International Journal of Bio-resource and Stress Management https://pphouse.org/ijbsm.php

Crossref

IJBSM December 2022, 13(12):1496-1503 Print ISSN 0976-3988 Online ISSN 0976-4038 **Article** AR3291

Review Article *Natural Resource Management*

DOI: HTTPS://DOI.ORG/10.23910/1.2022.3291

Canine Mammary Tumours: Advances in Classification, Grading and Expression of Biological Markers

Aditya Sharma[1] and Sheikh Uzma Farooq[2]

[1]Dept. of Veterinary Pathology, Khalsa College of Veterinary and Animal Sciences, Amritsar (143 001), India
[2]Dept. of Veterinary Pharmacology and Toxicology, Khalsa College of Veterinary and Animal Sciences, Amritsar (143 001), India

Open Access

Corresponding aditya555sharma@gmail.com

0000-0002-1987-5849

ABSTRACT

Mammary tumours are the most frequently encountered neoplasms in female dogs. Malignant types of canine mammary tumours are more frequent than benign mammarytumours. Due to vast histological diversity of canine mammary tumours their diagnosis is difficult and provides little prognostic information. The presence of cells with enlarged nuclei and prominent nucleoli often leads to the misdiagnosis of mammary carcinoma.Use of a histological grading system may be helpful for classification and prognosis of canine mammary tumours. Immunohistochemistry is an extraordinarily powerful tool in the armamentarium of the diagnostic surgical pathologist. It is widely used in diagnosis of cancers by detection of tumor specific antigens expression. Canine mammary tumours have two histological classification given by WHO, first in 1974 and it is modification occur in 1999. New histological classification of CMTs is based on modification of WHO criteria given by Goldschmidt. In Veterinary science, two slightly different Misdorp and Pen systems (modifications of the human method) are used for histological grading of the canine mammary gland carcinoma. These two grading system is based on the assessment of the three morphological features: tubule formation, nuclear pleomorphism and mitotic counts. Some potential prognostic cellular biomarkers have been investigated for canine mammary tumours, and are frequently used for their detection and prognosis. Most commonly used cellular biomarkers are human epidermal growth factor, progesterone and estrogen receptors, E-cadherin and P-cadherin and proliferation marker Ki67.

KEYWORDS: Mammary tumour, malignant, cadherins, immunohistochemistry, mitotic count, ki67

***Citation* (VANCOUVER):** Sharma and Farooq, Canine Mammary Tumours: Advances in Classification, Grading and Expression of Biological Markers. *International Journal of Bio-resource and Stress Management*, 2022; 13(12), 1496-1503. HTTPS://DOI.ORG/10.23910/1.2023.3291.

Data Availability Statement: Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

Conflict of interests: The authors have declared that no conflict of interest exists.

RECEIVED on 14th October 2022 RECEIVED in revised form on 05th December 2022 ACCEPTED in final form on 16th December 2022 PUBLISHED on 28th December 2022

1. INTRODUCTION

Mammary glands (MG) are distinguishing feature of mammals that provide appropriate nourishment and passive immunity to their offspring. They are epidermal appendages that possibly evolved over 300 million years ago, from ancient apocrine sweat glands. Mammary development can first be recognized during embryologic development by the appearance of 2 ventral linear thickenings (ridges) of ectoderm, below which are specialized regions of mesoderm. The ridges, also referred to as milk lines, extend from the axillary to the inguinal region. The ectodermal cells migrate along each milk line and coalesce to form a placode, which eventually becomes individual mammary glands. The formation of the placode is a complex interaction, involving several signal pathways between the epithelial cells of the ectoderm and mesenchymal cells of the mesoderm. In domestic animals, mammary gland tumours are rare except dogs and cats. Canine mammary tumours are the most common neoplasm in bitches. (Misdorp et al.,1999). Simple carcinoma is composed of only one cell type, either luminal epithelial cells or myoepithelial cells while as complex carcinoma is characterized by the presence of two cell population: Malignant epithelial component and Benign myoepithelial component. Mix-type carcinoma has a malignant epithelial component and benign mesenchymal component. The benign mammary tumours are ductal papillomas, fibroadenomas, mixed tumours and simple adenomas. It is also common to find more than one type of tumour in a same patient.There is a huge histological diversity of canine mammary tumours which makes their diagnosis difficult and provides little prognostic information. Soa histological grading system is very important for classification and prognosis of the mammary tumours (Pena et al., 2013). In Veterinary science two slightly different, Misdorp and Pena systems (modifications of the human method) are used for histological grading of the canine mammary gland tumours (Misdorp et al., 2002; Clemente at el., 2010). However Pena system has shown better predictive ability and prognostic indicator in canine mammary carcinomas. This grading system is based on the assessment of the following 3 morphological features: Tubule formation, Nuclear pleomorphism, Mitotic counts. Immunohistochemistry (IHC) is an extraordinarily powerful tool in the armamentarium of the diagnostic surgical pathologist. It is widely used in diagnosis of cancers by detection of tumour specific antigens expression. Some of the most commonly used IHC markers include calponin. p63, SMA, Basal keratin, Human epidermal growth factor Estrogen and Progesterone receptor, E- cadherin, P- cadherin and Proliferation marker Ki – 67 (Toledo et al., 2016). HER-2 (also known as *neu,* HER-2*/neu and c-erbB-2*) is a cell membrane receptor having an intracellular protein kinase domain, belonging to a family of membrane-bound protein kinases that include an epidermal growth factor receptor (EGFR). (Killeen, 2004). Criteria for HER2 Immunohistochemistry in canine mammary tumours is still a proposal based on human breast cancer system. The score is given in between 0 to 3+ depending upon the malignancy. Similar scoring patterns are described for estrogen and progesterone Immunohistochemistry though the staining patterns for malignancy are different. Cadherin is a superfamily of trans membrane's glycoproteins. They are located on the basolateral membrane in the adherent junctions Most studies of cadherin in tumorgenesis are focused on E-cadherin as it is the main cadherin expressed by epithelial cells (Klopfleisch et al., 2011). Reduced immunostaining of E-cadherin is associated with the malignant phenotype of cancer. Similarly P cadherin has a reduced immunostaining in malignancies while ki67 shows proliferation nature of tumour. Using all these markers in association or alone can aid in proper diagnosis and extent of tumour proliferation.

1.1. Histological classification of CMTs

Histological examination is gold standard for diagnosis and classifying the mammary gland tumours, (Sorenmo et al., 2011). Two histological classifications systems for canine mammary tumours have been published by World Health Organization: First in 1974 and Second in 1999. New histological classification of CMTs by Goldschmidt includes 5 new morphological subtypes of CMTs as compared to 1999namely Micropapillary invasive carcinoma, Comedocarcinoma, Ductal carcinoma, Intraductal papillary carcinoma and Carcinoma and Malignant myoepithelioma. (Goldschmidt et al., 2011).

1.2. Criteria of malignancy

A major problem in evaluating canine mammary neoplasms is identifying the true malignant neoplasms. The presence of cells with enlarged nuclei and prominent nucleoli often leads to the misdiagnosis of mammary carcinoma. The following are the most significant criteria for the diagnosis based on hematoxylin and eosin stainedsections: (Goldschmidt et al., 2011)

1) Tumour type.

2) Significant nuclear and cellular pleomorphism,mitotic index.

3) Presence of randomly scattered areas of necrosis within the neoplasm.

4) Peritumoural and lymphatic invasion.

5) Regional lymph node metastasis.

2. NEW HISTOPATHOLOGICAL CLASSIFICATION OF CMTS

2.1. Malignant epithelial neoplasms

2.1.1. Carcinoma–in situ

In situ carcinoma is neoplastic tissues that have welldemarcated nodules which have not extended through the basement membrane into the adjacent mammary tissue. It is densely cellular in nature and consist of closely packed cells arranged in irregular tubules (Antuofermo et al., 2007). The cells vary between polygonal to round and cuboidal, with scant amount of eosinophilic cytoplasm with a high nuclear:cytoplasmic ratio.

2.1.2. Carcinoma–simple types

In simple carcinoma there is only one cell type,either luminal epithelial cells or myoepithelial cells. (Misdorpet al.,1972).

2.1.3. Carcinoma–tubular

Tubular carcinomas are very common mammary carcinomas in the dog. In this type of tumour neoplastic cells are predominantly arranged in a tubular fashion (Misdorp et al. (1972). The lining of the tubules is often 1 to 2 cells thick, and cells are variable in their morphology. Nuclei may be hypochromic, normochromic, or hyperchromic in morphology (Goldschmidt et al., 2011).

2.1.4. Carcinoma–tubulopapillary

Tubulopapillary carcinoma is one in which the neoplastic tubules are arranged in a sessile or pedunculated papillary fashion (Misdorp et al., 1972). Thepapillae usually extend into tubular lumina andare supported by a fine fibrovascular connective tissue stroma (Goldschmidt et al., 2011).

2.1.5. Carcinoma–cystic-papillary

Here the papillae extend into marked dilated and cystic tubular lumina and are supported by a fibrovascular stroma (Goldschmidt et al., 2011).

2.1.6. Carcinoma–cribriform

Cribriform carcinoma is usually uncommon and hereneoplastic epithelial cells forma sievelike arrangement. (Goldschmidt et al.,2011).

2.1.6. Carcinoma–micropapillary invasive

Carcinoma micropapillary is an invasive form of neoplasm, well described in humans and hasan unfavorable prognosis. (Luna-more et al., 1994). This neoplasm has been recently described in dogs (Gama et al., 2008) and is characterized by an intraductal neoplastic population forming small intraluminal irregular aggregates and small papillae that do not have a supporting fibrovascular stalk. (Goldschmidt et al., 2011).

2.1.7. Carcinoma–solid

Solid carcinoma is one in which the cells are predominantly arranged in solid sheets, and are without lumina. (Misdorp et al., 1993). The cells are oval, and often have poorly demarcated cell margins with scant cytoplasm. Nuclei are oval and hyperchromatic with coarsely packed chromatin.

2.1.8. Comedocarcinoma

Comedocarcinoma is a neoplasm characterized by the presence of necrotic areas within the center of the neoplastic cell. There is abundant amorphous eosinophilic material admixed with cellular debris and necrotic Neutrophils with some macrophages. (Goldschmidt et al., 2011).

2.1.9. Carcinoma–anaplastic

This is most malignant of theall mammary carcinomas, showing diffuse invasion of connective tissue and lymphatic vessels by the cancerous cells.Neoplastic cells are often individualized or grouped in nests, oval/polygonal with moderate to abundant eosinophilic cytoplasm. (Goldschmidt et al., 2011). Nuclei are round to oval, occasionally indented with coarsely stippled chromatin.Intense inflammation is most striking feature of this tumour. (Misdorp et al.,1993).

2.1.10. Carcinoma–complex type

Complex-type carcinoma has a malignant epithelial component with benign myoepithelial component. (Misdorp et al., 1972). The neoplasm has presence of two cell populations supported by a fibrovascular stroma. The first population is of epithelial cells. They arearranged in irregular tubules lined by a single layer to several layers of cuboidal to columnar cells with a scant to moderate amount of eosinophilic cytoplasm. Necrosis of these epithelial cells may be focal or multifocal, and occasional foci of squamous differentiation may be present. The second population is composed of spindleshaped cells (myoepithelial cells) within the interstitium, arranged in irregular bundles within a fibrillar basophilic (myxoid) matrix. Such cells have poorly demarcated borders, scant to moderate homogeneous slightly eosinophilic cytoplasm, and round to ovoid central nuclei with finely stippled chromatin and a small central nucleolus (Goldschmidt et al., 2011).

2.1.11. Carcinoma and malignant myoepithelioma

This is characterized by presence of two different cell population,first is epithelial cell and second is myoepithelial cell. Both type of cell population is malignant in nature (Goldschmidt et al., 2011).

2.1.12. Carcinoma–mixed type

These exhibit a complex histological pattern as they consist of epithelial and mesenchymal component (Cassali et al., 2011). A mesenchymal component consists of cartilage and/or bone and/or adipose tissue. The epithelial component of neoplasm is malignant and mesenchymal component as benign. (Goldschmidt et al., 2011).

2.1.13. Ductal carcinoma

Ductal carcinoma is the malignant counterpart of the ductal

adenoma. The neoplastic cell population is arranged in cords and tubules that surround slitlike lumina and exhibit significant anisokaryosis and anisocytois. Mitotic index is high (Goldschmidt et al., 2011).

2.2. *Malignant epithelial neoplasms: special types*

2.2.1. Squamous cell carcinoma

Squamous cell carcinoma is composed solely of squamous epithelium. The neoplasm has its origin either from squamous cells of the teat duct or from ductal epithelial cells (Goldschmidt et al., 2011). Histologically, the neoplasm is similar to those that occur in the skin. Islands and cords of epithelial cells are visualized with the formation of keratin pearls.

2.2.2. Mucinous carcinoma

This tumour is not well described in the veterinary literature and is characterized by the presence of abundant extracellular mucinous material (Misdorp et al., 2002). The neoplastic epithelial mucus producing cells are usually individual cells but can form tubules and nests. The cells are perodic acid-schiff-diastase positive and are alcian blue positive (Goldschmidt et al., 2011).

2.2.3. Lipid-rich carcinoma

Lipid rich carcinoma is a mammary carcinoma characterized by cells with abundant vacuolated cytoplasm containing a large amount of neutral lipid (Misdorp et al., 2002). Nuclei are round to oval with clumped chromatin. Anisokaryosis and anisocytosis are moderate, with variable numbers of mitoses.

2.2.4. Malignant myoepithelioma

In malignant myoepithelioma, cells may be oval to spindle shaped. Cells have usuallypoorly demarcated borders and have moderate amount of eosinophilic or basophilic cytoplasm, with occasional intracytoplasmic clear vacuoles. Nuclei are round and central, with finely stippled chromatin and a single hyperchromatic nucleolus (Goldschmidt et al., 2011).

2.3. *Malignant mesenchymal neoplasms (Sarcoma)*

2.3.1. Osteosarcoma

Osteosarcoma is the most common mesenchymal neoplasm of the canine mammary gland, and ir spreads via hematogenous route,mainly to respiratory tract (Goldschmidt et al., 2011). It is characterized by osteoid formation by neoplastic cells. Pleomorphism and mitotic activity is usually high. (Cassali et al., 2011)

2.3.2. Chondrosarcoma

Chondrosarcoma is a rare mammary neoplasm (Goldschmidt et al., 2011). It is characterized by multiple chondroblasts in lacunae. Chondroblasts of various sizes and shapes are dispersed beneath the fibrous capsule (Serin and Aydogan et al., 2009).

2.3.3. Fibrosarcoma

Fibrosarcoma is characterized by proliferation of fusiform cells with a distinctly interwoven pattern. Canerous cells have indistinct cell borders, a small amount of eosinophilic fibrillar cytoplasm, oval to elongate nuclei that contain finely stippled chromatin and variably distinct nucleoli. Anisokaryosis and anisocytosis are moderate (Goldschmidt et al., 2011).

2.4. *Malignant mixed mammary tumour (Carcinosarcoma)*

These neoplasms are composed of cells that morphologically resemble epithelial cellsluminal epithelium and/or myoepithelial) and have various types of differentiation including adeno,squamous,mucinous and anaplastic(Misdorp et al., 1972).

2.5. Benign mammary neoplasms

2.5.1. Adenoma–simple

Simple adenomas are well-demarcated and have non-infiltrative nodular lesions composed of cells which are arranged in tubules and occasionally contain an amorphous amphophilic secretion. Anisokaryosis and anisocytosis are minimal with very few mitotic figures. (Goldschmidt et al., 2011).

2.5.2. Intraductal papillary adenoma (Duct Papilloma)

This neoplasm has a papillary, arborescent growth pattern supported by a fibrovascular stalk (Misdorp et al., 1999) Mitoses are rare.

2.5.3. Fibroadenoma

Fibroadenoma is a benign growth originating from the proliferation of epithelial and stromal elements (Cassali et al., 2011). Neoplastic mass consists of tubules which are lined by cuboidal or columnar cells, with nuclei that are round and uniform in nature.

2.5.4. Myoepithelioma

Myoepithelioma is a rare neoplasm composed of spindleshaped cells arranged in short bundles admixed with an extracellular fibrillar basophilic material (myxoid matrix). Anisokaryosis and anisocytosis are minimal, with low mitotic index.

2.5.5. Complex adenoma (Adenomyoepithelioma)

Adenomyoepithelioma is a benign tumour originating from the proliferation of epithelial and myoepithelial cells. (Cassali et al., 2011). Its differential diagnosis from complex well differentiated carcinomas can be difficult. The presence of a capsule, absence of necrosis and atypia and low mitotic activity is the basis for diagnosis. (Cassali et al., 2002).

2.5.6. Benign mixed tumour

Benign mixed tumours are characterized by benign proliferation of cells that are morphologically similar to

epithelial components and mesenchymal cells that produce cartilage and/or bone and/or adipose tissue possibly in combination with fibrous tissue (Misdorp et al., 1999).

2.6. Hyperplasia/Dysplasia

2.6.1. Duct ectasia

In duct ectasia, there is cystic dilation of large ducts with accumulation of necrotic debris, variable numbers of vacoulated macrophages mixed with lipid material, and cholesterol clefts in the ducts. Duct ectasia may be secondary to occlusion of duct lumina by intraductal neoplasms. (Goldschmidt et al., 2011).

2.6.2. *Lobular hyperplasia–regular (Adenosis)*

Regular lobular hyperplasia is a benign proliferation of the ularductules resulting in increased number of ductules and acini perlobule (Misdorp et al., 1999).

2.6.2. Lobular Hyperplasia with Secretory (Lactational) Activity

Lobular hyperplasia with secretory (lactational) activity is a non cancerous proliferation of intralobular ducts/ductules and acini resulting in increased numbers of ducts/ductules and acini per lobule.

2.6.3. Lobular hyperplasia with fibrosis (Interlobular Fibrous Connective Tissue)

Lobular hyperplasia with fibrosis is a benign proliferation of intralobular ducts/ductules and acini which results in increased numbers of ducts/ductules per lobule with increased amount of interlobular fibrous connective tissue. (Goldschmidt et al., 2011).

2.6.4. Epitheliosis–intraductal hyperplasia without atypia

Epitheliosis (intraductal hyperplasia without atypia) is a lesion that shows regular proliferation of epithelial cells within the lumen of ducts (Misdorp et al., 1999). The aggregates of cells often fill the duct.(Goldschmidt et al., 2011).

2.6.5. Fibroadenomatous change

Fibroadenomatous change is an uncommon lesion that shows proliferation of interlobular ducts and periductal stromal cells .The stroma is often edematous or myxomatous. The fibroblast nuclei and ductal cell nuclei shows pleomorphism and increased numbers of mitotic figures (Goldschmidt et al., 2011).

2.6.6. Gynecomastia

Gynecomastia refers to enlargement of the mammary glands in a male with duct ectasia and lobular hyperplasia. One or more glands can be affected. This change is mostly seen with Sertoli cell tumors (Goldschmidt et al., 2011).

2.7. Neoplasms of Nipple

Ductal adenoma and carcinoma are rare neoplasms that involve the tissue of the nipple with no neoplastic tissue beneath the mammary gland. The nipple is enlarged and firm. Within the neoplastic tissue, the ducts have by one or more layers of epithelial cells, and the lumina often appearsslitlike.(Goldschmidt et al., 2011).

2.8. Hyperplasia or dysplasia of the nipple

Nipple hyperplasia or dysplasia is a lentigo-like proliferation of melanocytic cells within the epidermis. The lesion is focal which surrounds the teat ducts. The epidermis is mildly hyperplastic in nature. In the basal cell layer,there is a increased number of melanocytes that often occur as a cluster of small groups.(Goldschmidt et al., 2011).

2.8.1. Histological grading of canine mammary tumour

Several histological malignancy grading systems have been proposed. In humans Elston and Ellis numeric method is worldwide used for grading of mammary tumours (Nottingham method) (Elston and Ellis, 1991).In Veterinary science two slightly different, Misdorp and Pena systems (modifications of the human method) are used for histological grading of the canine mammary gland tumours (Misdorp et al., 2002; Clemente at el., 2010). (Table 1). However Pena system has shown better predictive ability and prognostic indicator in canine mammary carcinomas. This grading system is based on the assessment of the following 3 morphological features:

- Tubule formation,
- Nuclear pleomorphism,
- Mitotic count

2.8.2. Expression of biological markers

Tumour markers or biological markers are the unique attribute that may reflect the neoplastic process by high or low level of expression relative to that of normal cells, offering use in diagnosis of cancer. Morphologic evaluation, together with immunophenotypic differences of CMTs can be used to help provide a definitive diagnosis.(Hicks et al., 2011). During mammary tumorigenesis, cell-specific differentiation markers are usually retained in cells and these markers are used to study the origin of the canine mammary tumors, mainly the epithelial and myoepithelial cell's role in the genesis of mixed tumours, based on its immunophenotypic characteristics (Pena et al., 2014). Normal canine mammary glands are lined by two cell layers, Inner/luminal population of secretory cells and outer layer known as basal/myoepithelial cell layer. The luminal epithelial cells are characterized by expression of low molecular weight luminal cytokeratins (CKs) including CK8, CK18, CK19 and CK7 (Reis-Filho and Tutt et al., 2008), whereas myoepithelial cells are characterized by expression of high molecular weight CKs such as CK5,

Table 1: Criteria for histological malignancy grade

Criteria for histological malignancy grade	Points	
A. Tubule formation	1	Formation of tubules in >75%
	2	Formation of tubules in 10%–75%
	3	Formation of tubules in (<10%) (minimal or no tubule formation)
B. Nuclear pleomorphism	1	Uniform or regular small nucleus and occasional nucleoli
	2	Moderate degree of variation in nuclear size and shape, hyperchromatic nucleus, presence of nucleoli (some of which can be prominent)
	3	Marked variation in nuclear size, hyperchromatic nucleus, often with ≥ 1 prominent nucleoli
C. Mitoses per 10 HPF	1	0–9 mitoses/10 HPF
	2	10–19 mitoses/10 HPF
	3	≥ 20 mitoses/10 HPF
Histological Malignancy Grade		Grade:
Total Scoring (A +B +C)	3–5	I (low, well differentiated)
	6–7	II (intermediate, moderately differentiated)
	8–9	III (high, poorly differentiated)

CK6 and CK17 in addition to other markers (Gamma et al., 2003) (Table 2).

Table 2: Markers commonly used to identify mammary myoepithelial cells (MECs) in canine mammary tumours (Pena et al., 2014)

Antibody	MECs	Myofibroblasts	Vessels	Tumor epithelial cells
Calponin	Strong	Weak-moderate	Strong	Rare
SMA	Strong	Moderate	Strong	Rare
p63	Strong	Negative	Negative	Rare
Basal keratins	Strong	Negative	Negative	Frequent

Other important markersused to diagnose the canine mammary tumour:

- Human epidermal growth factor
- Estrogen and Progesterone receptor
- E- cadherin and P- cadherin
- Proliferation marker Ki – 67

2.8.3. *Human epidermal growth factor*

HER-2 (also known as *neu,* HER-2*/neu* and *c-erbB-2*) is a cell membrane receptor with an intracellular protein kinase domain and belongs to a family of membrane-bound protein kinases that include an epidermal growth factor receptor (EGFR)(Killeen et al., 2004). Expression of the EGFR and c-ERB-2 epidermal growth factors in the mammary carcinomas of the female dog is related to the tumour development and progression with poor prognosis. (Horta et al., 2012). IHC for HER2 should be scored as 0, 1+, 2+, and 3+ based on guidelines proposed by the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) and only 3+ tumours are considered positive (Wolff et al., 2007) (Table 3).

Table 3: Interpretation criteria for HER2 Immunohistochemistry in canine mammary tumours:-A proposal Based on human breast cancer system

IHC Score	Interpretation criteria
3+	Strong, complete, homogeneous membrane labeling in >30% of cells
2+	Strong, complete membrane labeling in ≤30% of cells
0–1+	Weak or moderate heterogeneous complete membrane labeling in at least 10% of cells No labeling (0) or weak, incomplete membrane labeling (1+) in any percentage of cells

2.8.4. Estrogen and progesteron receptor

Both are steroid hormones and play important role in mammogenesis during puberty in mammals. There are two

known isoforms of the ER:-ERα and ERβ. (Queiroga et al., 2011). In CMT, lower expression of ERα is related with a worse prognosis however ERβ-positive tumours are more frequently benign than malignant. The higher expression of ERβ in malignant CMT with a lower grade of malignancy is suggestive of good prognosis (Martin de las et al., 2004). So based on immunohistochemical expression, benign and malignant tumourcan be differentiated. Benigntumours are positive for both ER & PR and Malignanttumours are positive for PR & negative for ER(Knight et al.,1977).

2.8.5. E- cadherin and P- cadherin

Cadherin is a superfamily of trans membrane's glycoproteins. They are located on the basolateral membrane in the adherent junctions Most studies of cadherin in tumorgenesis are focused on E-cadherin as it is the main cadherin expressed by epithelial cells (Klopfleisch et al., 2011). Reduced immunostaining of E-cadherin is associated with the malignant phenotype of cancer. Loss of its expression is related to cell detachment and favoring the tumor dissemination (Horta et al., 2012). P-cadherin is a glycoprotein with a structure similar toE cadherin. It is expressed on the myoepithelial cells of adult and normal mammary tissue. The selective expression of E and P-cadherin is important for differentiation of the mammary gland (Toledo et al., 2016) Aberrant expression of P-cadherin is associated with the infiltrative growth pattern of the tumor (Asproni et al., 2015).

2.8.6. Proliferation marker Ki – 67

The most studied cellular proliferation marker in tumours is the Ki-67,a nuclear protein with a molecular mass of 345 and 395 kD (double band),expressed in all phases of cellular cycle except G0 (Ranzi et al., 2015). Immunohistochemical technique is most common method for evaluation of Ki-67 monoclonal antibodies (Matsumoto et al., 2015, Thuroczy et al., 2007). Benign tumours have a low number of Ki-67 positive cells, while the malignant tumours have high number of Ki-67 positive cells. High rate of Ki-67 is positively correlated with metastasis, death from cancer, lower overall survival rate and low disease-free interval (Thuroczy et al., 2007).

3. CONCLUSION

Mammary tumours are the most frequently encountered neoplasms in female dog. There is a wide histogenic classification of mammary tumours based on expertise of WHO which makes their diagnosis more difficult. High precision is required by the pathologist in classifying the type of mammary tumour as well as to determine the malignancy associated with the tumour. Immunohistochemistry provides additional information about the tumour as the markers used in IHC can reveal the exact histogenic origin and metastatic potential of the tumour. Hence histopathology and immunohistochemistry when used together could serve as perfect diagnostic techniques in order to classify and determine the outcome of neoplasm.

4. REFERENCES

Antuofermo, E., Miller, M.A., Pirino, S., Xie, J., Badve, S., Mohammed, S.I., 2007. Spontaneous mammary intraepithelial lesions in dogs-a model of breast cancer. Cancer Epidemiology and Prevention Biomarkers 16(11), 2247–2256.

Asproni, P., Ressel, L., Millanta, F., Vannozzi, I., Poli, A., 2015. Co-localization of PTEN and E-cadherin in canine mammary hyperplasia and benign and malignant mammary tumors. Research in Veterinary Science 103, 113–118.

Cassali, G.D., Serakides, R., Gartner, F., Schmitt, F.C., 2002. Invasive micropapillary carcinoma of the dog mammary gland:a case reort. Arquivo Brasilerio de Medicina Veterinaria e Zootecina 54(4), 366–369.

Cassali, G.D., Lavalle, G.E., DeNardi, A.B., Ferreira, E., Bertagnolli, A.C., Estrela-Lima, A., Sobral, R.A., 2011. Consensus for the diagnosis, prognosis and treatment of canine mammary tumours. Brazilianjournal of Veterinary Pathology 4(2), 153–180

Clemente, M., Perez-Alenza, M.D., Illera, J.C., Pena, L., 2010. Histologic, immunologic and ultrastructural description of vasculogenic mimicry in canine mammary cancer. Veterinary Pathology 47, 265–274.

Elston, C.W., Ellis, I.O., 1991. Pathological prognostic factors in breast cancer, I: the value of histological grade in breast cancer: experience from a large study with long-term follow-up. Histopathology 19, 403–410.

Gama, A., Alves, A., Gartner, F., Schmitt, F.C., 2003. p63:a novel myoepithelial cell marker in canine mammary tissues. Veterinary Pathology 40(4), 412–420.

Gama, A., Alves, A., Schmitt, F.C., 2008. Clinicopathologic features of invasive micropapillary carcinoma (IMC) in dogs. Veterinary Pathology 45, 600–601.

Goldschmidt, M., Pena, L., Rasotto, R., Zappulli., V., 2011. Classification and grading of canine mammary tumors. Veterinary Pathology 48(1), 117–131.

Hicks, D. G., 2011. Immunohistochemistry in the diagnostic evaluation of breast lesions. Immunohistochemical Molecular Morphology 19, 501–505.

Horta, R.S., Costa, M.P., Lavalleii, G.E., Araujo, R.B., Cassali, G.D., 2012. Prognostic and predictive factors of canine tumours with immunohistochemistry's assistance. Ciência Rural 42(6), 308–311.

Killeen, A.A., 2004. Principles of molecular pathology. Humana Press Inc. Totowa, New Jersey, 185–209.

Klopfleisch, R., Von Euler, H., Sarli, G., Pinho, S.S., Gartner, F., Gruber, A.D., 2011. Molecular carcinogenesis of canine mammary tumors: news from an old disease. Veterinary Pathology 48(1), 98–116.

Knight, W.A., Livingston, R.B., Gregory, E.J., McGuire, W.I., 1977. Estrogen receptor as an independent prognostic factor for early recurrence in breast cancer. Cancer research 37(12), 4669–4671.

Luna-More, S., Gonzalez, B., Acedo, C., Rodrigo, I., Luna, C., 1994. Invasive micropapillary carcinoma of the breast:A new special type of invasive mammary carcinoma. Pathology Research and Practice 190(7), 668–674.

Martin de las Mulas, J., Reymundo, C., Espinosa de los Monteros, A., 2004. Calponin expression and myoepithelial cell differentiation in canine, feline and human mammary simple carcinomas. Veterinary and Comparative Oncology 2, 24–35.

Misdorp, W., Cotchin, E., Hampe, J.F., Jabara, A.G., Von Sandersleben, J., 1972. Canine malignant mammary tumours: II. Adenocarcinomas, solid carcinomas and spindle cell carcinomas. Veterinary Pathology 9, 447–470.

Misdorp, W., Cotchin, E., Hampe, J.F., Jabara, A.G., Von Sandersleben, J., 1993. Canine malignant mammary tumours: III. Special types of carcinomas, malignant mixed tumours. Veterinary Pathology 10, 241–256.

Misdrop, W., Else, R.W., Hellemen, E., Lipscomb, T.P., 1999. Histological classification of mammary tumours of the dog and the cat, 2nd ser., vol.7, Armed Force Institute of Pathology and World Health Organisation, Washington, DC.

Misdorp, W., 2002. Tumors of the mammary gland. In: Tumors in Domestic Animals, ed. Meuten DJ (4th Edn.). Iowa State Press, Ames, IA, pp. 575–606, 764.

Pena, L., De Andres, P.J., Clemente, M., Cuesta, P., Perez-Alenza, M.D., 2013. Prognostic value of histological grading in noninflammatory canine mammary carcinomas in a prospective study with two-year follow-up: relationship with clinical and histological characteristics. Veterinary Pathology 50(1), 94–105.

Pena, L., Gama, A., Goldschmidt, M., Ahadie, J., Benazzi, C.,Castagnaro, M., Milan, Y., 2014. Canine mammary tumours:a review and consensus of standard guidelines on epithelial and myoepithelial phenotypic markers, HER2 and hormone receptor assessment using immunohistochemistry. Veterinary Pathology 51(1), 127–145

Queiroga, F.L., Pires, I., Parente, M., Gregorio, H., Lopes, C.S., 2011. COX-2 over-expression correlates with VEGF and tumour angiogenesis in canine mammary cancer. Veterinary Journal 189(1), 77–82.

Ranzi, A.D., Silva, D.A., Graziottin, J.N., Annels, T.M., Bica, N., 2015. Immunohistochemistry biomarkers in nonmuscle invasive bladder cancer,applied immunohistochemistry and molecular morphology. Veterinary Journal 7, 198–121.

Reis-Filho, J.S., Tutt, A.N.J., 2008. Triple negative tumours:a critical review.Histopathology 52(1), 108–118.

Serin, G., Aydogan, A., 2009. Chondrosarcoma in the mammary gland of a bitch:a case reort. Veterinarni Medicina 11, 543–546.

Sorenmo, K., 2003. Canine mammary gland tumours. Veterinary clinics North America: small animal Practice 33, 573–596.

Thuroczy, J., Reisvaag, G.J., Perge, E., Tibold, A., Szilagyi, J., Balogh, L., 2007. Immunohistochemical detection of progesterone and cellular proliferation in canine mammary tumours. Journal of Comparative Pathology 137(3), 92-122.

Toledo, G.N., Silva, P.D.A., Borges, B.N., Monteiro, F.O.B., Feliciano, M.A.R., 2016. Specific cellular biomarkers for canines breast Cancer. Amazonion Journal 175(11), 1752–1760.

Wolff, A.C., Hammond, M.E., Hayes, D.F., 2007. Immunohistochemical testing of estrogen and progesterone receptors in breast cancer. Journal of Oncological Practices 6, 195–197.