) têm maior risco de desenvolver neoplasias mamárias (ANTUOFERMO et al., 2007; MOUSER et al., 2010).

Sorenmo e colaboradores (2009) demonstraram que tumores malignos podem se desenvolver a partir de um tumor benigno pré-existente. Sugerindo que lesões hiperplásicas, displásicas e benignas participam de etapas do processo de cancerização, sendo precursoras do câncer de mama (ANTUOFERMO et al., 2007; MOUSER et al., 2010; SORENMO et al., 2009). Isso pode ser explicado pelo fato de todo parênquima mamário estar exposto a mesma quantidade de hormônios, justificando o desenvolvimento de múltiplos tumores e a presença de vários estágios de transformação do epitélio mamário ao mesmo tempo (SORENMO et al., 2009).

As hiperplasias mamárias caninas apresentam alterações de expressão gênica envolvidas em proliferação e adesão celular, que estão relacionadas a homeostase celular, e quando desreguladas podem causar o câncer. Alterações no padrão de expressão de fatores de transcrição, ou nos genes envolvidos em manter a integridade do DNA e motilidade celular também podem indicar estágios iniciais de transformação neoplásica (RAO et al., 2009).

Em mulheres, maior risco de aparecimento de neoplasias malignas tem sido associado com biópsia prévia de lesões não neoplásicas. Sugerindo que a maioria dos tumores mamários em humanos também se origina de lesões benignas, consideradas com potencial de pré-malignidade (ARPINO; LAUCIRICA; ELLEDGE, 2005; HARTMANN et al., 2005).

O atual modelo de progressão do câncer de mama humano propõe um processo de passo múltiplo linear que inicia como atipia epitelial, progride para hiperplasia ductal atípica e evolui para carcinoma ductal *in situ*, progredindo para fase potencialmente letal do carcinoma ductal invasivo. Isso sugere que na carcinogênese da glândula mamária, atipia é um elo importante entre a lesão benigna e maligna (ARPINO; LAUCIRICA; ELLEDGE, 2005; BOMBONATI; SGROI, 2011).

Alterações como adenose, papiloma intraductal, papiloma com esclerose, hiperplasia ductal, hiperplasiaductal atípica e carcinoma*in situ* foram identificadas em associação com tumores mamários em cadelas. Lesões mamárias intraepiteliais sem atipia foram associadas a tumores benignos, enquanto as lesões com atipia (hiperplasia ductal atípica) e carcinoma ductal *in situ* geralmente foram associadas com neoplasias mamárias malignas (ANTUOFERMO et al., 2007). Além disso, o perfil molecular dessas lesões sugere que hiperplasia mamária atípica e o tecido não neoplásico adjacente aos tumores mamários podem ter importante papel no processo

de transformação neoplásica maligna e representar uma alteração pré-cancerosa (ANTUOFERMO et al., 2007; FERREIRA et al., 2012).

Mouser e colaboradores (2010) realizaram análise histológica e imunohistoquímica de lesões intraepiteliais, em cadelas sem evidencias clínicas(sem nódulos mamários palpáveis) ou histórico de doença mamária. Nesse estudo, 52%das cadelas apresentavam pelo menos um tipo de lesão intraepitelial, incluindo hiperplasia ductal e hiperplasia ductal atípica ou carcinoma ductal *in situ*, sendo que 21 cães (19,4%) tinham mais de um tipo de lesão intraepitelial. Porém, até onde se pode saber, não há estudos que tenham avaliado prevalência das lesões intraepiteliais, em mamas sem nódulos palpáveis, de cadelas acometidas por neoplasias mamárias.

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CAPÍTULO 2: Lesões Mamárias Não Neoplásicas Em Cadelas Portadoras De Neoplasia Mamária e Correlação Com Tipo e Grau Histológico

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(instruções aos autores no Anexo 1)

Correlation of non-neoplastic mammary lesions with type and histological grade of dogs with mammary neoplasm

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Summary

Hyperplasias and dysplasia of the canine mammary gland are evolutionary stages in the carcinogenesis, few studies describe these lesions, as well as their epidemiological characteristics and association with mammary neoplasms. The aim of this study was to determine the occurrence of non-neoplastic mammary lesions adjacent to mammary tumors and no palpable nodules located in the mammary chains of female dogs with neoplasias, as well as to verify the influence of the number of malignant neoplasms in the mammary chain, of histological grade and type of carcinoma in the frequency of these non neoplastic lesions. 314 samples of mammary tissue from 68 female dogs that were submitted to mastectomy due to mammary tumors. All mammary glands tissue, with or without a nodule, were submitted to histopathological analysis to evaluate the neoplastic and non neoplastic alterations located adjacent to the tumors and also in the mammarys without palpable nodules in the mammary chain. Fischer's exact test was applied to verify the association between the occurrence of non neoplastic changes and the histological grade and type of the neoplasms. The average age of the dogs was 9.8 years, with a minimum of 3 years. Of the mammary samples, 138 had palpable nodules and 176 had no palpable nodules. Histopathology 18 (13%) samples from mammarys with nodules presented non neoplastic lesions, and 120 (87%) presented neoplastic lesions. In the mammarys that didn't have palpable nodules, 145 (82.40%) samples presented non-neoplastic lesions, 25 (14.2%) samples had neoplasms, and in only six (3.4%) no alterations were observed in the mammary tissue. In the evaluation of non neoplastic lesions in the mammary tissue adjacent to the tumors, 253 lesions were observed, being DE (n= 126), LH (n = 37), ALH (n = 22), SLH (n = 18), LHF (n=11), EP (n = 20) and IP (n = 19). ALH occurred with a higher frequency of mammary tissue adjacent to carcinomas. However, there was no correlation of histological type and degree with the occurrence of non-neoplastic lesions. The 160 samples with hyperplastic and dysplastic lesions located in the mammarys without tumor of the same mammary chain were: ED (n = 131), LH (41), ALH (n = 13), LHS (n = 32), LHF (n=12), EP (n = 38) and PI (n = 37). In these mammarys IP and SLH were more frequent when the tumor was malignant and FLH and EP were more frequent when the tumor was benign and histological grade did not influence in the frequency of NNL. NNL were more frequent in the other mammarys when the bitch had more than one tumor in the mammary chain in the other mammarys in the mammary chain. In conclusion, the type and histological grade did not influence the frequency of non neoplastic lesions, but the histopathological analysis of mammary glands without nodules should not be neglected, since the non neoplastic changes of the gland adjacent to neoplasms and in the other mammarys without nodules in the mammary chain whith tumor were more frequently associated with malignant neoplasias when compared with the benign neoplasms.

Keywords: Hyperplasias, dysplasias, prevalence, female dog, mammary neoplasia.

Introduction

Mammary tumor progression model in women proposes that modifications in the mammary epithelial component lead to gradual cell transformation, starting with typical epithelial proliferation (typical hyperplasia), subsequent atypical transformation (atypical hyperplasia), modifying in non-invasive carcinoma, which could result in progression to tissue invasion and formation of distant organ metastases (Wellings and Jensen 1973; Lakhani 1999).

This model has been suggested for dogs by several authors who verified that hyperplastic changes in the canine mammary represent evolutionary stages in the carcinogenesis process, evaluating the expression of molecular markers such as estrogen, progesterone, Ki67 and HER2 receptors (Antuofermo et al., 2007) gene expression involved in cell proliferation (Rao et al., 2009, Carvalho et al., 2016) and epithelial cell adhesion proteins (Jakab et al., 2008).

Few studies describe non-neoplastic lesions (NNL), such as dysplasias and hyperplasias in dogs without neoplastic disease (Nelson et al. 1973; Mouser et al. 2010) or associated with mammary neoplasms (Antuofermo et al., 2007, Ferreira et al., 2010, Carvalho et al., 2010).

To the best of our knowledge, there have been no studies evaluating the prevalence of these lesions in mammarys without palpable nodules of female dogs affected by mammary neoplasms. In order to generate information that may contribute to the clarification of the role of these lesions in tumor progression, this study aimed to: 1) verify the occurrence of NNL in the mammary tissue adjacent to mammary tumors, as well as in mammarys with no palpable nodules located in mammary chains of dogs with neoplasias; 2) to verify if the type and histological grade of carcinoma influences the frequency of NNL in the adjacent mammary tissue and in other mammals of the mammary chain, and 3) to verify if the number of carcinomas in the mammary chain influences the frequency of NNL in the other mammarys.

Materials and methods

Animals and samples

Total of 314 samples of mammary tissue from dogs of the Animal Pathology Laboratory of the Federal University of Uberlândia (UFU) were studied. Samples came from 68 dogs of different races and ages, with mammary lesions, which were voluntarily referred for clinical and surgical care at the Veterinary Hospital of UFU.

After the clinical evaluation and according to the veterinary medical recommendation, the dogs were submitted to surgical treatment using partial mastectomy techniques, unilateral total mastectomy or bilateral total mastectomy, with removal of regional lymph nodes. As for the age group, dogs were grouped as: young (up to one year), adults (one to eight years) and elderly (more than eight years).

Immediately after surgical excision, the excised mammary chains were sent to the Pathology Laboratory and palpated to identify palpable nodules, which were measured using a Zaas® caliper. Samples were then collected from all excised mammarys (affected or not by nodules or palpable masses), containing skin and subcutaneous tissue. Each sample was identified with anatomical denomination of each mammary (thoracic mammals - M1 and M2, abdominal - M3 and M4 and inguinal - M5) and stored separately in a 10% buffered formalin solution.

Histopathological analysis

Samples were processed and histological sections of 4μm were stained with the hematoxylin and eosin (HE) technique. Diagnosis of mammary lesions was performed in a double-blind study by two pathologists according to Goldschmidt et al. (2011).

Neoplastic lesions, as well as non-neoplastic lesions (NNL), were evaluated. These were grouped, according to location, into: 1) lesions in the mammary adjacent tissue to the tumors; 2) lesions in the other mammarys without tumor of the same mammary chain. Neoplastic lesions were grouped into benign and malignant, and these were grouped into complex and simple carcinomas. Histological histology of mammary carcinomas was attributed according to Elston & Ellis (1991).

Statistical analysis

Statistical evaluation was performed using GraphPadInstat® software. Contingency analysis was performed by Fischer's exact test to verify the association between the occurrence of NNL adjacent to the tumors and in the mammarys without palpable nodules located in the mammary chain of dogs with neoplasms with the type and histological grade of the neoplasias.

Results

We obtained information on the age of 64 dogs, and none of the dogs were young. The mean age was 9.8 years, ranging from 3 to 16 years. The frequency of NNL was similar in adult and elderly dogs (Table 1).

Intraepithelial neoplasms (hyperplasias and dysplasias) were identified in the mammary adjacent tissue to the tumors and in the mammary tissue without tumor of the same mammary chain, including ductal ectasia (ED), lobular hyperplasia regular (LH), lobular hyperplasia with atypia (ALH), regular hyperplasia with secretory activity (LHS), lobular hyperplasia with fibrosis (LHF), epitheliosis (EP) and intraductal papillomatosis (IP) (Fig. 1) (Table 1).

Ductal ectasia was the most frequent alteration, followed by LH, PI and ALH, both in the mammary adjacent tissue of tumors and in the mammary tissue without tumor of the same mammary chain.

Of the mammarys evaluated (n = 314), 138 had palpable nodules and 176 had no palpable nodules. At the histopathological examination, 18 (13.04%) samples from mammarys with nodules had NNL, and 120 (87%) had neoplastic lesions. In the mammarys that did not have palpable nodules, 145 (82.40%) samples presented NNL, 25 (14.2%) samples presented neoplasms and in only six (3.4%) no alterations were observed in the mammary tissue. Considering 308 samples with neoplastic or non-neoplastic lesions, neoplastic proliferations were identified in 145 samples and NNL were found in 163 samples.

Of neoplastic lesions, 44 were benign and 104 were malignant. Among the benign tumors the simple adenoma was the most frequent and among malignant the complex carcinoma. The benign neoplasms identified were: simple adenoma (n = 18), benign mixed tumor (n = 13), complex adenoma (n = 9), intraductal papillary adenoma (n = 3) and myoepithelioma (n = 1). 60 complex carcinomas were diagnosed: complex carcinoma (n = 34), carcinoma in mixed tumors (n = 25) and carcinosarcoma (n = 1); (n = 9), carcinoma in situ (n = 8), solid carcinoma (n = 8), papillary carcinoma (n = 3), papillary carcinoma adenovasal carcinoma (n = 3), intraductal papillary carcinoma (n = 2), comedocarcinoma (n = 1) and micropapillary carcinoma (n = 1).

Of the 18 (13.04%) samples from mammarys with nodules that presented non-neoplastic lesions, considering that some mammarys presented more than one lesion, were evidenced ED (n = 16), EP (n = 7), PI 5), LH (n = 5), ALH (n = 1), LHF (n = 1), LHS (n = 1).

In the case of mammarys without palpable nodules that presented neoplastic proliferations (n = 25), histological types identified were: carcinoma in situ (n = 5), tubular

carcinoma (n = 2), solid carcinoma (n = 1), simple adenoma (n = 9), complex adenoma (n = 2), benign mixed tumor (n = 2), complex carcinoma (n = 3), and mixed tumor carcinoma (n = 1).

Histological grade was assigned to 96 carcinoma samples, with the exception of carcinoma in situ, of which 60 were complex carcinomas and 36 were simple carcinomas. Most of the complex carcinomas were found to be Grade I (66.7%), 30% grade II and 3.3% grade III; while simple carcinomas showed grade II (43.2%) more frequently, followed by grade I (29.6%) and grade III (9.3%).

In the evaluation of the non-neoplastic lesions in the mammary adjacent tissue of tumors, 253 lesions were observed, considering that in a simple sample with mammary neoplasia, more than one type of adjacent non-neoplastic lesion was identified. The most frequent non-neoplastic lesion was ED (n = 126), followed by LH (n = 37), ALH (n = 22), LHS (n = 18), LHF and PI (n = 19) (Table 2). However, there was no association between type (p = 0.6503) and histological grade with frequency (p = 0.4826) of NNL.

Of the 68 dogs evaluated, 16 had mammarys without palpable nodules associated with only one type of benign neoplasia, 17 had mammarys without palpable nodules associated with only one type of malignant neoplasia, and 35 had mammarys without palpable nodules associated with more than one type of malignant neoplasia.

Considering all mammarys without palpable nodules, regardless of being associated with only one type of benign neoplasia (n = 16), with only one type of malignant neoplasm (n = 17) or with more than one type of neoplasm = 35), we identified 160 samples with NNL. Considering that more than one type of alteration was observed in a single sample, ED (n = 131) was the most frequent lesion, followed by LH (41), ALH (n = 13), LHS (n = 32), LHF (n = 12), EP (n = 38) and PI (n = 37).

In the evaluation of the relationship between the type and histological grade of the tumor and the NNL in the other mammarys in the mammary chain associated to only one type of neoplasia, it was verified that the IP and LHS were more frequent in the other mammarys in the mammary chain when the tumor was malignant and LHF and EP were more frequent when the tumor was benign. However, the histological grade did not influence the frequency of NNLs in the other mammarys in the mammary chain. Table 3 summarizes the occurrence of NNL of mammarys without nodules located in mammary chains with only one type of benign or malignant neoplasia.

We also evaluated the frequency of NNLs located in mammary mammarys that had only one type of malignant neoplasia in relation to the frequency of NNL in the mammarys located in the mammary chain with more than one neoplasia (Table 4). It was found that in all cases there was at least one type of malignant neoplasm associated and it could be associated with other malignant neoplasm (s) or benign neoplasm (s). It was observed that the NNL were more frequent in the other mammarys when the bitch had more than one tumor in the mammary chain.

Discussion

The minimum age of dogs affected by neoplasms and NNL in this study was three years, and the frequency of NNL was similar between adult and old dogs. Warner (1976) evaluated the frequency of dysplasias in dogs from six months to four years of age, and found this change only in dogs older than two years of age, more frequently in dogs three to four years of age, suggesting that two-year-old is probably the age of onset of development of these lesions coinciding with the full growth of the mammary gland of dogs soon after the first or second estrous cycle. In the present study, LH, PI and ALH were frequent, both in the mammary adjacent tissueto the tumors and in the mammary tissue without tumor of the same mammary chain.NNL were described in dogs with no history of mammary neoplasia, and 51.9% of the dogs presented at least one NNL (ductal hyperplasia, with or without atypia, or carcinoma in

situ) and 19.4% had two or more NNLs simultaneously and about 22.2% of the dogs had atypical hyperplasia (Mouser et al., 2010).

Although ED is the most frequent alteration in the present study, references regarding this NNL are rare. ED affects castrated and uncastrated dogs of various ages, and there is no association with increased risk of mammary cancer in dogs with this alteration, but ED can occur concomitantly with neoplasms (Miller et al., 2001).

In the evaluation of mammarys with palpable nodules (less than 0.5 cm), 18 (13.04%) were diagnosed as hyperplasias and dysplasias, among them ectasiaductal, epitheliosis, papillomatosis, regular atypical lobular hyperplasia with secretory activity and with fibrosis. Previous studies have reported the occurrence of non-neoplastic mammary nodules, but with a much higher frequency (56%) (Cameron and Faulkin 1971). Premalignant lesions such as adenoma with atypia, lobular hyperplasia, ectasiaductal carcinoma, in situ carcinoma, epitheliosis, and secretory hyperplasia have been identified in previously palpable tumors and are considered precursors of malignant mammary tumors, due to the fact that malignant tumors are often larger than the benign ones (Sorenmo et al., 2009).

In the present study neoplastic alterations, both malignant and benign, were evidenced in mammarys without palpable nodules. Mouser et al. (2010) identified neoplasms in dogs without evidence of nodules, but only benign neoplasms. These authors reported that adult dogs develop spontaneous intraepithelial lesions even in the absence of tumors. In the present study, in which dogs had mammary neoplasms, 82.40% of mammarys without palpable nodules presented non-neoplastic lesions, and in only six (3.4%) no alterations were observed in the mammary tissue. The occurrence of hyperplasia / dysplasias in mammary-feeding palpable nodules associated with the presence of neoplasms in the mammary chain (14.25%), as observed in this study, reinforces the hypothesis of other authors (Antuofermo et al. et al., 2010; Foster 2013) that carcinomas may develop from intraepithelial lesions.

Ductal or lobular hyperplasia may progress to dysplasia and then to a neoplasm and from a benign adenoma to non-invasive carcinoma and, in the final stage, to invasive forms (Foster 2013). Studies have shown that dogs with precancerous histological lesions (hyperplasia, atypical hyperplasia and carcinoma in situ) are at higher risk of developing mammary cancers (Antuofermo et al., 2007; Mouser et al. 2010).

Non-neoplastic mammary lesions are detected adjacent to mammary neoplasms (Antuofermo et al., 2007, Ferreira et al., 2012, Carvalho et al., 2016). However, in the present study, both histological type and grade did not influence the frequency of non-neoplastic lesions. Intraepithelial lesions without atypia were associated with benign tumors, whereas lesions with atypia (atypical ductal hyperplasia and ductal in situ carcinoma) were generally associated with malignant mammary neoplasms (Antuofermo et al., 2007). The ALH occurred with a higher frequency of the mammary adjacent tissue to the carcinomas. This data corroborates reports that this type of lesion is a precursor of invasive mammary carcinomas in dogs (Ferreira et al., 2012) and this hypothesis has also been supported by studies in humans (Rao et al. 2002, 2009; Arpino et al. 2005; Hartmann et al. 2005; Bombonati e Sgroi 2011).

In the case of the other mamma without nodules located in the mammary chain with tumor, it was verified that the NNL, PI and LHS, were more frequent in the other mamma in the mammary chain when the tumor was malignant and the LHF and EP were more frequent when the tumor was benign.

Growth factors produced by the tumor may contribute to the formation of a highly proliferative tumor microenvironment, thus influencing the adjacent non-neoplastic mammary gland in a paracrine manner (Carvalho et al., 2016). It is not yet known if these growth factors could also act on other mamma in the mammary chain.

Sorenmo et al. (2009) have demonstrated that malignant tumors can develop from a pre-existing benign tumor. We suggest that hyperplastic, benign and dysplastic lesions participate in stages of

the cancerization process, being precursors of mammary cancer (Antuofermo et al., 2007, Sorenmo et al., 2009).

Pre-cancerous / dysplastic / hyperplastic changes in mamma without palpable masses, located in the mammary chain with at least one neoplasia, were not previously reported. There was a high frequency (82.4%) of these alterations, thus, due to the possibility of intraepithelial lesions evolving to malignant neoplasias (Antuofermo et al., 2007, Mouser et al., 2010), this study reinforces the recommendation of complete mastectomy, even in dogs that present only one node in the chain, due to the chance of recurrence (Stratmann et al., 2008).

Regarding the chance of recurrence, 58% of dogs that were treated with regional mastectomy of a simple mammary tumor developed a new tumor in the remaining mammary of the same mammary chain, demonstrating that there is a greater risk of developing a new mammary neoplasm in other dogs glands previously diagnosed with mammary carcinoma (Stratmann et al., 2008).

In previous studies the pathological and immunophenotypic characteristics of intraepithelial and hyperplastic lesions adjacent to mammary tumors were compared with similar lesions in humans, suggesting that the dog may be a suitable model for the study of the progression and biological behavior of these lesions in women (Antuofermo et al. 2007; Ferreira et al., 2010). Mammary-palpable mammarys with mammary-chain NNL of dogs undergoing amastectomy is an easily accessible material that could be used in molecular studies on neoplastic progression in dogs and as a model for women.

Antuofermo et al. (2007) demonstrated a high prevalence of intraepithelial lesions adjacent to malignant mammary tumors when compared to benign ones, and these intraepithelial lesions are considered to be at high risk for progression to an invasive carcinoma. In the present study, in the evaluation of the relationship between the number of tumors in the mammary chain and the frequency of NNLs in the other mammarys in the mammary chain, it was observed that the NNL were more frequent when dog had more than one tumor in the mammary chain. However, further studies are needed to determine the role of NNLs in tumor progression and the process of canine cancerization

Conclusions

Non-neoplastic alterations of the mammary gland occur frequently, both in adjacent tissue to mammary neoplasms, and in mammarys without palpable nodules present in the neoplasia chain. The type and histological grade do not influence the frequency of NNL. When the bitch has more than one mammary carcinoma, the frequency of NNLs in the other mammarys without palpable nodes present in the neoplasia chain is higher. Thus, the histopathological analysis of all mammarys of dogs with mammary tumors submitted to mastectomy should be performed for investigation of non-neoplastic and neoplastic lesions.

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Table 1. Number of dogs with non-neoplastic lesions according to age and location (adjacent to the tumor and in the other breasts of the mammary chain).

Tipo NNL	Age		Localization	
	Adult (3 - 8 years) (n=28)	Elderly (> 8 years) (n=36)	Adjacente to tumor (n=68)	without palpable nodules in the chain (n=68)
DE	28 (100%)	31 (86,1%)	64 (94,1%)	62 (91,2%)
LHR	12 (42,9%)	15 (41,7%)	25 (36,8%)	28 (36,8%)
ALH	4 (14,3%)	7 (19,5%)	18 (26,4%)	18 (26,5%)
LHS	7(12%)	3 (8,3%)	11 (16,2%)	12 (17,6%)
LHF	4 (14,3%)	3 (8,3%)	8 (11,8%)	5 (7,4%)
EP	10 (35,7%)	13 (36,1%)	17 (25%)	23 (33,8%)
IP	14 (50%)	11 (30,6%)	20 (29,4%)	26 (38,2%)

NNL: Non-neoplastic lesion; DE: ductal ectasia; LH: regular lobular hyperplasia; ALH: lobular hyperplasia with atypia; LHS: regular hyperplasia with secretory activity; LHF: lobular hyperplasia with fibrosis; EP: epitheliosis; IP: intraductal papillomatosis.

Table 2. Frequency of non-neoplastic lesion in the mammary adjacent tissue to the tumors, according to histological type and grade**.**

Histological type	Type of non-neoplastic lesion						
	DE (n=126)	LH (n=37)	ALH (n=22)	LHS (n=18)	LHF (n=11)	EP (n=20)	IP (n=19)
Benign Neoplasms	38,1% (48)	24,3% (9)	18,2% (4)	27,8% (5)	18,2%(2)	20% (4)	21% (4)
Simple adenoma	15	3	2	-	2	1	2
Benign mixed tumor	10	5	1	-	-	2	-
Adenoma Complex	20	1	-	3	-	1	2
Myoepithelioma	1	-	1	-	-	-	-
Intraductal Papillary Adenoma	2	-	-	2	-	-	-
Malignant Neoplasms	61,9% (78)	75,7% (28)	82% (18)	72,2% (13)	81,8% (9)	80% (16)	79% (15)
Tubulo-papillary carcinoma	7	3	3	2	-	2	1
Tubular carcinoma	7	2	2	-	1	2	2
Carcinoma in situ	6	3	3	3	2	1	-
Solid carcinoma	3	2	-	1	-	1	2
Papillary carcinoma	2	1	-	-	-	-	-
Adenoescamous carcinoma	3	-	-	1	-	-	-

Intraductal Papillary Carcinoma	2	-	-	-	-	-	-
Comedocarcinoma	1	1	-	-	1	-	-
Micropapillary carcinoma	-	-	1	-	-	1	-
Complex carcinoma	24	10	6	6	2	6	5
Carcinoma–mixed type	22	6	3	-	3	3	5
Carcinossarcoma	1	-	-	-	-	-	-
Histological grade							
Grade I	43	14	9	4	6	6	10
Grade II	24	11	6	5	1	8	4
Grade III	2	-	1	-	-	2	1

DE: ductal ectasia; LH: regular lobular hyperplasia; ALH: lobular hyperplasia with atypia; LHS: regular hyperplasia with secretory activity; LHF: lobular hyperplasia with fibrosis; EP: epitheliosis; IP: intraductal papillomatosis.

Table 3. Frequency of non-neoplastic lesion in mamma without palpable nodules in the same mammary chain, according to the type and histological grade of the neoplasia.

Histological type	Type of non-neoplastic lesion						
	ED (n=79)	LH (n=23)	ALH (n=6)	LHS (n=14)	LHF (n=4)	ED (n=16)	IP (n=17)
Benign Neoplasms	51,9% (41)	52,2% (12)	50% (3)	28,6% (4)	75% (3)	69,7% (11)	35,3% (6)
Simple adenoma	13	3	1	-	-	3	-
Benign mixed tumor	18	8	-	-	3	7	3
Adenoma Complex	4	1	1	-	-	1	-
Intraductal Papillary Adenoma	5	-	1	4	-	-	3
Malignant Neoplasms	48,1% (38)	47,8% (11)	50% (3)	71,4% (10)	25% (1)	31,3% (5)	64,7% (11)
Tubulo-papillary carcinoma	5	-	-	1	-	1	1
Tubular carcinoma	-	-	-	-	-	-	-
In situ carcinoma	2	-	-	4	-	-	2
Solid carcinoma	2	2	-	-	-	-	1
Papillary carcinoma	4	1	-	1	-	2	2
Adenoescamous carcinoma	-	-	-	-	-	-	-
Intraductal Papillary Carcinoma	2	-	-	-	-	-	-
Comedocarcinoma	-	-	-	-	-	-	-
Micropapillary carcinoma	1	1	-	-	-	-	-
Complex carcinoma	10	4	-	1	-	1	4
Mixed type carcinoma	12	3	3	3	1	1	1
Histological grade							
Grade I	17	7	6	2	-	4	4
Grade II	16	2	-	3	4	6	6
Grade III	2	3	-	-	-	-	1

DE: ductal ectasia; LH: regular lobular hyperplasia; ALH: lobular hyperplasia with atypia; LHS: regular hyperplasia with secretory activity; LHF: lobular hyperplasia with fibrosis; EP: epitheliosis; IP: intraductal papillomatosis.

Table 4. Frequency of non-neoplastic lesions in breasts without palpable nodules according to the amount of malignant tumor types in the mammary chain.

Number of malignant tumors in the chain	Type of non-neoplastic lesion						
	ED	LH	ALH	LHS	LHF	EP	IP
One type	42,2% (38)	37,9% (11)	30% (3)	35,7% (10)	11,1% (1)	18,5% (5)	35,5% (11)
More than one type	57,8% (52)	62,1% (18)	70% (7)	64,3% (18)	88,9% (8)	81,5% (22)	64,5% (20)

DE: ductal ectasia; LH: regular lobular hyperplasia; ALH: lobular hyperplasia with atypia; LHS: regular hyperplasia with secretory activity; LHF: lobular hyperplasia with fibrosis; EP: epitheliosis; IP: intraductal papillomatosis.

Figures

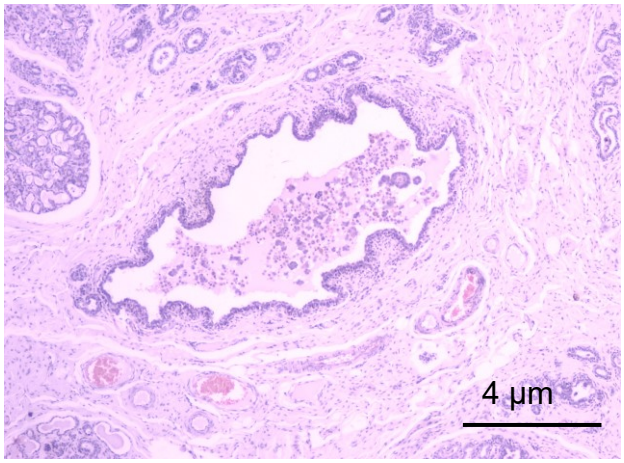

Fig. 1. EctasiaDuctal showing dilation of the ducts with accumulation of necrotic debris and eusinophilic material in the lumen. HE. Bar, 4µm.

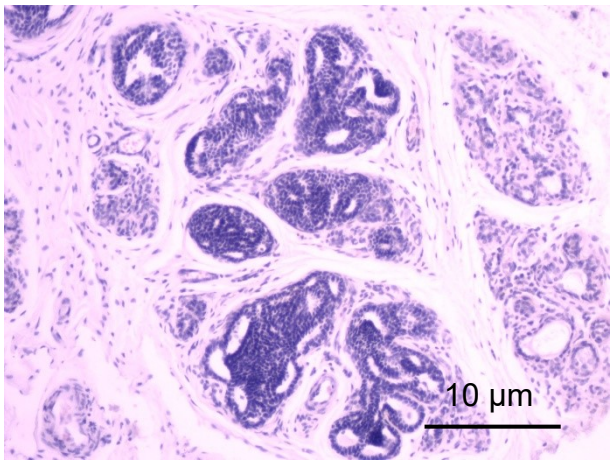

Fig.2. Epitheliosis showing regular proliferation of epithelial cells into the lumen of the ducts, with cell aggregates filling the lumen of the ducts, cells exhibit hyperchromatic nucleoli with small nuclei. . HE. Bar, 10µm.

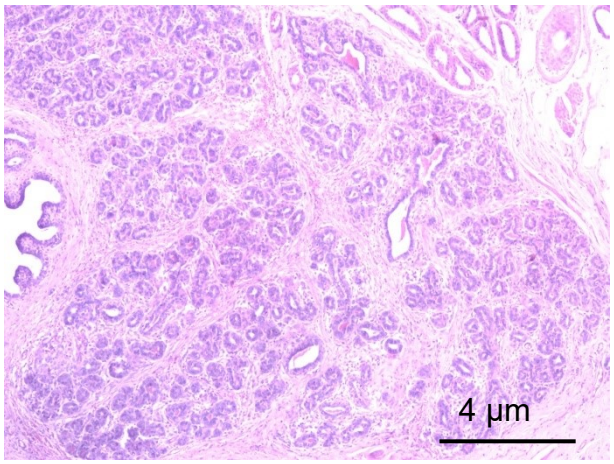

Fig.3. Lobular hyperplasia with fibrosis showing large amount of interlobular fibrous connective tissue. HE. Bar, 4μm.

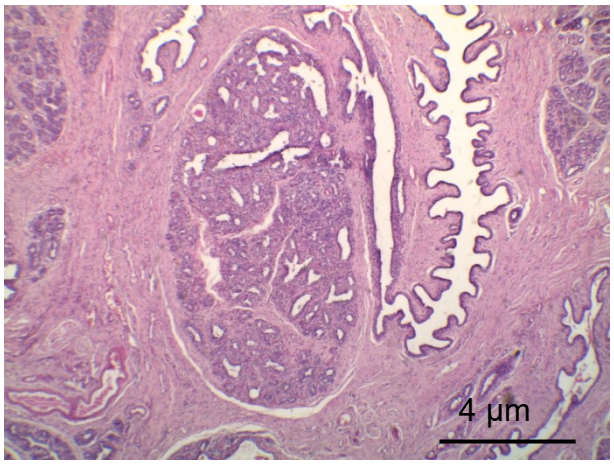

Fig.4. Lobular hyperplasia with atypia showing epithelial cells with cellular atypia, including epithelial hyperplasia, hyperchromatic nuclei, anisocariasis and anisocytosis.HE. Bar, 4μm.

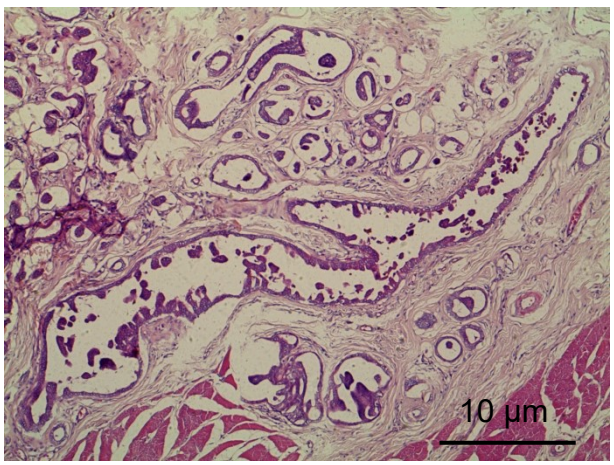

Fig.5. Papillomatosis showing multifocal intraluminal papillary epithelial proliferation.HE. Bar, 10μm.

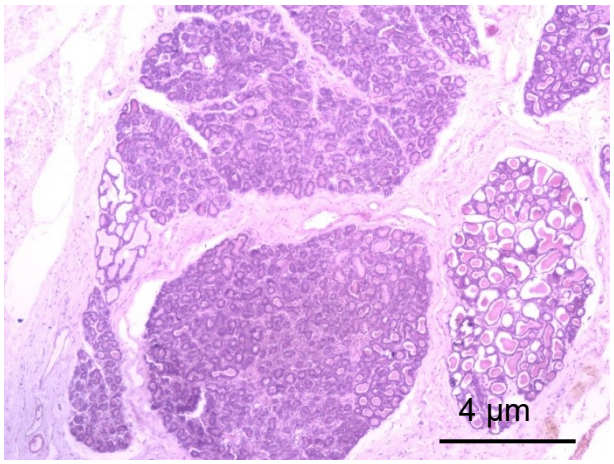

Fig.6. Lobular hyperplasia with secretory activity showing epithelial cells with secretory activity and accumulation of secretion in the lumen of the acini.HE. Bar, 4µm.

Anexo 1 – Instruções aos autores da Revista Journal of Comparative Pathology

JOURNAL OF COMPARATIVE PATHOLOGY

AUTHOR INFORMATION PACK

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DESCRIPTION

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