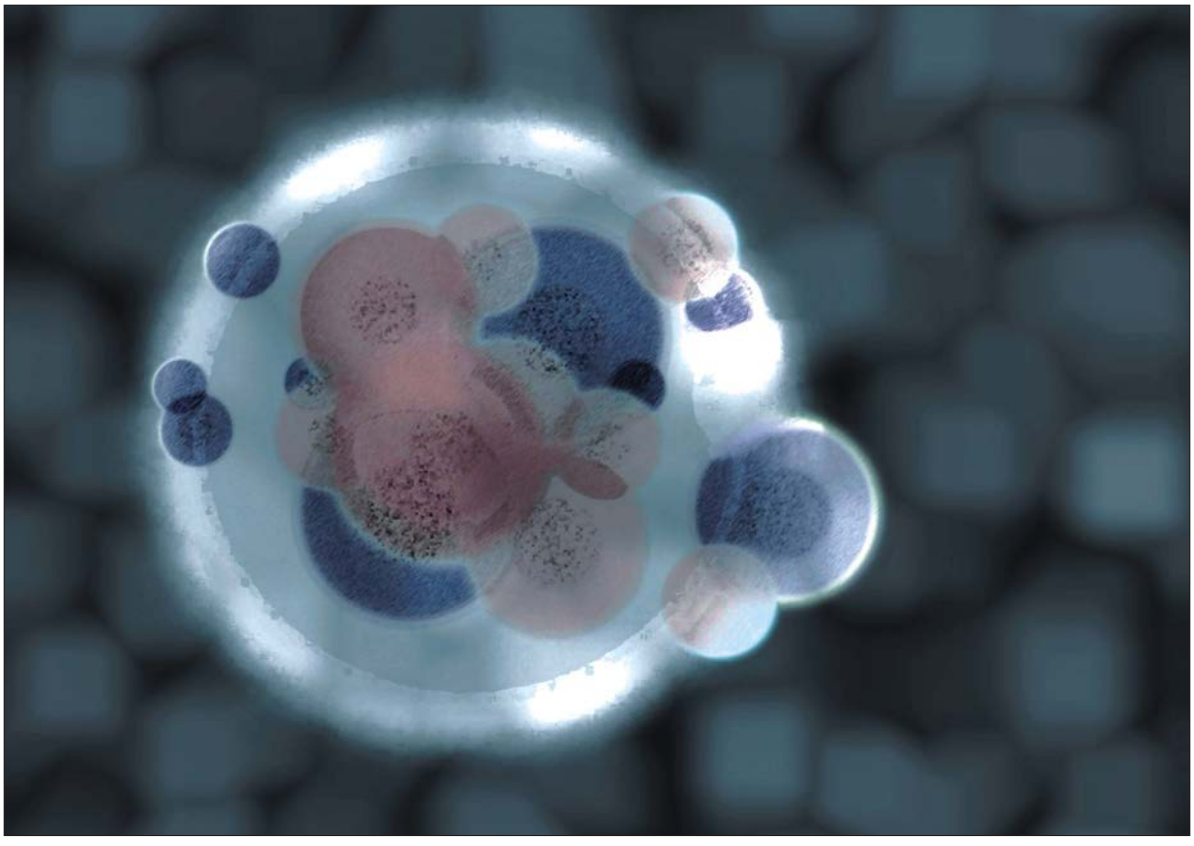

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Grading in Canine Mammary Gland Carcinoma

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Abstract: Objectives of this study were to describe classification and grading in canine mammary carcinoma. The histological diagnosis was made on the basis of the current WHO classification for canine mammary tumors and then tumors were graded histologically in accordance with the Elston and Ellis method for human breast tumors and based on the assessment of three morphological features: tubule formation, nuclear pleomorphism and mitotic counts. The mammary carcinomas of 33 cases were classified to 17 (51.5%) simple carcinoma, 12 (36.4%) complex carcinoma and 4 (12.1%) carcinoma arising in benign tumor. The histological grade of these cases were as follows: grade I, 11 (33.3%); grade II, 7 (21.2%); Grade III, 15 (45.5%). Present results illustrated good relationship between tumors grading and histological type also revealed that most grade II and III of these tumors were classified as simple one. Despite of many methods for grading such as Gilbertson and Misdorp method used in this research was less complicated and more comparable with human medicine. So, this routine use of this method would help the pathologist and clinician for more accurate prognosis and treatment in canine mammary carcinoma and facilitate comparative studies of canine and human researches.

Key words: Dog, mammary carcinoma, grading

INTRODUCTION

Mammary neoplasms in dogs are second in frequency after skin tumors and they are the most common types of tumors in the bitch. According to the histological diagnosis, between 41 and 53% of the mammary tumors that occur in the bitch are considered malignant (Misdorp, 2002). Major problem in canine mammary carcinoma is accurate prognosis for post surgical cases (Benjamin *et al*., 1999; Karayannopoulou *et al*., 2005) and reliable prognostic factors are of great importance for estimating the clinical outcome (Misdorp, 2002; Martin De Las Mulas *et al*., 2005). Since mammary tumors are common in dogs, many attempts have been made to improve their histological classification in order to predict their biological behavior (Dutra *et al*., 2004). The histologic heterogeneity observed in canine mammary carcinoma presents considerable difficulties in the design of a classification system that will assure reproducibility of a prognostically meaningful categorization. These difficulties are reflected by the number of classification system proposed so far (Gilbertson *et al*., 1983). On the other hand, establishing an animal model of spontaneous mammary carcinoma is paramount for testing new treatment and preventive modalities before human clinical trials. Unlike laboratory rodents, dogs share a common environment with people and, therefore, may be exposed to some of the same carcinogens. As in humans, advancing age, progesterone treatment, obesity in early life and diet also increased the risk of mammary tumors in the dog. They are also more outbreeding than laboratory rodents, yet certain breeds are at increased risk for developing mammary tumors. Because dogs have a shorter life span than people, it is possible to study mammary carcinoma and invasive tumors that develop after a few years instead of decades (Millanta *et al*., 2002; Pena *et al*., 2003; Nieto *et al*., 2003; Gama *et al*., 2004; Dutra *et al*., 2004; Karayannopoulou *et al*., 2005; Antuofermo *et al*., 2007; Cadieu and Ostrander, 2007; Munson and Moresco, 2007).

Grading of neoplasm represents an attempt to quantitated characteristics and a grade of any cancer is one of the most important pieces of information needed by clinician who treat cancer (Elston and Ellis, 1991). Without grading, it has been difficult and more often impossible to compare results of studies by different investigator and to arrive at valid conclusions about the biologic characteristics of different types of mammary carcinoma (Gilbertson *et al*., 1983). Nottingham method, a modification of the original Bloom and Richardson method

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by Elston *et al*. (1982), has been successfully applied and its value as an independent prognostic factor demonstrated by a study of a large number of patients, with long- term follow-up (Elston and Ellis, 1991). The aim of present study was to express and describe the validity of the grading by Elston and Ellis method in canine mammary carcinoma accompanying with classification of the mammary carcinoma. Despite common rate of canine mammary carcinoma in Iran, there was no attempt to grade these in literature and assumed this is the first report.

MATERIALS AND METHODS

Animals: Thirty three tissue samples of mammary carcinomas were studied. They were obtained from bitches that underwent surgery at the Veterinary Hospital and sent to Department of Pathology or euthanized or took out from the archive of Department of Pathology, Veterinary School of Tehran University, from September 2006 till September 2007. The animals, aged 4-15 years, had not been undergone chemotherapy or radiotherapy. Eleven of the cases were followed up for one year after resection.

Histopathology and classification: Samples from tumors were fixed in 10% buffered formalin and embedded in paraffin. Sections were cut at 4 µm and stained with Haematoxylin and Eosin. The histological diagnosis was made on the basis of the current WHO classification for canine mammary gland tumors (Misdorp *et al*., 1999; Misdorp, 2002). Those displaying mixed features were classified according to the most frequent histological differentiation. The tumors were assessed on a double-headed microscope by two observers, one pathologist with a special interest in mammary gland tumors and other a trainee pathologist. Where variance between the observers occurred on a particular feature, a consensus decision was reached by discussion.

Grading studies: The grading system we used is the Elston Grade which is short name of Elston modification of the Bloom-Richardson grading system and Nottingham method. Assessment of this Semiquantitative method for histological grades is by evaluating the cancer for three parameters, tubules, nuclear variation and mitotic activity and then sum the designated points in order to produce a possible between 3 to 9 points and score ranges equate to roman numeral grades (Elston and Ellis, 1991).

Tubule formation: For evaluating tubule formation, qualitatively tubular structures must exhibit clear central lumina; quantitatively all parts of each tumor blocks were scored and the proportion occupied by such tubular structures was assessed semiquantitatively. A score of one point is allocated when more than 75% of the area is composed of definite tubules. Two points are appropriate for tumors in which between 10 and 75% of the area shows tubule formation. Where tubules occupy 10% or less of the tumor, three points are assigned (Elston and Ellis, 1998).

Nuclear pleomorphism: When the tumor nuclei are small, with little increase or variations in size compared with normal nuclei and have regular outlines and uniformity of nuclear chromatin, one point is appropriate. A score of 2 points is given when the nuclei are larger than normal, have more open vesicular nuclei with visible, usually single, nucleoli and there is a moderate variation in size and shape. A marked variation in size and shape, especially when very large and bizarre nuclei are present, scores 3 points; furthermore nuclei are vesicular with prominent enlarged and often multiple nucleoli (Elston and Ellis, 1998).

Mitotic counts: Mitotic activity is best assessed at the periphery of tumor, where active growth is most likely and a minimum of 10 high power fields are assessed. No hyperchromatin or pyknotic nuclei were counted because these cells may be undergoing necrosis or apoptosis. Only structures which could not be misinterpreted as anything expect mitotic figures were counted. Up to 7 mitosis per 10 high fields are scored 1 point, 8-16 are scored 2 points and more than 17 are scored 3 points (Elston and Ellis, 1998). Scoring was originally performed using an Olympus BX-40 microscope fitted with a 10x eyepiece and a 40x objective, which provide a field area of 0.239 mm^2.

After adding up together the scores of each category a number between 3 and 9 would be obtained, then Elston Grade allocated on the following basis: 3 to 5 points formed Elston grade I, which are well differentiated, 6-7 points formed Elston grade II, which are moderately differentiated and 8 to 9 points formed Elston grade III, which are poorly differentiated.

RESULTS

Thirty three malignant mammary carcinomas were classified according to the World Health Organization criteria to 17 (51.5%) simple carcinoma, with one type of cell, 12(36.4%) complex carcinoma, with both epithelial and myoepithelial components which arranged in a more or less stellate, reticulated pattern and 4 (12.1%) carcinoma arising in benign tumor, with foci of atypical cells in benign mixed tumors. Simple carcinomas were subclassified to 12 (70.6%) tubulopapillary carcinoma, with formation of tubules with papillary projections, 4 (23.5%) solid carcinoma, with arranging in solid sheets, cords, or nests and 1 (5.9%) Cribriform carcinoma, with solid shape accompanying by small apertures like a sieve.

The histological grading of the 33 cases were as follows: grade I, 11 (33.3%), with clear lumen formation (Fig. 1) which occupied more than 10% of area, cells with regular outline and uniform and less than 7 mitosis in 10 field; grade II, 7 (21.2%) and grade III, 15 (45.5%), most tumor area included no lumen formation and cell with pleomorphic shape, vesicular nuclei with visible, multiple nucleoli and multiple bizarre and more than 17 mitosis in 10 field (Fig. 2). The relationship between tumors grading and histological type and subclassification of simple

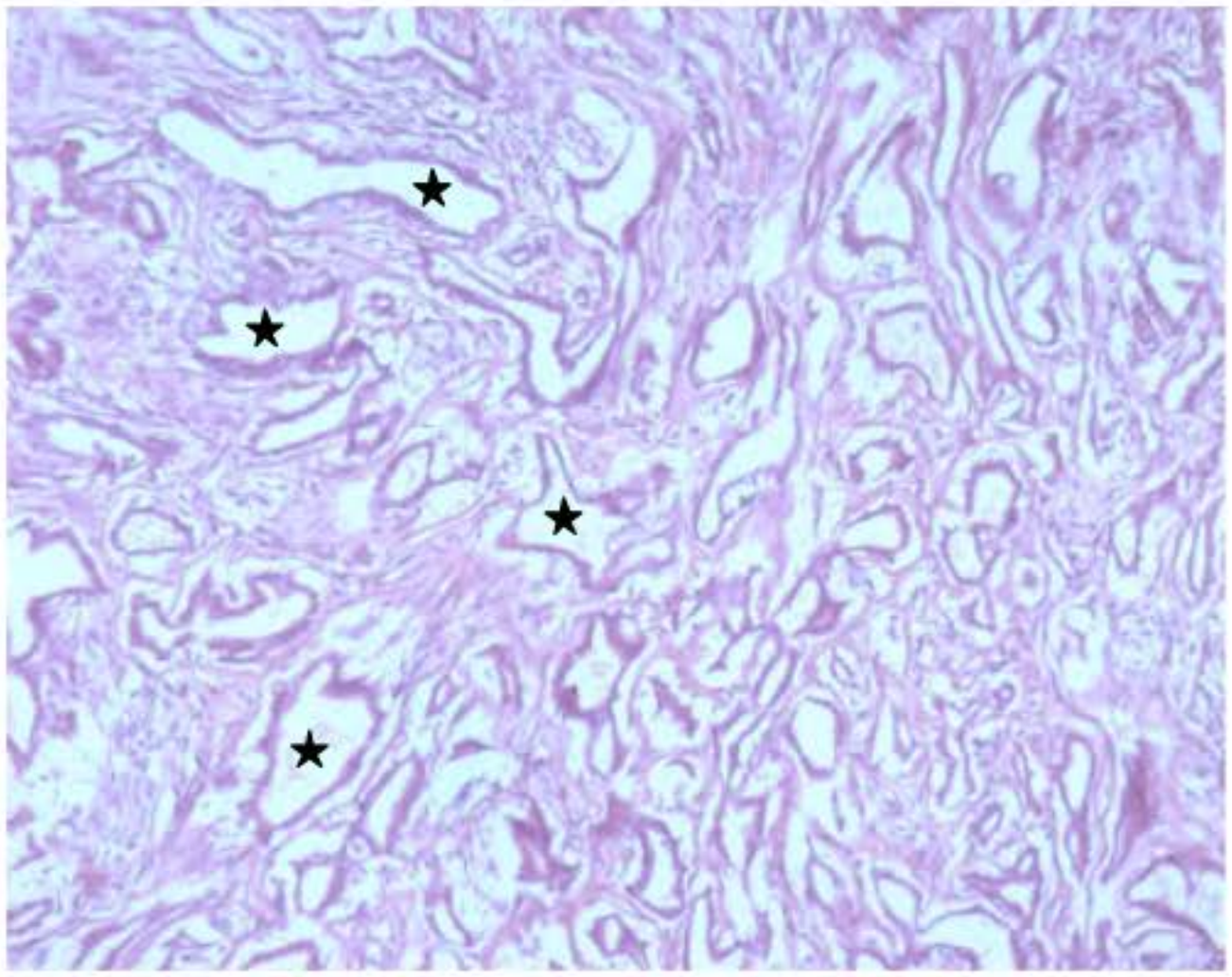

Fig. 1: Simple carcinoma, subclassification of tubulopapillary carcinoma. This tumor was graded I, well differentiated. Note the tubule with clear lumen formation (stars), (H and E, x132)

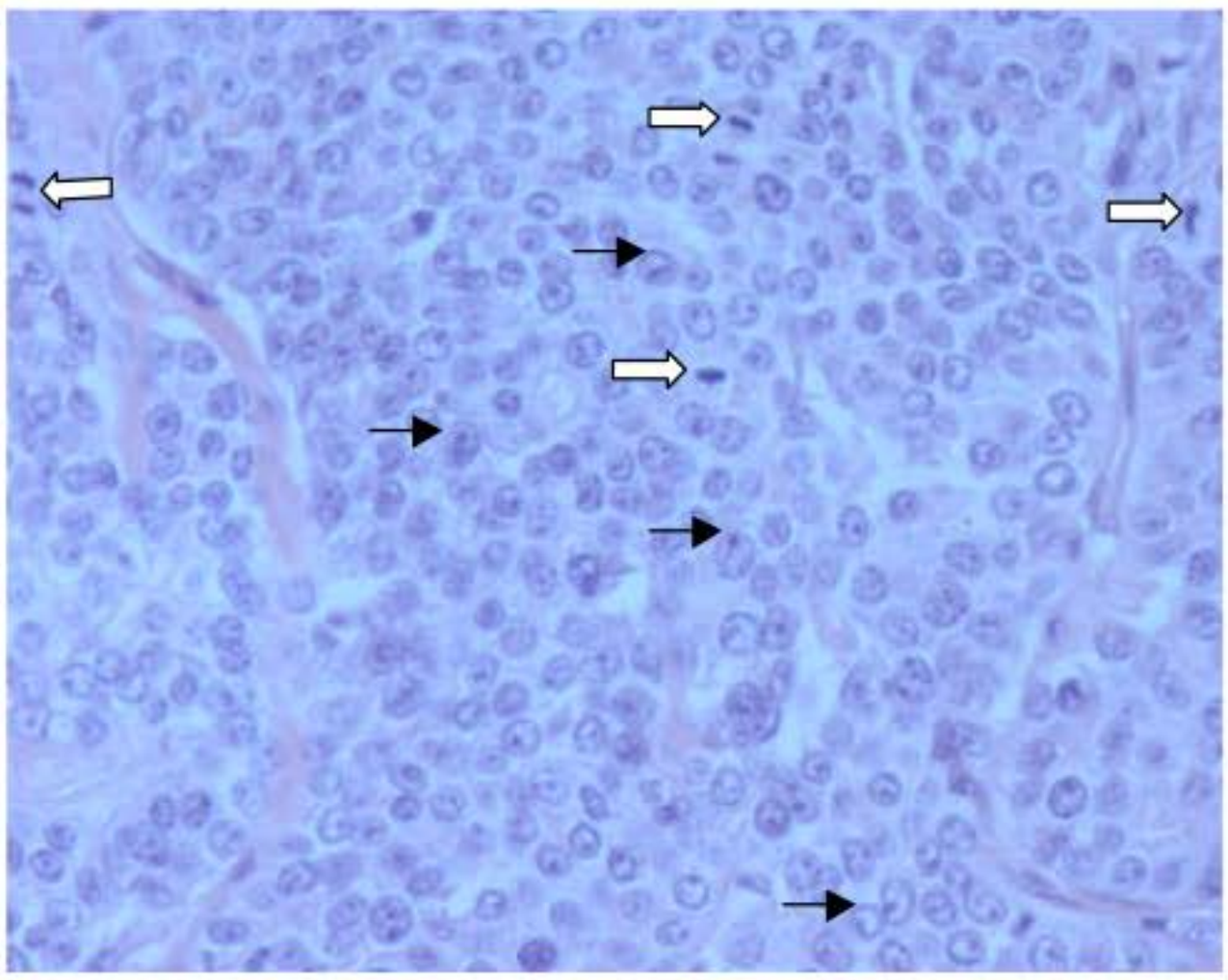

Fig. 2: Simple carcinoma, subclassification of Solid Carcinoma with grade III. Note to lack of tubule formation, large vesicular nuclei with prominent nucleoli (Black arrows) and different mitotic figures (White arrows) and high mitotic index (H and E, x528)

Table 1: Relationship between histological grading and tumor type in 33 dogs with mammary carcinoma

Histological type	Grade I	Grade II	Grade III	Total
Simple carcinoma	3 (17.6%)	4 (23.5%)	10 (58.8%)	17 (100%)
Complex carcinoma	5 (41.7%)	3 (25%)	4 (33.3%)	12 (100%)
Carcinoma arising in benign tumor	3 (75%)	0	1 (25%)	4 (100%)
Total	11 (33.3%)	7 (21.2%)	15 (45.5%)	100

Table 2: Relationship between histological grading and subclassification of simple carcinomas in 17 dogs with simple mammary carcinoma

Histological type	Grade I	Grade II	Grade III	Total
Tubulopapillary carcinoma	3 (25%)	3 (25%)	6 (50%)	12 (100%)
Solid carcinoma	0	0	4 (100%)	4 (100%)
Cribriform carcinoma	0	1 (100%)	0	1 (100%)
Total	3 (17.6%)	4 (23.5%)	10 (58.8%)	100

carcinomas is presented in Table 1 and 2, respectively. One year after the resection of the 11 cases, 8(72.7%) dogs were alive while 3(27.3%) had died as a consequence of canine mammary carcinoma. All three cases were in grade III but 2 had tubulopapillary carcinoma and 1 had Solid carcinoma.

DISCUSSION

In this attempt, relation between morphological classification and histologic grade was assessed in 33 canine mammary carcinomas according to the latest WHO classification and semi-quantitative method of Elston and Ellis. Simple carcinomas and complex carcinomas were common in dogs and it confirms other reports from Misdorp (1999, 2002).

Malignant mixed mammary tumor is familiar expression for pathologist and clinician and some pathologists use it to describe a mixed tumor with a single malignant component, epithelial or mesenchymal and etc. Benjamin *et al*. (1999) stated that term of malignant mixed tumor is a false term since confusion exists about using of this term also Gartner *et al*. (1999) reinforced the role of myoepithelial cells in mesenchymal metaplasia in mixed tumors. Benjamin *et al*. (1999) called these tumors carcinoma or sarcoma in a mixed tumor in their reclassification that is much more accurate and understandable designation and that has biological significance. The WHO scheme also used carcinosarcoma as a synonym for malignant mixed tumor, despite the connotation of carcinosarcoma, which implies that both carcinomatous and sarcomatous components must be involved (Benjamin *et al*., 1999). In new classification of WHO in 1999 and 2001 the use of the term malignant mixed tumor was discarded and replaced by carcinoma and sarcoma in benign tumors.

In the present study, a correlation between histological type and grade was evident (Table 1). Complex carcinomas and carcinoma arising in benign tumor were grade I or II but simple carcinomas (the most malignant type) were usually grade III or II that is in agreement with Benjamin *et al*. (1999) and Misdorp *et al*. (1999) and Karayannopoulou *et al*. (2005). Similar observations were reported in human patients by Elston and Ellis (1991).

In literature there are different methods for grading, Misdorp (Restucci *et al*., 2000), Gilbertson (Preziosi *et al*., 1995; Pena *et al*., 1998; Sarli *et al*., 2002), Lagadic and Estrada (Martin De Las Mulas *et al*., 2005) and Elston and Ellis (Millanta *et al*., 2002; Pena *et al*., 2003; Nieto *et al*., 2002; Gama *et al*., 2004; Dutra *et al*., 2004; Munson and Moresco, 2007) and many researchers have commented on the difficulty of comparing results from different reported studies of canine mammary cancer because of the diversity of opinions concerning the biologic characteristics of different morphologic forms. Karayannopoulou *et al*. (2005) reported that in canine mammary carcinomas Elston Grade was significantly related to prognosis, especially in cases of simple carcinoma and this is in agreement with our findings. Castagnaro *et al*. (1998) expressed good predictive value for Elston Grade in respect of grade I and III in feline mammary tumors. The Elston and Ellis method has been adopted for use in the breast cancer pathological data sets and is the system recommended by the International Union against Cancer and WHO. Their next challenge for the future is not to search for further prognostic factors but to identify those, which in addition to hormone receptor and c-ErbB-2 status, can be used to predict response to specific therapies (Elston and Ellis, 2002). Millanta *et al*. (2002), Nieto *et al*. (2002), Pena *et al*. (2003), Gama *et al*. (2004), Dutra *et al*. (2004) and Munson and Moresco (2007) utilized Elston method grading in canine mammary gland tumors researches. So Elston grade is more expedient and practical between researchers to compare data.

The most accurate evaluation of nuclear pleomorphism is by use of morphometry or computer image analysis, which are expensive and time consuming process and impractical for routine diagnostic practice (Elston and Ellis, 1998) but we used normal epithelial cells adjacent to the tumor cells as reference point. In the cases

with no normal cells, we found inflammatory cells such as lymphocytes for comparing with tumor cells, with taking into account their smaller size than epithelial cells. In mitotic counting, only mitotic figures counted without hyperchromic nuclei (prophase stage) suspected apoptotic cells or intratumoral lymphocytes so excluding hyperchromic nuclei is important for avoiding overgrading. In mitotic count another mandatory point is attention to high power field of microscope. As we know high power field varies up to six fold from microscope to microscope. Elston and Ellis standardized field area and by using this convention comparing data is possible. After measuring of the field diameter at the appropriate magnification the correct point score is obtained by plotting the actual mitotic count per 10 fields against diameter as shown in Fig. 3 (Elston and Ellis, 1998).

The measurement of only one of the parameters (tubule formation, nuclear pleomorphism, mitotic count) associated with histological grade is unlikely to provide powerful prognostic information (Elston and Ellis, 1991; Misdorp, 2002; Karayannopoulou *et al*., 2005). Complete histological grading is therefore preferable to nuclear grading for accurate prognosis.

The first prerequisite for accurate histological grading is careful specimen preparation. From the time of the surgical or biopsy procedures onward, the tissue specimen must be properly handled (no squeezing or pressing which might distort the microscopic features), properly fixed, carefully sliced and portion trimmed to proper size and thickness so that the various processing chemicals will be able to optimally perform each of their jobs, properly chemically processed, expertly microtome-sectioned into tissue ribbons, carefully mounted on slides and passed through a chemical series which removes the wax and rehydrate the tissues and carefully stained. If tissues are roughly handled, cut too tick, or over- stained with dye, overgrading becomes a risk. Delay in fixation, improperly defatted and dehydrated prior to wax infiltration, or if wax is insufficiently removed prior to staining, and/or under stained, undergrading becomes a risk. Pathologist and histotechnologist form a team who combine forces to perform series of activities to accurate grading (Elston and Ellis, 1998).

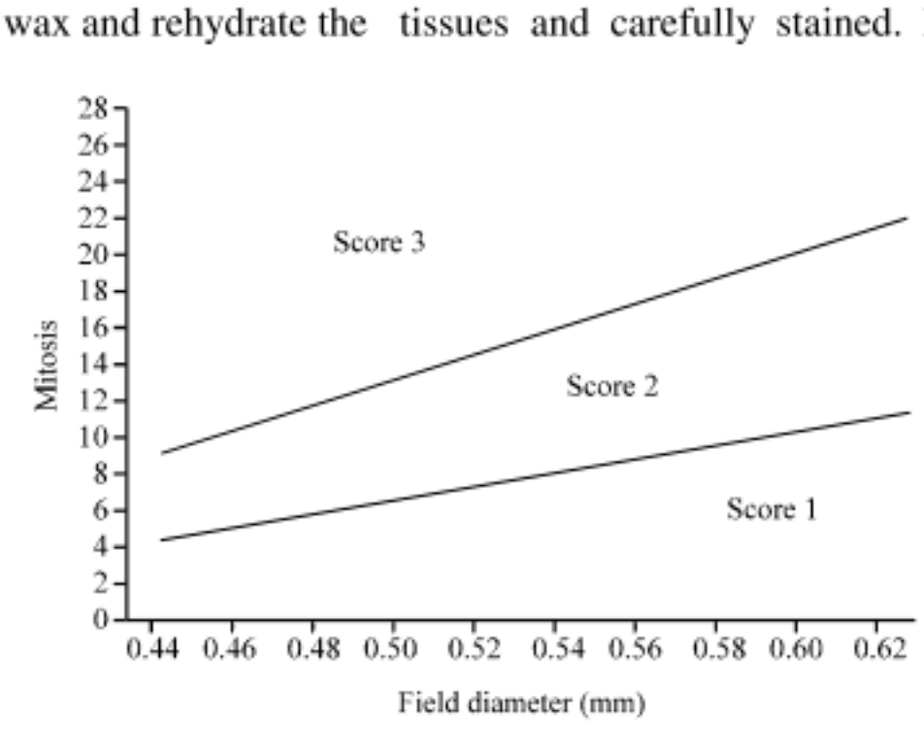

Fig. 3: A plot of the actual mitotic count per 10 fields against diameters

In conclusion grading is a simple and practical method for prognosis and so to decide better treatment. Furthermore, it will be useful for future researches to adjust histologic behavior with other methods such as immunohisochemical studies and different therapeutic researches in canine mammary tumors and human breast cancers. Most of pathologists who have research in canine mammary gland tumor use this method but in our country this is the first study in this area and authors hope to this method be routine between pathologists and clinicians.

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