

# Reproduction in Domestic Animals

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was detected in 65% of benign tumors and 35% of malignant tumors. Central flow was only observed in malignant tumors and mixed flow was detected in 35% of benign tumors and 25% of malignant tumors. The results of this preliminary study show that the presence of both vascular ring sign and central flow were commonly seen in malignant tumors while peripheral flow was more commonly detected in benign tumors. Further cases are needed to use the color flow Doppler ultrasonography patterns to distinguish benign from malignant tumors.

### Abstract P151

#### Morphology and Viability Assessment of Leukocytes in Low Somatic Cell Count Milk of Dairy Cows

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Somatic cell count (SCC) is one of the most common parameters for detection of subclinical mastitis. Udder quarters with SCC <100 000 are generally considered healthy. Differential leukocyte counts of milk cells give insight into the current immune status of an individual udder quarter. Since assays using monoclonal antibodies are time consuming and costly a rapid two stain flow cytometric method was established using acridin orange and propidium iodide for morphological differentiation of viable milk cells. Three groups of 29 healthy heifers (group A: SCC 1000–19 000; group B: 20 000–49 000; group C: 50 000–100 000) were used. In total 355 milk samples were examined bacteriologically, by Fossomatic<sup>®</sup> and by flow cytometry (FACScan<sup>®</sup>). Briefly, three major populations were differentiated morphologically (forward scatter/side scatter): polymorphonuclear granulocytes (PMN), lymphoid cells and MPh/epithelial cells. As a control 210 samples were counterstained with monoclonal antibodies against epitopes on bovine PMN, lymphocyte subsets and MPh/epithelial cells. The percentage of PMN differed between and increased across the three different low somatic cell count groups: group A 38%, group B 47% and group C 62%. Percentage of lymphoid cells decreased ( $p < 0.001$ ) with increasing SCC: group A 56%, group B 48% and group C 32%. The population of macrophages did not differ and was similar across the three groups (5–6%). This study shows that the percentage of PMN and lymphoid cells in milk cells are a highly sensitive parameter of early changes in individual udder quarters. Although, milk with SCC 100.000 is classified as healthy, increasing amounts of inflammatory cells can be seen in low SCC milk (<50 000/ml).

### Abstract P152

#### A Case of Mammary Gland Carcinoma and Malignant Myoepithelioma in a Bitch

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Mammary tumours are the most common form of the tumours in the bitch. They affect mainly middle-aged and elderly female dogs. In this case report, a 10-year-old female

mixed breed dog, spayed before first heat, was admitted to a private veterinary practice with two mammary nodules on the right mammary chain. Complete unilateral mastectomy was performed and the formalin-fixed surgical specimen was submitted to pathology service. Routine hematoxylin and eosin histopathological evaluation was performed. On the 1st abdominal gland, the microscopic exam revealed a well circumscribed, unencapsulated nodule, compatible with lipoma. The 2nd abdominal gland showed a neoplastic spindle cell population, loosely arranged in an abundant myxoid, bluish colour matrix with cartilaginous type matrix areas. These cells presented round pale nuclei, increased contour, prominent nucleoli and nuclear pleomorphism. Multinucleated giant cells and high mitotic rate were also seen. Some sections showed epithelial proliferations arranged in a tubulopapillary pattern. Since the myoepithelial component with malignant characteristics predominated as a distinct feature, the diagnosis of carcinoma and malignant myoepithelioma was established, according to Goldschmidt (*Davis Foundation European Symposium*, University of Munich, Germany, 27–29 Aug 2007), since this diagnosis was not possible according to previous classifications. As the myoepithelial component is benign in almost all tumours, this is considered a rare lesion in the dog.

### Abstract P153

#### Comparison of Milk Yield and Reproductive Indices between Romanian Sheep Breeds (Merinos of Palas and Merinos of Transilvania)

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The aim of this study was to compare the milk performance at the first and second lactations, and reproductive indices at the first and second parturition between indigenous sheep breeds (Merinos of Palas and Merinos of Transilvania). The number of animal studied was 200 for every breed. The sheep of Merinos of Palas breed (MP) were compared with contemporary sheep from Merinos of Transilvania breed (MT) lambed in the same period. Both breeds were kept in the same conditions and with the same feeding. The first lactation records were analyzed according to the following linear model:  $Y_{ij} = \mu + H_i + S_j + e_{ij}$ . The second lactation records were analyzed according to the following linear model:  $Y_{ijk} = \mu + H_i + S_j + J_k + e_{ijk}$ . The difference in milk production between MP and MT breeds, at first and second lactations, were not different. The reproductive indices recorded were: fecundity, fertility, number of lambs to wean and the potential reproductive capacity. All reproductive indices were normal and not different, except for the potential reproductive capacity. The alive at birth, at the first and second parturition, was 123.16% and 127.75% for the Merinos of Transilvania, respectively 134.24% and 139.91% for the Merinos of Palas.

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## INTRODUCTION

The mammary gland is the most commonly affected organ for tumours in the bitch. Mammary tumours mainly affect middle-aged and elderly female dogs. In this case report, a 10 year-old female mix breed dog, spayed before first heat, showed to private veterinary practice with two mammary nodules on the right mammary chain.

The classification of canine mammary neoplasms has been based mainly on standard histopathology and only to a lesser extent on histogenesis (Misdorp *et al.*, 1999). Most canine mammary tumours show epithelial proliferation, which may be benign or malignant, and myoepithelial, usually benign. Myoepithelial cells combine phenotypic features of epithelial cells and contractile ones, which give a typical immunophenotype.

## MATERIALS AND METHODS

- Complete unilateral mastectomy.
- Formalin-fixed, in 10% buffered formalin, surgical specimen was submitted to pathology service.
- Routine histopathological evaluation: all samples were paraffin embedded, sectioned at 3 µm, and stained with hematoxylin and eosin (H&E) for histopathologic evaluation.
- Immunohistochemical staining was performed using the labeled-(strept)avidin-biotin-peroxidase method (LAB-SA) (Histostain®-Plus kit Zymed). Primary antibodies, pre-treatments, dilution and incubation time are showed in Table 1.

Table 1 – Labeled-(strept)avidin-biotin-peroxidase method

LAB-SA METHOD			
ANTIBODIES	PRE-TREATMENT	DILUTION	INCUBATION TIME
Monoclonal Cytokeratins (CK) 5, 6, 8, 17 and 19, Clone MNF116 (DAKO)	Trypsin 20' @ 37°C	1: 50	Overnight @ room temperature
Monoclonal Anti-Vimentin (VIM), Clone V9 (DAKO)	10' @ 98°C in citrate buffer	1: 50	
Monoclonal Muscle Actin (MA), Clone H4F35 (DAKO)	10' @ 98°C in citrate buffer	1: 50	

## RESULTS

### HISTOPATHOLOGY

- On the 1<sup>st</sup> abdominal gland, the microscopic exam revealed a well circumscribed, unencapsulated nodule, compatible with lipoma.
- The 2<sup>nd</sup> abdominal gland showed epithelial proliferations arranged in a tubulopapillary pattern (Fig. 1) with neoplastic areas of spindle cell population, loosely arranged in an abundant myxoid, bluish color matrix (Fig. 2) with cartilaginous type matrix areas. These cells presented round pale nuclei, increased contour, prominent nucleoli and nuclear pleomorphism (Fig. 3). Multinucleated giant cells and high mitotic rate were also seen.

### IMMUNOHISTOCHEMISTRY

- The epithelial component revealed strong cytoplasmic immunoreactivity to CK (Fig. 4).
- The neoplastic spindle cell population revealed weakly reaction to CK, a strong cytoplasmic immunoreactivity to VIM (Fig. 5) and most of the cells revealed strong cytoplasmic immunoreactivity to MA (Fig. 6).

## DISCUSSION AND CONCLUSIONS

- Since the myoepithelial component with malignant characteristics predominated as a distinct feature, the diagnosis of carcinoma and malignant myoepithelioma was established, according to Goldschmidt (2007), since this diagnosis was not possible according to previous classifications.
- As the myoepithelial component is benign in almost all tumours, this is considered a rare lesion in the dog.
- However more studies, using specific myoepithelial markers, are expected to confirm the diagnosis and differentiate from other types of canine mammary tumors, such as carcinosarcoma.

### PHOTOS

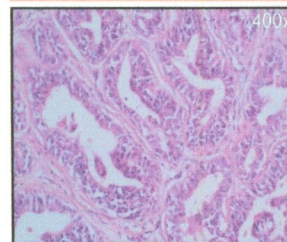


Fig. 1 Proliferation of cells resembling luminal epithelial cells, showing a tubular arrangement. H&E.

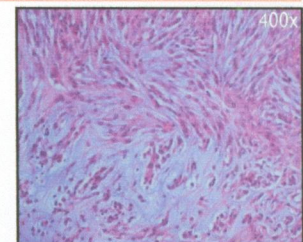


Fig. 2 Areas of spindle cell population, loosely arranged in an abundant myxoid, bluish color matrix. H&E.

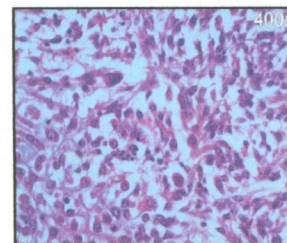


Fig. 3 Spindle cell population, loosely arranged. Pleomorphism is notary. H&E.

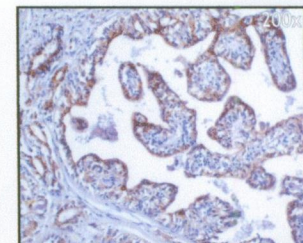


Fig. 4 Epithelial proliferations arranged in a tubulopapillary pattern. Strong cytoplasmic reaction to CK. LAB-SA anti-CK MNF116.

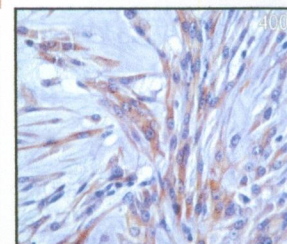


Fig. 5 Spindle cell population. Strong cytoplasmic reaction to VIM. LAB-SA anti-VIM.

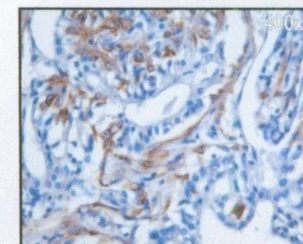


Fig. 6 Spindle cell population. Strong cytoplasmic reaction to MA in most neoplastic cells. LAB-SA anti-MA.

## REFERENCES