

## Ultrasonographic characteristics of benign mammary lesions in bitches

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**ABSTRACT:** The aim of this study was to evaluate the applicability of B-mode, Doppler, acoustic radiation force impulse elastography and contrast-enhanced ultrasound exams in the differentiation of neoplastic and non-neoplastic benign mammary lesions in bitches. This research was conducted as a prospective secondary observational cohort (2014–2016) study, which included 36 mammary lesions, evaluated physically and by ultrasound (B-mode, Doppler, contrast-enhanced ultrasound and acoustic radiation force impulse) exams prior to mastectomy and histopathological classification as neoplastic or non-neoplastic lesions. All ultrasonographic parameters studied were compared between histopathological classifications using Fisher’s or Student’s tests and differences were considered significant when  $P < 0.05$ . Out of 36 benign mammary lesions evaluated, 25 were classified as neoplastic and 11 as non-neoplastic. The qualitative and quantitative parameters evaluated using the different ultrasound methods were not effective ( $P > 0.05$ ) in differentiating between neoplastic and non-neoplastic mammary masses in bitches. Nevertheless, some B-mode variables, such as longitudinal length ( $P = 0.0292$ ), width/length ratio ( $P = 0.0001$ ) and width/height ratio ( $P = 0.0001$ ) showed limited efficacy in the differentiation of mammary lesions types. In conclusion, ultrasonographic evaluation of benign canine mammary lesions did not allow differentiation between neoplastic and non-neoplastic tissues and only a few B-mode variables may aid in the prediction of specific tumour types.

**Keywords:** Doppler; acoustic radiation force impulse; ARFI elastography; contrast-enhanced ultrasound; dogs; neoplastic

Mammary tumours are among the most common cancers in women and bitches, causing high morbimortality rates (Nyman et al. 2006; Gokhale 2009). These lesions have been extensively studied in bitches as an experimental model of human breast cancer (Feliciano et al. 2012). The prevalence of benign mammary lesions is dependent on the geographic region and on several epidemiological, sociological and cultural conditions such as life expectancy, nutrition, contraceptive administration and treatment delay (Oliveira et al. 2010). In this

sense, the mean prevalence of benign mammary lesions in developed countries is approximately 50% (Bostock 1986), while in less developed countries, like Brazil, this incidence varies between 10 and 30% (Oliveira et al. 2010). Canine mammary tumours account for approximately 50% of all tumours in female dogs and 82% of all tumours in the reproductive tract (Oliveira et al. 2010).

The presumptive diagnosis of mammary lesions is commonly based on clinical signs, history and on physical and cytological exams (Cassidy et al.

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2015). Biopsies are not usually made of mammary lesions due to the invasiveness of this procedure and because it may lead to inappropriate diagnostic delay (Masciadri and Ferranti 2011). Benign mammary lesions comprise a heterogeneous group of tissue alterations including developmental abnormalities, inflammatory lesions, fibrocystic changes, stromal lesions and neoplasms; only neoplastic lesions are associated with an increased risk of subsequent mammary cancer (Guray and Sahin 2006; Cassidy et al. 2015). In this context, non-invasive pre-surgical differentiation between neoplastic and non-neoplastic benign lesions can prevent unnecessary invasive surgery. However, a rapid, simple, non-invasive and safe technique that can be used to confirm this diagnosis is lacking (Gonzales de Bulnes et al. 1998).

Ultrasound exams have become an important tool for the evaluation of neoplasms, as they can complement diagnostic information, predict the malignancy of mammary neoplasms in women and bitches (Davoudi et al. 2014; Feliciano et al. 2017), aid in canine mammary carcinoma classification (Feliciano et al. 2018) and in the differentiation of neoplastic and non-neoplastic lesions in women (Masciadri and Ferranti 2011). However, to the best of our knowledge, no studies describing the ultrasonographic characteristics of benign mammary lesions in bitches have been reported in the literature. Thus, the aim of this study was to evaluate the applicability of B-mode, Doppler, acoustic radiation force impulse (ARFI) elastography and contrast-enhanced ultrasound (CEUS) exams for the differentiation of neoplastic and non-neoplastic benign mammary lesions in bitches.

## MATERIAL AND METHODS

**Ethical aspects.** This study follows the guidelines of the Brazilian Council for Control of Animal Experimentation and was approved by the Ethics Committee in the Use of Animals of the School of Agrarian Sciences and Veterinary Medicine, Univ. Estadual Paulista, Jaboticabal-SP, Brazil (protocol No. 023705/12). Pet owners provided free and informed consent to the inclusion of their animals.

**Experimental design.** In this prospective secondary observational cohort (2014–2016) study, data from our previous study describing physical and ultrasound examinations performed prior to

mastectomy and histopathological classification of canine mammary masses diagnosed as benign lesions were analysed (Feliciano et al. 2017). With these inclusion criteria, a total of 36 masses from 28 bitches were enrolled. The following clinical characteristics were evaluated: localisation, growth time and palpation consistency and surface.

Ultrasound exams were performed by a single experienced veterinary sonographer prior to mastectomy, and subsequent histopathological classification as a neoplastic or non-neoplastic lesion and tissue typing were conducted according to Misdorp et al. (1999) and Cassali et al. (2013). Each mammary mass was evaluated using different ultrasonographic methods (B-mode, Doppler, ARFI elastography and CEUS) in the order described below, according to Feliciano et al. (2017), and using Acuson S2000® equipment (Siemens, Munich, Germany) with a 9.0 MHz linear transducer.

**B-mode ultrasonography.** Mammary lesions were evaluated based on echotexture (homogeneous or heterogeneous), echogenicity (hypo-, hyperechoic, or mixed with solid or liquid components; in relation to the adjacent and normal mammary tissue), contours (defined or undefined), invasiveness (present or absent) and other findings (presence of cystic, anechoic and hyperechoic areas or acoustic shadowing). Length (cm), width (cm), and width/length ratio in longitudinal section and height (cm), width (cm), and width/height ratio in transverse sections were obtained.

**Doppler.** In the colour Doppler of lesions, neovascularisation (presence or absence), localisation (peripheral, central or diffuse) and the type of vessel (perinodular, vessels around mass parenchyma; mosaic, random vascular points into the parenchyma or network) were evaluated in the masses.

The vascular index of neovascularisation was obtained using spectral Doppler: systolic velocity (cm/s), diastolic velocity (cm/s), resistive index (systolic velocity – diastolic velocity)/systolic velocity), characteristic (arterial or turbulent) and pattern (high, intermediate, or low resistivity) of blood flow (Feliciano et al. 2012). The angle between the Doppler beam and the vessel's long axis did not exceed 60°. Colour gain was adjusted to reduce excessive colour noise when blood flow was too slow. A 2–4-mm gate with apertures was positioned at the centre of the vessel to measure the flow's spectral trace, spectral curve and vascular indexes, which were obtained automatically following

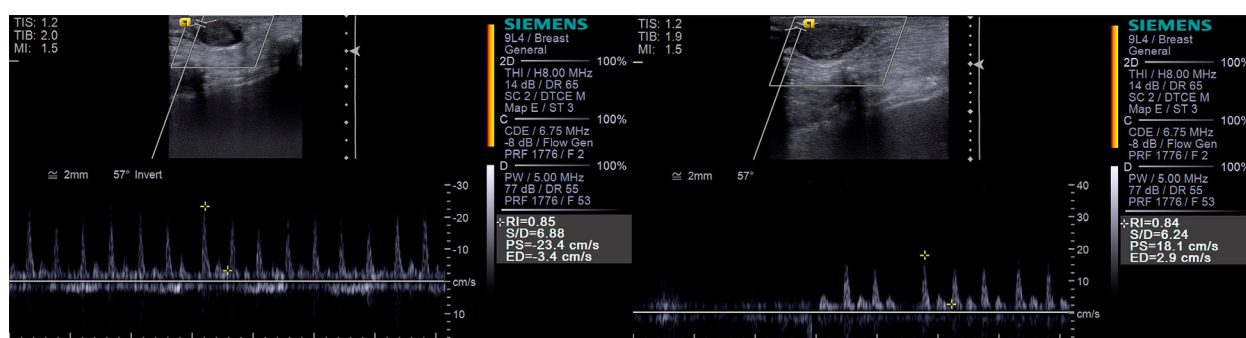


Figure 1. Doppler ultrasonography image of a fibroadenoma in a female dog. Spectral Doppler ultrasonographic image of mass with tumour neovascularisation and blood flow velocity waveforms in the benign lesion

software identification of the ultrasonic scanner for each waveform. A minimum of three subsequent waves were used in the evaluation (Figure 1).

**Contrast-enhanced ultrasonography.** Contrast-enhanced ultrasonography (CEUS) was performed using contrast-specific software (Cadence®, Siemens, Munich, Germany) and after delineation of the mass area, the probe was held steadily and the adjustable parameters such as depth, gain, mechanical index and focal zones were optimised and maintained. Contrast agent (SonoVue®, Bracco, Milan, Italy) was immediately administered as an intravenous bolus (0.1 ml, followed by 5 ml saline flush) via a catheter in the cephalic vein. Video clips were obtained for 5 min following bolus injection of contrast and recorded in the internal storage system for each mass assessed.

Microbubble perfusion and the dynamic enhancement of the image of each lesion were analysed based on the presence or absence of contrast (Figure 2); wash-in time (seconds), enhancement peak (seconds), and wash-out time (seconds); and enhancement characteristics: (1) enhancement level relative to surrounding normal mammary tissue (hyper-, iso- or hypo-enhancement), (2) pattern (centripetal, centrifugal or diffuse), (3) localisation (central, peripheral or diffuse), (4) internal homo-

geneity (homogeneous or heterogeneous) and (5) perfusion type (discreet, moderate or increased) (Tagawa et al. 2016).

**Acoustic radiation force impulse elastography.** Elastographic analyses were performed using the VTIQ method of ARFI (virtual touch tissue imaging quantification, 2D-SWE technique) (Feliciano et al. 2014; Tang et al. 2015). Qualitative ARFI generated elastograms that were evaluated according to deformability (deformable or not deformable); whitish tones (bluish areas, soft or deformable tissues) corresponded to more elastic tissues and darker tones (reddened areas, hard or non-deformable tissues) to more rigid tissues. Quantitative ARFI consisted of a software function that determined shear wave velocity once the calliper was positioned on the mass parenchyma. Six measurements of different randomly selected areas in each tissue (cranial, caudal, central, superficial, deep and medium regions) were used to determine the mass mean shear wave velocity (SWV m/s) (Figure 3).

**Histopathological classification.** Samples of the mammary tumours were collected after mastectomy for histopathological evaluation. Multiple tissue fragments were fixed in 10% formaldehyde solution in phosphate buffer (pH 7.4) and routinely processed for histopathology analysis prior

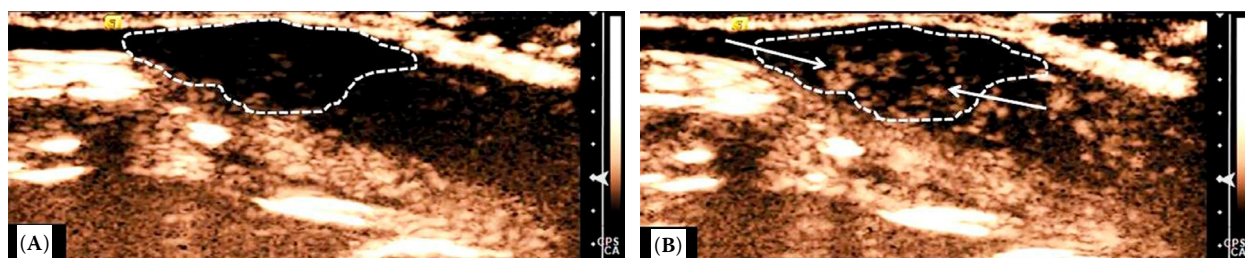


Figure 2. Contrast-enhanced ultrasonography image 15 seconds after contrast application to a simple adenoma in a female dog. (A) Absence of contrast in tumour (dashed line) and (B) peak enhancement and diffuse enhancement (dashed line and arrows)



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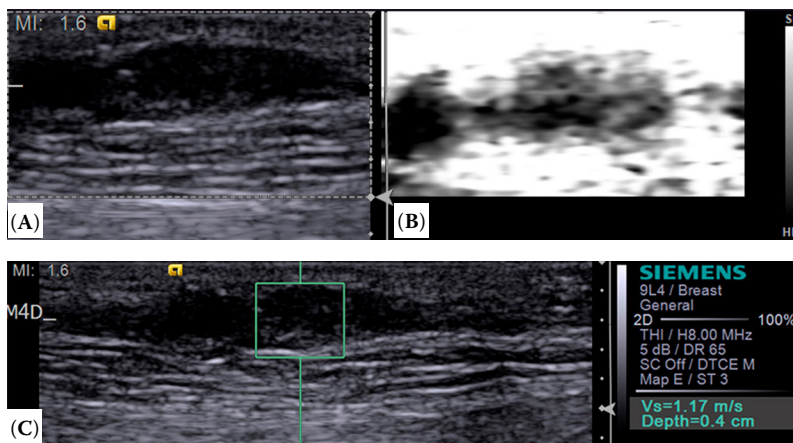


Figure 3. Acoustic radiation force impulse (ARFI) elastography image showing stiffness characteristics in a benign mixed tumour in a female dog. Image of the qualitative ARFI: (A) B-mode of the mammary tumour; and (B) elastogram with characteristics of the stiffness of the lesion: deformable and heterogeneous with soft (white) and hard areas. (C) Image of the quantitative ARFI in the mammary tumour with the calliper in the parenchyma, which was used to measure the shear wave velocity

to paraffin embedding. Tissue sections (5 µm) were mounted onto glass slides and stained with haematoxylin and eosin (HE). These lesions were analysed by a single, experienced pathologist under a light microscope, classified as neoplastic or non-neoplastic and were typed according to World Health Organization guidelines (Misdorp et al. 1999; Cassali et al. 2013).

**Statistical analysis.** Statistical analysis was performed using the software R, version 3.3.0 (R® foundation for statistical computing, Austria). Qualitative ultrasound variables were compared between neoplastic and non-neoplastic histopathological classifications using Fisher's test, while quantitative variables were compared using Student's test and differences were considered significant when  $P < 0.05$ . For ultrasonographic parameters that showed significance, the cut-off point, sensitivity, specificity, accuracy and area under the curve were calculated using histopathological classification as a reference for receiver-operating characteristic curve analysis in a logistic regression model aimed at assessing and comparing the diagnostic performance of each technique.

## RESULTS

The following breeds of bitches were enrolled in this study: ten mixed breeds (36%), six Poodles (21%), four Pitbulls (14%) and eight other breeds (29%) (Dobermann, German shepherd, Pinscher, Labrador retriever and Rottweiler). The mean weight was  $17.8 \pm 12.9$  kg. Thirty-one (86%) of the lesions showed slow evolution (> six months) and five (14%) exhibited fast evolution (< six months). Twenty-eight (78%) lesions were of regular aspect

and eight (22%) were irregular. As to consistency, 31 (86%) were considered firm and five (14%) floating. Histopathological exams revealed 19 (59%) benign mixed tumours, seven (19%) lobular hyperplasias, four (11%) simple adenomas, three (8%) ductal hyperplasias and three (9%) other neoplasms (tubular adenoma, fibro adenoma and acinar hyperplasia).

From the 36 evaluated lesions, 25 (69%) were histopathologically classified as neoplastic and 11 (31%) as non-neoplastic (Table 1). The mean age of these animals was  $10.7 \pm 2.5$  years; however, according to the lesion type, the mean age was  $10.5 \pm 2.8$  years for the patients with neoplastic lesions and  $11.3 \pm 1.3$  years for those with non-neoplastic lesions. Considering the localisation, 27 (75%) were preceding from the right mammary chain and nine (25%) from the left one; the most affected mammary gland was the abdominal caudal (10, 28%), followed by the thoracic caudal (nine, 25%), abdominal cranial (eight, 22%), inguinal (seven, 19%) and thoracic cranial (two, 6%).

The qualitative and quantitative parameters evaluated using different ultrasonographic methods

Table 1. Histopathological classification of benign canine mammary tumours (Misdorp et al. 1999; Cassali et al. 2013)

Type	Diagnosis	Quantity
Neoplastic	simple adenoma	4
	tubular adenoma	1
	fibroadenoma	1
	benign mixed tumour	19
Non-neoplastic	acinar hyperplasia	1
	ductal hyperplasia	3
	lobular hyperplasia	7
Total		36

Table 2. Values of qualitative variables (%) evaluated using different ultrasonographic methods (B-mode, Doppler and acoustic radiation force impulse (ARFI) elastography) in benign canine mammary tumours

Variables	Parameters	Neo- plastic	Non-neo- plastic	<i>P</i> -value
<b>B-mode ultrasonography</b>				
Echotexture	homogeneous	48	55	1.0000
	heterogeneous	52	45	
Echogenicity	hypoechoic	48	55	0.2249
	mixed	52	45	
Contours or margins	defined	100	100	1.0000
	undefined	0	0	
Invasiveness	present	0	0	1.0000
	absent	100	100	
<b>Doppler ultrasonography</b>				
Vascularization	present	68	70	1.000
	absent	32	30	
Localization	peripheral	88	43	0.1879
	central	6	0	
	diffuse	6	57	
Vessel type	perinodular	18	14	0.0819
	mosaic	71	29	
	network	12	57	
Characteristics	arterial	100	100	1.0000
	turbulent	0	0	
Patterns	high resistivity	24	29	0.2589
	intermediary	71	43	
	low	6	29	
<b>ARFI elastography</b>				
Deformability	deformable	64	73	0.7148
	not deformable	36	27	

(B-Mode, Doppler, CEUS and ARFI elastography) were not statistically significant ( $P > 0.05$ ) from each other for differentiation between neoplastic and non-neoplastic benign mammary lesions (Tables 2 and 3).

Quantitative ultrasonographic parameters were subsequently compared between mammary lesion types (Table 4), and there were no significant differences ( $P > 0.05$ ) for the variables studied using Doppler (Figure 1), CEUS (Figure 2) and ARFI elastography (Figure 3). However, some B-mode ultrasound parameters were different between lesion types: tubular width in the longitudinal section was lower in adenoma than in fibroadenoma, ductal hyperplasia, lobular hyperplasia and mixed

Table 3. Mean  $\pm$  SD of quantitative variables evaluated using different ultrasonographic methods (B-mode, Doppler, contrast-enhanced ultrasonography and acoustic radiation force impulse (ARFI) elastography) in benign canine mammary tumours

Variables	Parameters	Neoplastic	Non-neo-plastic	P-value
<b>B-mode ultrasonography</b>				
Measures	longitudinal width (cm)	1.51 $\pm$ 1.14	1.99 $\pm$ 1.55	0.6141
	longitudinal length (cm)	0.76 $\pm$ 0.92	0.82 $\pm$ 0.88	0.9867
	width/length ratio	0.49 $\pm$ 0.42	0.42 $\pm$ 0.18	0.5475
	transverse height (cm)	1.41 $\pm$ 0.99	1.89 $\pm$ 1.51	0.6835
	transverse width (cm)	0.69 $\pm$ 0.82	0.80 $\pm$ 0.61	0.2772
	width/height ratio	0.48 $\pm$ 0.32	0.48 $\pm$ 0.13	0.3996
<b>Doppler ultrasonography</b>				
Vascular indexes	systolic velocity (cm/s)	20.27 $\pm$ 12.13	13.89 $\pm$ 4.97	0.2057
	diastolic velocity (cm/s)	5.23 $\pm$ 3.49	4.80 $\pm$ 2.16	0.8406
	resistive index	0.74 $\pm$ 0.09	0.65 $\pm$ 0.10	0.0813
<b>Contrast-enhanced ultrasonography</b>				
Perfusion times	wash-in time (s)	13.00 $\pm$ 19.00	10.50 $\pm$ 4.00	0.6985
	wash-out time (s)	81.00 $\pm$ 41.00	81.00 $\pm$ 1.00	0.7671
	time to peak (s)	20.00 $\pm$ 20.00	88.00 $\pm$ 12.00	0.7609
<b>ARFI elastography</b>				
Shear wave velocity	SWV (m/s)	1.40 $\pm$ 0.64	1.62 $\pm$ 0.67	0.3482

benign tumour ( $P = 0.0001$ ), while the value of fibroadenoma was lower than acinar hyperplasia ( $P = 0.02$ ) (Figure 4). The acinar hyperplasia width/height ratio in the longitudinal section was lower ( $P = 0.0001$ ) than the other benign lesions. Also, the tubular adenoma width/height ratio in the transversal section was lower ( $P = 0.0001$ ) than in the other benign lesions.

## DISCUSSION

Ultrasonographic evaluation of benign mammary lesions in bitches did not allow differentiation between neoplastic and non-neoplastic masses and

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Table 4. Mean  $\pm$  SD of quantitative variables evaluated using different ultrasonography methods (B-mode, Doppler, contrast-enhanced ultrasonographic and acoustic radiation force impulse (ARFI) elastography) in benign canine mammary tumours using histopathological classification as a reference

Histopathologic diagnosis	Simple adenoma	Tubular adenoma	Fibroadenoma	Acinar hyperplasia	Ductal hyperplasia	Lobular hyperplasia	Benign mixed tumor
<b>B-mode ultrasonography</b>							
Long width (cm)	0.94 $\pm$ 0.29 <i>P</i> = 0.3019	0.56 $\pm$ 0.00	1.75 $\pm$ 0.00	3.33 $\pm$ 0.00	2.20 $\pm$ 2.12	1.60 $\pm$ 1.37	1.66 $\pm$ 1.26
Long length (cm)	0.35 $\pm$ 0.19 <sup>abc</sup> <i>P</i> = 0.0292	0.20 $\pm$ 0.00 <sup>a</sup>	0.64 $\pm$ 0.00 <sup>c</sup>	0.23 $\pm$ 0.00 <sup>ab</sup>	1.06 $\pm$ 1.12 <sup>bc</sup>	0.79 $\pm$ 0.89 <sup>bc</sup>	0.88 $\pm$ 1.03 <sup>bc</sup>
Width/length ratio	0.36 $\pm$ 0.12 <sup>b</sup> <i>P</i> = 0.0001	0.36 $\pm$ 0.00 <sup>b</sup>	0.37 $\pm$ 0.00 <sup>b</sup>	0.07 $\pm$ 0.00 <sup>a</sup>	0.47 $\pm$ 0.14 <sup>b</sup>	0.47 $\pm$ 0.14 <sup>b</sup>	0.54 $\pm$ 0.47 <sup>b</sup>
Transv height (cm)	0.69 $\pm$ 0.24 <sup>a</sup> <i>P</i> = 0.1637	0.98 $\pm$ 0.00 <sup>a</sup>	2.62 $\pm$ 0.00 <sup>a</sup>	2.89 $\pm$ 0.00 <sup>a</sup>	2.06 $\pm$ 2.07 <sup>a</sup>	1.59 $\pm$ 1.42 <sup>a</sup>	1.52 $\pm$ 1.04 <sup>a</sup>
Transv width (cm)	0.42 $\pm$ 0.39 <sup>a</sup> <i>P</i> = 0.0552	0.15 $\pm$ 0.00 <sup>a</sup>	0.85 $\pm$ 0.00 <sup>a</sup>	0.85 $\pm$ 0.00 <sup>a</sup>	0.94 $\pm$ 0.85 <sup>a</sup>	0.71 $\pm$ 0.60 <sup>a</sup>	0.77 $\pm$ 0.91 <sup>a</sup>
Width/height ratio	0.69 $\pm$ 0.76 <sup>b</sup> <i>P</i> = 0.0001	0.15 $\pm$ 0.00 <sup>a</sup>	0.32 $\pm$ 0.00 <sup>b</sup>	0.29 $\pm$ 0.00 <sup>b</sup>	0.50 $\pm$ 0.13 <sup>b</sup>	0.49 $\pm$ 0.13 <sup>b</sup>	0.45 $\pm$ 0.14 <sup>b</sup>
<b>Doppler ultrasonography</b>							
Systolic velocity (cm/s)	17.60 $\pm$ 0.00 <i>P</i> = 0.756	NA	23.4 $\pm$ 0.00	10.9 $\pm$ 0.00	13.75 $\pm$ 8.70	14.7 $\pm$ 4.51	20.24 $\pm$ 12.92
Diastolic velocity (cm/s)	7.3 $\pm$ 0.00 <i>P</i> = 0.8374	NA	3.40 $\pm$ 0.00	2.9 $\pm$ 0.00	5.2 $\pm$ 1.98	5.07 $\pm$ 2.57	5.21 $\pm$ 3.65
Resistive index	0.59 $\pm$ 0.00 <i>P</i> = 0.1413	NA	0.85 $\pm$ 0.00	0.73 $\pm$ 0.00	0.59 $\pm$ 0.12	0.65 $\pm$ 0.11	0.74 $\pm$ 0.09
<b>Contrast-enhanced ultrasonography</b>							
Wash-in (s)	6.00 $\pm$ 0.00 <i>P</i> = 0.3618	NA	NA	NA	14.00 $\pm$ 0.00	7.00 $\pm$ 0.00	19.00 $\pm$ 0.00
Peak (s)	11.00 $\pm$ 0.00 <i>P</i> = 0.2851	NA	NA	NA	18.00 $\pm$ 0.00	18.00 $\pm$ 0.00	25.5 $\pm$ 0.00
Wash-out (s)	95.00 $\pm$ 0.00 <i>P</i> = 0.3207	NA	NA	NA	81.00 $\pm$ 0.00	95.00 $\pm$ 0.00	67.5 $\pm$ 0.00
<b>ARFI elastography</b>							
SWV (cm/s)	1.29 $\pm$ 0.76 <i>P</i> = 0.3637	2.04 $\pm$ 0.00	0.53 $\pm$ 0.00	1.52 $\pm$ 0.00	1.11 $\pm$ 0.91	1.85 $\pm$ 0.52	1.44 $\pm$ 0.78

Long = longitudinal, NA = not available, SWV = shear wave velocity, Transv = transversal

showed limited efficacy for the comparison of benign histopathological types. B-mode variables enabled limited differentiation of some types of benign mammary neoplasms; however, Doppler, ARFI elastography and CEUS evaluations failed to reveal any differences between different types of benign mammary lesion. Based on the results of this study, the B-mode method did show some utility in predicting the type of benign mammary mass based on the differentiation criteria of meas-

urements of longitudinal length, width/length ratio and width/height ratio.

Conventional ultrasonography revealed that all benign masses had regular contours, and 50% showed homogenous parenchymal echotexture and 50% were heterogeneous (hyperechogenic with cystic areas). This result corroborates those of Bastan et al. (2009), Soler et al. (2016), Calas et al. (2007), Feliciano et al. (2012) and Feliciano et al. (2017) who found regular margins in most benign

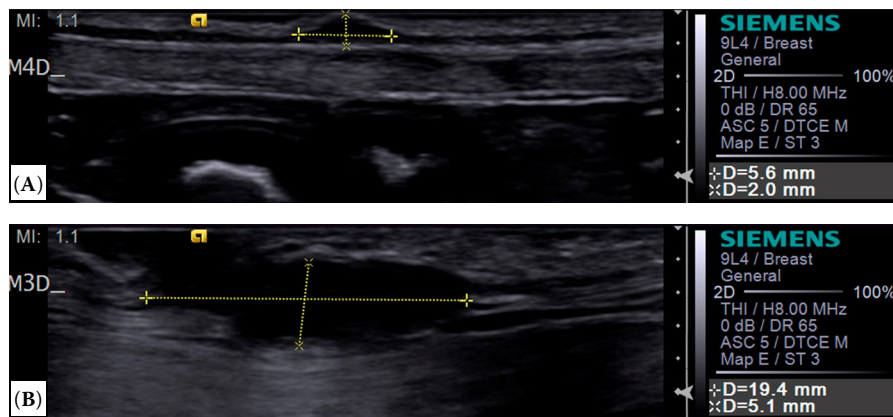


Figure 4. B-mode image of a tubular adenoma (A) and lobular hyperplasia (B) in female dogs. Longitudinal section of the mammary lesions (dotted lines) for measurement of length and width

masses. However, in these studies homogeneous echotexture was observed in the majority of benign masses evaluated. Invasiveness of adjacent tissues was absent in this study in agreement with Soler et al. (2016), who evaluated benign masses in humans.

Colour Doppler performed in the current study revealed that the presence of vascularisation in benign mammary masses does not correlate with neoplastic tissue. Vascularisation was present in 69% of the benign lesions evaluated; the most common vascular pattern was peripheral and the resistive index was  $0.71 \pm 0.10$  in line with the findings of Soler et al. (2016). These authors studied benign tumours in women and observed vascularisation in 75% of cases; the most common vascular pattern was peripheral and the resistive index  $0.74 \pm 0.03$ . In contrast, Feliciano et al. (2012) observed vascularisation in only 46% of benign masses evaluated; nonetheless, the systolic velocity reported by this author (19.91 cm/s for benign neoplasms) was close to our value (20.27 cm/s).

Davoudi et al. (2014) and Schmillevitch et al. (2009) evaluated benign mammary lesions using Doppler ultrasonography in humans and found that 70% and 50% of these lesions were fibroadenomas, respectively. In the first study, the mean area of the fibroadenoma (width/height ratio) was found to  $15.65 \pm 7.27 \text{ cm}^2$ , and the mean value of resistive index was  $0.65 \pm 0.00$ . In the second study, the mean resistive index in benign tumours was found to be  $0.61 \pm 0.00$ . In the present study, only 1% of masses were fibroadenomas and the mean area (width/height ratio) and resistive index were  $0.32 \pm 0.00 \text{ cm}^2$  and  $0.85 \pm 0.00$ , respectively.

ARFI elastography enables the quantitative evaluation of tissue stiffness based on shear wave velocity (waves that return from target tissues) estimation. This modern tool showed the highest diagnostic

efficacy for differentiating malignant and benign mammary masses in women and bitches (Tozaki et al. 2011a; Guiling et al. 2013; Feliciano et al. 2014; Ricci et al. 2014; Zhou et al. 2014; Tang et al. 2015; Gong et al. 2016; Liu et al. 2016; Feliciano et al. 2017); however, in our study, this tool was not effective in differentiating between benign mammary types. The elastographic properties of some benign tumours, such as those of fibroadenomas, remain unclear (Ricci et al. 2014). Besides, just like Tozaki et al. (2011b), this study had limitations in relation to the sensitivity of the technique to movement artefacts and the fixed-box dimension of the target region of interest. In addition, this technique has not been described for the differentiation of benign neoplastic and non-neoplastic masses in any another veterinary or medical study.

Contrast-enhanced ultrasound proved ineffective in the differentiation of benign mammary types; however, it proved to be useful for the identification of tumour macro- and micro-circulation. Most reports on the diagnostic efficacy of CEUS for the characterisation of mammary masses in humans (Liu et al. 2008; Wan et al. 2012; Xia et al. 2014; Wang et al. 2016) have described this technique as an acceptable predictor of malignancy. Our results provide some important diagnostic information for exams of canine mammary lesions. A high degree of contrast enhancement was described in benign masses (Wan et al. 2012); this variable was proportionally higher in the benign masses analysed in the present study and might be correlated with inflammation, which is frequent in this lesion type.

Nyman et al. (2006) evaluated twenty-six dogs with 11 benign mammary tumours using B-mode and colour Doppler ultrasonography. Of these, eight were neoplastic (six complex adenomas, one simple adenoma and one benign mixed tumour)



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and three were non-neoplastic (hyperplasia). The mean length and width were 1.50 and 0.80 cm, respectively. These findings are similar to our results, in which we made the same histopathological diagnosis. Further, mean length and width was 1.51 and 0.76 cm, respectively, in benign neoplastic tumours (simple adenoma, tubular adenoma, fibroadenoma and mixed benign tumour) and 1.99 and 0.82 cm, respectively, in non-neoplastic tumours (acinar hyperplasia, ductal hyperplasia and lobular hyperplasia).

In conclusion, ultrasonographic evaluation of benign canine mammary lesions did not allow differentiation between neoplastic and non-neoplastic tissues and only a few B-mode variables may aid in the prediction of specific tumour types.

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## REFERENCES

- Bastan A, Ozenc E, Pir Yagci I, Baki Acar D (2009): Ultrasonographic evaluation of mammary tumors in bitches. *Kafkas Universitesi Veteriner Fakultesi Dergisi* 1, 81–86.
- Bostock DE (1986): Canine and feline mammary neoplasms. *British Veterinary Journal* 142, 506–515.
- Calas MJG, Koch HA, Dutra MVP (2007): Breast ultrasound: evaluation of echographic criteria for differentiation of breast lesions. *Radiologia Brasileira* 40, 1–7.
- Cassali GD, Lavallo GE, Ferreira E, Estrela-Lima A, De Nardi B, Ghever C, Sobral RA, Amorim RL, Oliveira LO, Sueiro FAR, Beserra HEO, Bertagnolli AC, Gamba CO, Damasceno KA, Campos CB, Araujo MR, Campos LC, Monteiro LN, Nunes FC, Horta RS, Reis DC, Luvizotto MCR, Magalhaes GM, Raposo JB, Ferreira AMR, Tanaka NM, Grandi E, Ubukata R, Batschinski K, Terra EM, Salvador RCL, Jark PC, Delecrodi JER, Nascimento NA, Silva DN, Silva LP, Ferreira KCRS, Frehse MS, di Santis GW, Silva EO, Guim TN, Kerr B, Cintra PP, Silva FBF, Leite JS, Mello MFV, Ferreira MLG, Fukumasu H, Salgado BS, Torres R (2013): Consensus for the diagnosis, prognosis and treatment of canine mammary tumors. *Brazilian Journal of Veterinary Pathology* 7, 38–69.
- Cassidy J, Bissett D, Roy AJ, Spence OBE, Payne M, Morris-Stiff G (eds) (2015): *Oxford Handbook of Oncology*. 4<sup>th</sup> edn. Oxford. Xxii + 873.
- Davoudi Y, Borhani B, Rad MP, Matin N (2014): The role of doppler sonography in distinguishing malignant from benign breast. *Journal of Medical Ultrasound* 22, 92–95.
- Feliciano MAR, Silva MAM, Vicente WRR (2012): Conventional and Doppler ultrasound for the differentiation of benign and malignant canine mammary tumors. *Journal of Small Animal Practice* 53, 332–337.
- Feliciano MAR, Maronezi MC, Pavan L, Castanheira TL, Simoes APR, Carvalho CF, Canola JC, Vicente WRR (2014): ARFI elastography as a complementary diagnostic method for mammary neoplasia in female dogs – preliminary results. *Journal of Small Animal Practice* 55, 504–508.
- Feliciano MAR, Uscategui RAR, Maronezi MC, Simoes APR, Silva P, Gasser B, Pavan L, Carvalho CF, Canola JC, Vicente WRR (2017): Ultrasonography methods for predicting malignancy in canine mammary tumors. *PloS One* 12, doi: 10.1371/journal.pone.0178143.
- Feliciano MAR, Uscategui RAR, Maronezi MC, Maciel GS, Avante ML, Senhorello ILS, Mucedola T, Gasser B, Carvalho CF, Vicente WRR (2018): Accuracy of four ultrasonography techniques in predicting histopathological classification of canine mammary carcinomas. *Veterinary Radiology and Ultrasound*, doi: 10.1111/vru.12606.
- Gokhale S (2009): Ultrasound characterization of breast masses. *The Indian Journal of Radiology and Imaging* 19, 242–247.
- Gong JJ, Wan ZX, Yao MH, Zhao LX, Xu G, Liu H, Wu R (2016): Conventional ultrasound, ultrasound elasticity imaging, and acoustic radiation force impulse imaging for prediction of malignancy in breast masses. *International Journal of Clinical and Experimental Medicine* 9, 8108–8117.
- Gonzales de Bulnes A, Garcia Fernandez P, Mayenco Aguirre AM, Sanchez de la Muela M (1998): Ultrasonographic imaging of canine mammary tumours. *Veterinary Record* 143, 687–689.
- Guiling L, De-Wei L, Yu-Xiao F, Yi-Jiang S, Zhu-Jun D, Jian G, Yan X, Tian-Sheng Y, Li Y, Kai-Fu T (2013): Performance of shear wave elastography for differentiation of benign and malignant solid breast masses. *PloS One* 8, doi: 10.1371/journal.pone.0076322.
- Guay M, Sahin AA (2006): Breast cancer benign breast diseases: Classification, diagnosis, and management. *The Oncologist* 11, 435–449.
- Liu H, Jiang YX, Liu JB, Zhu QL, Sun Q (2008): Evaluation of breast lesions with contrast-enhanced ultrasound using the microvascular imaging technique: Initial observations. *Breast* 17, 532–539.



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- Liu BX, Zheng YL, Shan QY, Lu Y, Lin MX, Tian WS (2016): Elastography by acoustic radiation force impulse technology for differentiation of benign and malignant breast lesions: a meta-analysis. *Journal of Medical Ultrasonics* 43, 47–55.
- Masciadri N, Ferranti C (2011): Benign breast lesions: Ultrasound. *Journal of Ultrasound* 14, 55–65.
- Misdorp W, Else RW, Hellmen E, Lipscomb TP (eds) (1999): *Histological Classification of Mammary Tumors of the Dog and Cat*. World Health Organization International Classification of Tumors of Domestic Animals. Armed Forces Institute of Pathology. 59 pp.
- Nyman HT, Nielsen OL, McEvoy FJ, Lee MH, Martinussen T, Hellmen E, Kristensen AT (2006): Comparison of B-mode and Doppler ultrasonographic findings with histologic features of benign and malignant mammary tumors in dogs. *American Journal of Veterinary Research* 67, 985–991.
- Oliveira JC, Kommers GD, Masuda EK, Marques BMFPP, Figuera RA, Irigoyen LF, Barros CSL (2010): A retrospective study of 1.647 mammary tumors in bitches (in Portuguese). *Pesquisa Veterinaria Brasileira* 30, 177–185.
- Ricci P, Maggini E, Mancuso E, Lodise P, Cantisani V, Catalano C (2014): Clinical application of breast elastography: State of the art. *European Journal of Radiology* 83, 429–437.
- Schmillevitch J, Guimaraes Filho HA, De Nicola H, Gorski AC (2009): Utilization of vascular resistance index in the differentiation between benign and malignant breast nodules. *Radiologia Brasileira* 42, 241–244.
- Soler M, Dominguez E, Lucas X, Novellas UR, Gomes-Coelho KV, Espada Y, Agut A (2016): Comparison between ultrasonographic findings of benign and malignant canine mammary gland tumors using B-mode, colour Doppler, power Doppler and spectral Doppler. *Research in Veterinary Science* 107, 141–146.
- Tagawa M, Kanai E, Shimmo G, Kano M, Kayanuma H (2016): Ultrasonographic evaluation of depth-width ratio (D/W) of benign and malignant mammary tumors in dogs. *Journal of Veterinary Medical Science* 78, 521–524.
- Tang L, Xu HX, Bo XW, Liu BJ, Li XL, Wu R, Dan-Dan L, Lin F, Xiao-Hong X (2015): A novel two-dimensional quantitative shear wave elastography for differentiating malignant from benign breast lesions. *International Journal of Clinical and Experimental Medicine* 8, 10920–10928.
- Tozaki M, Isobe S, Fukuma E (2011a): Preliminary study of ultrasonographic tissue quantification of the breast using the acoustic radiation force impulse (ARFI) technology. *European Journal of Radiology* 80, e182–e187.
- Tozaki M, Isobe S, Yamaguchi M, Ogawa Y, Homma K, Saito M, Joo C, Fukuma E (2011b): Ultrasonographic elastography of the breast using acoustic radiation force impulse technology: preliminary study. *Japanese Journal of Radiology* 29, 452–456.
- Wan C, Du J, Fang H, Li F, Wang L (2012): Evaluation of breast lesions by contrast enhanced ultrasound: qualitative and quantitative analysis. *European Journal of Radiology* 81, e444–e450.
- Wang YM, Fan W, Zhao S, Zhang K, Zhang L, Zhang P, Ma R (2016): Qualitative, quantitative and combination score systems in differential diagnosis of breast lesions by contrast-enhanced ultrasound. *European Journal of Radiology* 85, 48–54.
- Xia HS, Wang X, Ding H, Wen JX, Fan PL, Wang WP (2014): Papillary breast lesions on contrast-enhanced ultrasound: morphological enhancement patterns and diagnostic strategy. *European Journal of Radiology* 24, 3178–3190.
- Zhou J, Zhan W, Dong Y, Yang Z, Zhou C (2014): Stiffness of the surrounding tissue of breast lesions evaluated by ultrasound elastography. *European Journal of Radiology* 7, 1659–1666.

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