

Detection of Progesterone Receptor as a Method of Diagnosing Mammary Cancer in Female Dogs

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Abstract

Introduction: Canine mammary gland tumor is the most common type of neoplasia in non-ovariectomized bitches. Approximately 50% of tumors are malignant. Neoplasms originating from the mammary gland represent the most common neoplastic disease in canines in Veterinary Medicine. **Aim:** Relate the expression of the receptor to progesterone (PR) with the tumor stage of canine mammary carcinoma. **Material and Methods:** Analytical-cross-sectional study, samples of paraffinized tumor tissue obtained from 30 canine patients with breast cancer were used. The expression of PR was performed by immunohistochemical labeling, using murine anti-PR (anti-PR Biocare brand). A descriptive analysis was carried out with the results using the SPSS program. **Results:** The predominant histological subtype of breast cancer was tubular carcinoma with 12 patients, followed by papillary cystic carcinoma with 6 patients, solid carcinoma 5, carcinosarcoma 4 and comedo-carcinoma 3. There was a significant trend between breast cancer subtypes, histological grade G1. Among the histopathological findings, the degree of invasion is related to the presence of tumor cells in adjacent lymph nodes, which is why it is a prognostic indicator. The expression of PR in the tumor tissue samples it was 42.8% positive versus 57.14% negative, of which 75% correspond to G1, 8.3% to G2 and 16.6% to G3. With respect to the relationship of the expression of PR vs type of tumor, it was found that 50% correspond to tubular carcinoma, 33.3% to papillary cystic carcinoma, 8.3% to solid carcinoma and 8.3% to comedocarcinoma. **Conclusion:** The hormone receptor was negative in more than half of the patients and histological grade is significantly associated with tumor subtypes, this study emphasizes the need to introduce

receptor testing into our routine clinical practice to offer the best treatment for breast cancer.

Keywords

Cancer, Mammary Gland, Progesterone, Immunohistochemistry, Progesterone Membrane Receptor Component 1 (PGRMC 1)

1. Introduction

Cancer is the leading cause of death worldwide: in 2020, almost 10 million deaths were attributed to this disease according to the WHO (1). So in veterinary medicine, it is no exception, it also has a high impact; resulting in high morbidity and mortality rates. In the dog breed, the most common cancer is breast cancer.

The mammary gland is a sweat gland located in the subcutaneous tissue whose main function is to nourish neonates and confer passive immunity as they develop as epithelial buds from the mammary ridges and extend from the axilla to the inguinal region. They are classified as thoracic, cranial abdominal, middle and caudal, and inguinal and are found exclusively in mammals and form the glandular tissue of the breast [1].

The mammary gland is a modified apocrine gland, characterized by a tubuloalveolar structure and canine females usually have 5 pairs of mammary glands, although 4 or 6 mammary pairs have been seen in some animals and each of them characterized by a mammary body formed by 8 to 14 lobes, connective tissue, skin, mammary papilla and the nipple [2].

1.1. Mammary Gland Cancer in Canines

Canine mammary gland tumor (CMT) is the most common type of neoplasia in dogs, approximately 50% of tumors are malignant originating from the mammary gland and it is the most common neoplastic disease in female canines [3].

These tumors vary in size from millimeters to centimeters, and at least 50% of cases present multiple masses, mainly in the caudal glands. It is important to take into consideration that the canine mammary gland is a hormone-dependent organ whose activity Cyclic is associated with consecutive phases of development and regression that differ between individual glands and also within each gland [4].

Mammary gland neoplasms are defined as masses of various sizes and shapes that emerge in any of the anatomical regions that make up this organ. An essential requirement for their appearance is that this mass is composed of glandular connective tissue, therefore, the cell growth presents an autonomous and uncontrolled growth, these abnormal growths of tumor cells in canines are grouped into various categories according to the structures involved and the degree of malignancy present, the most frequent are five: dysplasias, mixed tumors, benign tumors different from the type mixed, carcinomas and sarcomas [5].

1.2. Risk Factors that Predispose Neoplasms in the Mammary Gland

Sex is the first risk factor; since they are females, they are predisposed to presenting this disease due to the tropism for the mammary gland and the presence of natural estrogens that have a solid relationship with the incidence of mammary neoplasms in female canines, demonstrated by the fact that in Bitches castrated at an early age before the first estrous cycle have an incidence of 0.05%, which increases by 8% or 26% if they are castrated after the first or second heat, respectively; If they are neutered later, the risk of developing malignant tumors is the same as that of a non-neutered dog [6] [7].

Diet should be considered as a risk factor for breast cancer. It has been shown that the consumption of foods with high percentages of fat and obesity at an early age are predisposing components, since they alter the availability and concentration of sex hormones when compared to canines that they consume low-fat diets and have an adequate weight [8].

Another risk factor is age, since malignant mammary tumors appear in dogs with an average age of 8 to 11 years and in younger dogs, the tumors are usually benign and in dogs under 5 years of age, it is very rare for them to occur at least that these females have been treated with progestogens [9].

Hormonal influence as a risk factor according to the study published by Schneider *et al.* in 1969, dogs subjected to ovariary hysterectomy (OVH) before the first estrus had a 0.5% risk of developing mammary tumors, while those operated on between the 1st and 2nd estrus, and after the 2nd estrus, showed 8% and 26% risk, respectively. Although no benefit was found in this study operating after the 2nd estrus, other studies showed that later OVH could also be protective, although not as significant [10].

The administration of exogenous hormones (progestogens and estrogens) increases the risk of developing mammary tumors. It was observed that there is a significant increase in benign tumors over malignant ones in bitches treated with progestogens, although this relationship is reversed in bitches treated with a combination of progestogens and estrogens [11].

1.3. Clinical Manifestation of the Disease

Many dogs have more than one mammary tumor and the 4th and 5th mammary pairs are the most affected. Curiously, the 1st mammary pair is not usually involved initially. The tumors can be small or large, fixed or mobile, ulcerated, single or multiple, regional lymph nodes. They may or may not be enlarged and distant metastases are infrequent, except for inflammatory breast carcinoma. They may also present a highly aggressive neoplasia with a fulminant course. These tumors encompass several mammary glands, presenting edema, inflammation, pain, and may cause systemic signs such as anorexia, weight loss or weakness [12].

1.4. Clinical Tumor Staging

The ideal and most useful staging system is one that directly correlates the clini-

cal stage with the patient’s evolution, and therefore has an impact on treatment. Although none of the currently used staging systems for mammary tumors have been validated by the American College of Veterinary Pathologists, several studies suggest that the staging systems offer prognostic significance and therefore guide clinicians when it comes to make therapeutic decisions [9].

Two staging systems have been proposed and published for canine mammary tumors: the original World Health Organization system and the modified WHO system, and as shown in **Table 1**, both WHO staging systems with a comparison. Direct influence of TNM criteria in assigning stages. As shown, there are important differences between the two systems. According to the modified system, stages I - III classify dogs based on tumor size, stage IV represents dogs with lymph node metastasis, and stage V represents distant metastasis [3] [13].

In contrast, the original staging system assigned the same stage of disease, namely stage II, to a dog with a small tumor and a positive regional lymph node (N1_b) as it did to a dog with a larger tumor (T2: 3 - 5 cm) regardless of lymph node status (N0_b or N1_b), suggesting that a larger tumor has the same prognostic importance as a positive lymph node. Additionally, according to the original WHO staging system, a dog with a tumor large (T3) would be assigned a status III, regardless of lymph node status (any N), again suggesting that tumor size is more important than lymph node status [7].

Table 1. Staging systems for tumors in domestic animals according to the WHO and the modified one [13].

Original WHO Staging System ⁵⁷				Modified WHO ^{77a}			
Stage I	T1a, b, c	N0	M0	Stage I	TI	N0	M0
Stage II	T0	NI	M0	Stage II	T2	N0	M0
	T1a, b, c	NI	M0				
	T2a, b, c	N0 or NIa	M0				
Stage III	T3a, b, c	AnyN	M0	Stage III	T3	N0	M0
	AnyT	AnyNb					
Stage IV	AnyT	AnyN	MI	Stage IV	AnyT	NI	M0
Stage V				Stage V	AnyT	AnyN	MI

Abbreviations	
T: Primary tumor (a, not fixed; b) fixed to skin; c, fixed to muscle)	T: Primary tumor
T0: No evidence of tumor	
TI: < 3 cm maximum diameter (a, b, c)	TI: < 3 cm maximum diameter
T2: 3-5 cm maximum diameter (a, b, c)	T2: 3 - 5 cm maximum diameter

Continued

T3: > 5 cm maximum diameter (a, b, c)	T3: > 5 cm maximum diameter
T4: Any T, inflammatory carcinoma	
N: Regional lymph node status (a, not fixed; b, fixed)	N: Regional lymph node status
Assessed by clinical exam or hitopathology	Assessed by histology or cytology
N0: No metastasis	N0: No metastasis
NI: Metastasis ipsilateral lymph node (a, b)	NI: Metastasis
N2: Metastasis bilateral lymph nodes (a, b)	
M: Distant metastasis	M: Distant metastasis
M0: No distant metastasis	M0: No distant metastasis
MI: Distant metastasis detected	MI: Distant metastasis detected

^aExcluding inflammatory carcinoma.

1.5. Histopathological Analysis of Neoplasms of the Mammary Gland

Mammary gland tumors manifest in canines in 42% as the main cancer pathology and the histopathological study provides the degree of infiltration, malignancy, tubular formation that includes, differentiation or degree of defined anaplasia, as well as the nuclear appearance and the index. Mitotic (MI) which helps us give an adequate diagnosis and treatment [3] [12].

Histologically, the canine mammary tumor is classified into malignant epithelial tumors (carcinomas), special types of carcinomas, malignant mesenchymal tumors (sarcomas), carcinosarcomas and benign neoplasms [3] [7].

1.6. The Hormone Progesterone

Progesterone (P4) is a steroid hormone secreted by the corpus luteum (CL) and the placenta that plays a fundamental role in reproductive events and the establishment and maintenance of pregnancy. The concentration of progesterone in the circulation is determined by a balance between its production and metabolism, whose responsible organ is the liver, progesterone (C₂₁H₃₀O₂) is a steroid hormone belonging to the group of progestogens, responsible for nidation and maintenance of pregnancy. Its main source of progesterone is the corpus luteum of the ovary, Although it has been isolated from the adrenal cortex and placenta of some mammals, its chemical structure is 21 carbon atoms, made up of four

fused rings, with ketone, aldehyde groups and two methyl branches. It is also the precursor of all steroid hormones, it is an intermediate. In the biosynthesis of androgens, estrogens and corticosteroids, it is hydrophobic and is synthesized into pregnenolone, a cholesterol derivative that has a molecular mass of 314.46 g/mol [14].

1.7. Expression of the Progesterone Receptor Membrane Component 1 Is Associated with Malignant Phenotypes of Breast Cancer

Histopathological examination is the main means of diagnosis for a mammary gland tumor and molecular biomarkers are currently used routinely, such as component 1 of the progesterone membrane receptor (PGRMC1), which is genetically overexpressed in a wide range of tumors of various types. Species, compared to healthy tissues, was first observed as a gene induced during dioxin-stimulated tumorigenesis in rat liver and was also part of a six-gene signature associated with non-genotoxic carcinogens [15].

The progesterone membrane receptor component 1 PGRMC1 was also detected in the plasma and serum of patients with lung and kidney cancer, respectively, in which its level was higher compared to healthy patients and recently in women affected by high stages of breast cancer, experiments showed that PGRMC1 promotes tumor cell proliferation, resistance to apoptosis caused by chemotherapy, invasion and metastasis in different tumor cell lines. Synthetic progestogens combined with estrogens in this cancer therapy. Furthermore, recent findings have shown that PGRMC1 can trigger estrogen-dependent proliferation of breast cancer cells, indicating that PGRMC1 could not only predict risk using progestins but also different estrogens [10] [15].

2. Materials and Methods

In the present work, 30 female canine breast cancer patients were used from whom tumor tissue was collected to perform an immunohistochemistry study to determine tumor type, grade, and PR expression.

2.1. Sample Collection

The tumor tissue samples were washed with saline solution, and stored in formalin until embedded in paraffin to make 4-micron-thick microtome sections and fixed on electrocharged slides.

2.2. Determination of Progesterone Receptor by Immunohistochemistry

Immunohistochemical labeling of the PR was performed on a semi-automated device (IntelliPATH FLX).

The paraffin-embedded tissue samples were cut on a microtome into 4 µm-thick sections. Antibody incubation was carried out using murine monoclonal antibody (anti-PR Biocare cat. no. ACA 424 A). Immunodetection was visualized using di-

aminobenzidine tetrahydrochloride (DAB; Sigma-Aldrich; Merck Millipore, Darmstadt, Germany). Slides were counterstained with hematoxylin for 2 min (Cat No. BSB 0024; Bio-SB, Inc.) and mounted with Entellan® (Cat. No. 107960; Merck-Millipore).

3. Results

Histopathological and morphological analysis of the tissues determined that the predominant histological subtype of breast cancer was tubular carcinoma (TC), followed by papillary cystic carcinoma (CPQ), solid carcinoma (CS), carcinosarcoma (CSAR) and comedocarcinoma (CC) respectively as seen in **Figure 1**.

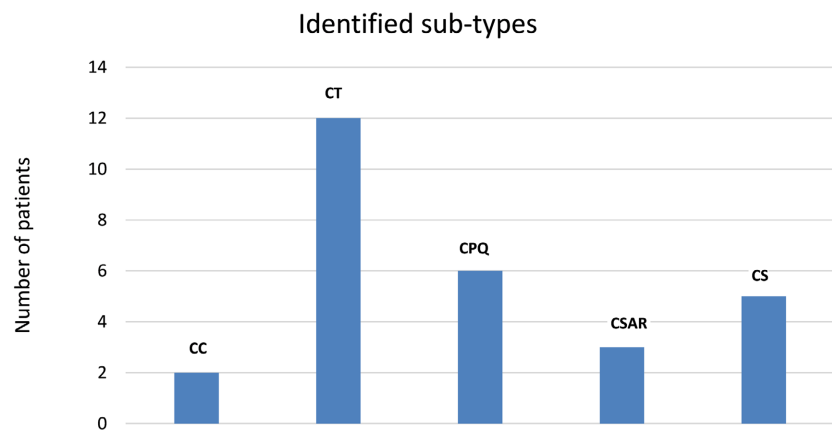


Figure 1. The number of patients and the predominant breast cancer subtypes of 30 patients are observed, where tubular carcinoma (TC) was the predominant one, followed by papillary cystic carcinoma (PCC) and solid carcinoma (SC).

3.1. Degree of Tumor Progression

According to the Elston and Ellis classification, the tumors were classified as grade 1 in 17 patients (56.6%), as grade 2 in 3 patients (10.7%), and as grade 3 in 10 patients (35.7%). The characteristics of the patients are summarized in **Table 2**, there was a significant trend between breast cancer subtypes ($p < 0.025$), in which the G1 histological grade predominates.

Table 2. Distribution of neoplasms according to tumor grade.

	n	%
Tumor Grade		
G1	17	56.6
G2	3	10.7
G3	10	35.7.6

Among the histopathological findings, the degree of invasion is related to the presence of tumor cells in adjacent and distant lymph nodes, so the presence of

metastases in lymphoid niches is a prognostic indicator, as seen in **Table 3**.

Table 3. Values for the count of lymph nodes contaminated with tumor cells.

	n	%
Lymph node metastasis		
YES (1 - 3)	16	53.3
YES (more than 3)	6	20
NO	8	26.6

3.2. Expression of the Progesterone Receptor (PR)

The PR expressions in the tissues of the 30 patients determined that 42.8% were positive versus 57.14% that were negative and of these, 75% correspond to G1, 8.3% to G2 and 16.6% to G3, with respect to The relationship of PR expression vs tumor type was found to be 50% tubular carcinoma, 33.3% papillary cystic carcinoma and 8.3% solid carcinoma and finally 8.3% comedocarcinoma **Table 4**.

Table 4. Count values of patients who tested positive for PR expression, cancer subtype and degree of progression.

Expression of PR		Cancer subtype and tumor grade
Positive patients	42.8%	50% Carcinoma tubular 33.3% Papillary cystic carcinoma 8.3 % Solid carcinoma 8.3% Comedocarcinoma
Negative patients	57.14%	75 % Son G1 8.3 %s Son G2 16.6 % Like G3

3.3. Progesterone Receptor Immunolabeling

As we can see in **Figure 2**, the different types of cancerous tumors found in the mammary glands are shown and revealing those with the immunohistochemistry and eosin and hematoxylin technique that were negative and positive to the PR progesterone receptor.

4. Discussions

This study determined that the immunohistochemical (IHC) technique that was used to mark the expression of PR applied in tissues from non-ovariectomized animals did allow the expression of the receptor to be assessed in samples from patients with breast cancer, in our country currently the access IHC is still limited by the high cost of the test, as well as the limited availability of laboratories that provide this service for veterinary use, but this technique could be used as a prognostic method to classify patients with this condition. The relationship of low PR ex-

pression with a poor prognosis has been described, while high expression is associated with more favorable outcomes [16].

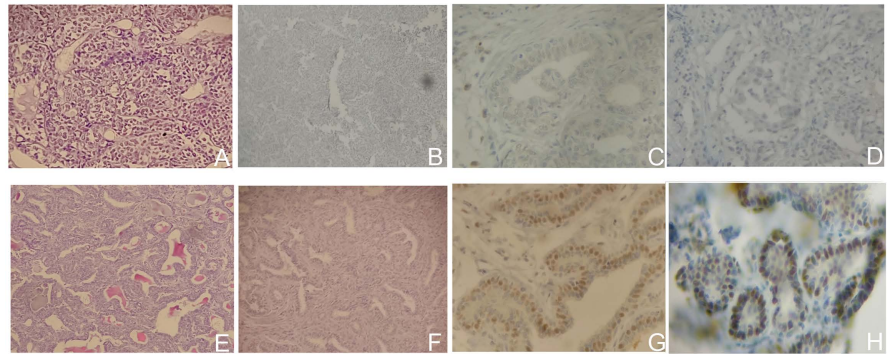


Figure 2. Photomicrographs of neoplastic canine breast tissue. “A” hematoxylin and eosin stain of neoplastic tissue presenting low-grade tubular carcinoma. “B” negative immunolabeling for PR 10×. “C” negative immunolabeling for PR 40×. “D” negative immunolabeling control for PR. “E” hematoxylin and eosin stain of neoplastic tissue presenting high-grade tubular carcinoma. “F” positive immunolabeling for RP 10×. “G” positive immunolabeling for PR 40×. “H” positive immunolabeling control for PR.

Our study revealed that 35% of breast cancer patients have well-differentiated tumors, 71% had positive lymph nodes, and the majority had stage T2 or T3 tumors. These unfavorable clinicopathological characteristics, such as high grade, lymph node involvement, and advanced stage, are consistent with what has been reported by other studies [14].

The advanced state of the disease in patients occurs precisely due to the delay in an accurate, timely diagnosis and adequate treatment of patients and largely also due to the delay in consultation and the lack of clinics with trained personnel for management. of this type of patients in the present study. In this study, it was observed that 60% of the cases, patients who did not express the PR, presented affection in the lymphatic line in 60%, exceeding the 40% of patients who were positive with expression of the PR, coinciding with the reports of the same observation that had already been made by different authors [11].

Our findings in the current study have implications for the management of breast cancer in dogs, in fact, it is common for patients with breast cancer to be treated blindly with tamoxifen and yet 60% of our patients are negative for the expression of PR, hormonal therapy would not be appropriate due to the decreased sensitivity of hormone receptors [16].

This study demonstrated interesting observations about breast cancer in dogs, however, some limitations should be mentioned, such as the lack of availability of the cellular marker in most cases, as well as completing a screening in which other associated markers could be identified such as the estrogen receptor (ER), Her 2neu and Ki67 and with this have a better overview of the expression of biomarkers to provide better diagnosis, prognosis and management of canine patients with breast cancer.

5. Conclusion

We report on the distribution of molecular subtypes of breast cancer and their associations with some clinicopathological features in dogs. Breast carcinoma in our patients are high-grade tumors and are diagnosed at an advanced stage. The hormone receptor was negative in more than half of the patients. Histological grade is significantly associated with tumor subtypes. This study emphasizes the need to introduce receptor testing into our routine clinical practice to offer the best breast cancer treatment.

Conflicts of Interest

The authors declare that they have no conflict of interest with respect to the publication of this article.

References

- [1] Peña, L., Gama, A., Goldschmidt, M.H., Abadie, J., Benazzi, C., Castagnaro, M., *et al.* (2013) Canine Mammary Tumors. *Veterinary Pathology*, **51**, 127-145. <https://doi.org/10.1177/0300985813509388>
- [2] Rasotto, R., Berlato, D., Goldschmidt, M.H. and Zappulli, V. (2017) Prognostic Significance of Canine Mammary Tumor Histologic Subtypes: An Observational Cohort Study of 229 Cases. *Veterinary Pathology*, **54**, 571-578. <https://doi.org/10.1177/0300985817698208>
- [3] Sanchez, F. (2005) Incidence, Classification and Therapy of Mammary Tumors in Canines. Master's Thesis, Pedagogical and Technological University of Colombia.
- [4] Kim, T., Yang, I.S., Seung, B., Lee, S., Kim, D., Ha, Y., *et al.* (2020) Cross-Species Oncogenic Signatures of Breast Cancer in Canine Mammary Tumors. *Nature Communications*, **11**, Article No. 3616. <https://doi.org/10.1038/s41467-020-17458-0>
- [5] Canadas, A., França, M., Pereira, C., Vilaça, R., Vilhena, H., Tinoco, F., *et al.* (2018) Canine Mammary Tumors: Comparison of Classification and Grading Methods in a Survival Study. *Veterinary Pathology*, **56**, 208-219. <https://doi.org/10.1177/0300985818806968>
- [6] Jaillardon, L., Abadie, J., Godard, T., Campone, M., Loussouarn, D., Siliart, B., *et al.* (2015) The Dog as a Naturally-Occurring Model for Insulin-Like Growth Factor Type 1 Receptor-Overexpressing Breast Cancer: An Observational Cohort Study. *BMC Cancer*, **15**, Article No. 664. <https://doi.org/10.1186/s12885-015-1670-6>
- [7] Seung, B., Cho, S., Kim, S., Bae, M., Lim, H., Kwak, S., *et al.* (2021) Impact of Histological Subtype on Survival in Canine Mammary Carcinomas: A Retrospective Analysis of 155 Cases. *Journal of Comparative Pathology*, **186**, 23-30. <https://doi.org/10.1016/j.jcpa.2021.05.002>
- [8] Alenza, D.P., Rutteman, G.R., Peña, L., Beynen, A.C. and Cuesta, P. (1998) Relation between Habitual Diet and Canine Mammary Tumors in a Case-Control Study. *Journal of Veterinary Internal Medicine*, **12**, 132-139. <https://doi.org/10.1111/j.1939-1676.1998.tb02108.x>
- [9] Shafiee, R., Javanbakht, J., Atyabi, N., Kheradmand, P., Kheradmand, D., Bahrami, A., *et al.* (2013) RETRACTED ARTICLE: Diagnosis, Classification and Grading of Canine Mammary Tumours as a Model to Study Human Breast Cancer: An Clinico-Cytohistopathological Study with Environmental Factors Influencing Public

Health and Medicine. *Cancer Cell International*, **13**, Article No. 469.

<https://doi.org/10.1186/1475-2867-13-79>

- [10] Toniti, W., Buranasinsup, S., Kongcharoen, A., Charoonrut, P., Puchadapirom, P. and Kasorndorkbua, C. (2009) Immunohistochemical Determination of Estrogen and Progesterone Receptors in Canine Mammary Tumors. *Asian Pacific Journal of Cancer Prevention*, **10**, 907-911.
- [11] Liu, D., Xiong, H., Ellis, A.E., Northrup, N.C., Rodriguez, C.O., O'Regan, R.M., *et al.* (2014) Molecular Homology and Difference between Spontaneous Canine Mammary Cancer and Human Breast Cancer. *Cancer Research*, **74**, 5045-5056. <https://doi.org/10.1158/0008-5472.can-14-0392>
- [12] Sorenmo, K.U., Rasotto, R., Zappulli, V. and Goldschmidt, M.H. (2010) Development, Anatomy, Histology, Lymphatic Drainage, Clinical Features, and Cell Differentiation Markers of Canine Mammary Gland Neoplasms. *Veterinary Pathology*, **48**, 85-97. <https://doi.org/10.1177/0300985810389480>
- [13] Owen, L. (1980) TNM Classification of Tumors in Domestic Animals. ED World Health Organization. Organisation mondiale de la Santé.
- [14] Burrai, G.P., Tanca, A., De Miglio, M.R., Abbondio, M., Pisanu, S., Polinas, M., *et al.* (2015) Investigation of HER2 Expression in Canine Mammary Tumors by Antibody-Based, Transcriptomic and Mass Spectrometry Analysis: Is the Dog a Suitable Animal Model for Human Breast Cancer? *Tumor Biology*, **36**, 9083-9091. <https://doi.org/10.1007/s13277-015-3661-2>
- [15] Ji, S., Wu, A. and Yang, H. (2012) Expression of Progesterone Receptor Membrane Component 1 Is Associated with Malignant Phenotypes of Breast Cancer. *Journal of Southern Medical University*, **32**, 635-638.
- [16] Kaszak, I., Ruszczak, A., Kanafa, S., Kacprzak, K., Król, M. and Jurka, P. (2018) Current Biomarkers of Canine Mammary Tumors. *Acta Veterinaria Scandinavica*, **60**, Article No. 66. <https://doi.org/10.1186/s13028-018-0417-1>