

1 USE OF CONTRAST-ENHANCED ULTRASONOGRAPHY IN CHRONIC PATHOLOGIC  
2 CANINE TESTES

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24 Giessen, Germany.

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26

## 27 ABSTRACT

28

29 Contrast-enhanced ultrasound with sulphur hexafluoride microbubbles was performed in seven  
30 healthy dogs without a history of reproductive pathology and with histologically confirmed normal  
31 testes and in 42 dogs with chronic scrotal anomalies. All dogs underwent orchiectomy and  
32 histological examination. Enhancement patterns and perfusion parameters (peak intensity and  
33 regional blood flow) of testes of healthy dogs and testes with chronic lesions were compared.  
34 Fourteen non-pathologic and 60 pathologic testes were considered. Forty testes were neoplastic (24  
35 interstitial cell tumours, 9 seminomas, 7 Sertoli cell tumours), 20 were non-neoplastic (16 testicular  
36 degenerations, 2 chronic orchitis, 1 testicular atrophy, 1 interstitial cell hyperplasia). In healthy dogs  
37 the contrast medium flow had a rapid homogeneous wash-in and wash-out, with a short peak phase.  
38 With contrast ultrasound, testes that were inhomogeneous with a hyperenhancing pattern were  
39 associated with neoplasia (sensitivity: 87.5%, specificity: 100%). Lesions with persistent inner  
40 vessels and a hypo-to-isoechoic background were significantly associated with seminomas  
41 (sensitivity: 77.8%, specificity: 100%). Testes with non-neoplastic lesions were characterized by a  
42 scant/moderate homogeneous enhancement. Perfusion parameters were higher in neoplastic lesions.  
43 Contrast ultrasound was a feasible diagnostic tool in the assessment of testicular lesions, with  
44 hyperenhancement being an important feature in the diagnosis of malignancy.

45

46 Key words: contrast-ultrasound, testis, neoplasia, chronic lesions, dog

47

## 48 INTRODUCTION

49

50 Testicular disease is common in the dog and diagnostic B-mode ultrasound is frequently used for  
51 breeding soundness examination and also for the diagnosis of testicular abnormalities (England  
52 1991). Whilst B-mode ultrasound is useful for detecting parenchymal lesions as well as measuring

53 testicular volume (England 1991, England 1995), for many organ systems it has limited ability to  
54 differentiate malignant from benign disease; conditions which frequently have different  
55 vascularisation and might be differentiated using contrast-enhanced ultrasound assessment of  
56 vascular perfusion.

57 In male dogs, testicular tumours are common, with an overall prevalence ranging between 6% and  
58 12%, second in frequency only to skin tumours, and have a higher incidence when compared to  
59 other species, including humans (Johnston et al 1991, Lawrence and Saba 2012). The most frequent  
60 testicular neoplasm is interstitial cell tumour, followed by seminoma and Sertoli cell tumour  
61 (Johnston et al 1991, Pugh and Konde 1991). Metastatic neoplasia affecting the testis is rare. Non-  
62 neoplastic lesions are less common than neoplasia ~~andbut~~ include acute or chronic orchitis,  
63 epididymitis, testicular torsion, spermatocele, varicocele, hydrocele, sperm granuloma, testicular  
64 degeneration and atrophy (Johnston et al 1991, Pugh and Konde 1991).

65 In men, ultrasonography is used extensively for the evaluation of intrascrotal lesions, and the most  
66 important goal of this diagnostic technique is the differentiation of malignant scrotal masses  
67 (Horstmann et al 1992, Lock et al 2011, ~~Horstmann et al 1992~~, Bertolotto et al 2011, Valentino et al  
68 2011).

69 In dogs, B-mode ultrasound and colour and power Doppler ultrasonography have been described  
70 for normal testes (Pugh and Konde 1990, Pugh and Konde 1991, ~~Pugh and Konde 1990~~, Gunzel-  
71 Apel et al 2001). There have been useful B-mode descriptions of testicular tumours and other  
72 scrotal abnormalities including hydrocele, testicular atrophy, inguinal hernia and infectious orchitis  
73 (Pugh and Konde 1991, England 1995, ~~Pugh and Konde 1991~~, Ober et al 2004). Testicular  
74 neoplasms are described as lesions with different echogenicity, without a single characteristic  
75 ultrasonographic appearance (~~England 1995~~, Johnston et al 1991, Pugh and Konde 1991, England  
76 1995). Although no specific features have been reported associated with particular tumour types,  
77 three Sertoli cell tumours in undescended testes were reported to have a similar appearance, being  
78 hypoechoic with large anechoic areas (Pugh and Konde 1991).

79 Recently, contrast-enhanced ultrasound with second-generation contrast media has been introduced  
80 to clinical practice in human and veterinary medicine. The modern contrast agents are blood pool  
81 agents and do not leave the intravascular space. They comprise phospholipid-coated microbubbles  
82 containing gases of high molecular weight and low solubility in water, which provide better  
83 resistance to pressure and prolonged persistence in blood (Nyman et al 2005). The microbubbles  
84 have a non-linear response (rhythmic size changes of the bubble are not equivalent), when  
85 insonated with acoustic frequencies from 1 MHz to above 3 MHz and have low acoustic pressure  
86 (low Mechanical Index, MI). The non-linear response generates fundamental and harmonic  
87 components. The harmonic component strongly increases the backscattered signal, compared with  
88 the signal received from the bubbles in the fundamental frequency. Low MI imaging has the  
89 advantage of being minimally destructive to the bubbles and allows real-time imaging of the  
90 vascularity to the level of capillaries (O'Brien et al 2004).  
91 ~~This new technology has proved to be a significant development for ultrasonographic examination~~  
92 ~~because of the ability to image the microvasculature of tissues and organs in real time.~~ Contrast  
93 ultrasound in dogs is particularly useful for the detection and characterisation of lesions of the liver,  
94 kidneys, spleen, and prostate gland (O'Brien et al 2004, Nyman et al 2005, Ohlerth et al 2007,  
95 Rossi et al 2008, Haers and Saunders 2009, Haers et al 2010,~~Nyman et al 2005, O'Brien et al~~  
96 ~~2004, Ohlerth et al 2007, Rossi et al 2008,~~ Vignoli et al 2011). In humans there are few reports  
97 describing the use of contrast ultrasound in testicular lesions, but it has been shown that the  
98 ~~technique may facilitate detection of changes in testicular microcirculation in cases of varicocele~~  
99 ~~and segmental infarction Bertolotto et al 2011, Caretta et al 2010).~~ Contrast ultrasound is more  
100 accurate than grey-scale and Doppler ultrasound for confirmation of diagnosis in acute scrotal  
101 disease, particularly infarction, trauma and torsion, as well as for detection of changes of  
102 microcirculation in cases of varicocele and for identifying testicular masses (~~Caretta et al 2010,~~  
103 Bertolotto et al 2011, Valentino et al 2011, Lock et al 2011).

104 ~~Commonly neoplastic testicular masses have increased vascularization and this feature may be~~  
105 ~~important in the diagnosis of neoplasia (Lock et al 2011).~~

106 To date there have been no studies assessing testicular perfusion with contrast ultrasound in dogs.  
107 The aim of this study was to describe the contrast-enhanced ultrasonographic features of chronic  
108 testicular lesions, and to evaluate whether contrast-ultrasound could provide useful information for  
109 differentiation of lesion type.

110

## 111 MATERIALS AND METHODS

112

113 The study was multicentric. Two groups of dogs were evaluated: a control group and a pathologic  
114 group. Cases of the control group were examined at the Department of Veterinary Clinical Science  
115 of the University of Naples. Inclusion criteria for the control group were: (1) clinically healthy male  
116 adult dogs, (2) no history of reproductive pathology, (3) absence of macroscopic, ultrasonographic,  
117 and microscopic lesions in the testes. Ethical approval for the study was given by the University of  
118 Naples.

119 Cases of the pathologic group were examined at the Department of Veterinary Medical Science of  
120 the University of Parma and at the Private Practice Clinica Veterinaria dell'Orologio. Dogs were  
121 included if a focal or diffuse testicular lesion was detected by palpation and/or by ultrasound  
122 examination, further confirmed by histologic examination. Dogs with lesions in undescended testes  
123 were excluded from the study.

124 Seven clinically healthy adult dogs that were to be castrated by owner's request (age ranged  
125 between 2 and 4 years, body weight ranged between 6 and 37 kg), and 42 adult intact male dogs  
126 with scrotal anomalies (age ranged between 1.5 and 12 years and body weight between 5 and 45 kg)  
127 were enrolled in this multicentric study over a four-year period (2009-2012). Prior to the  
128 ultrasonographic evaluation, a complete general physical examination, serum chemistry profile and  
129 complete blood cell count were performed. Reproductive status was not evaluated. Informed

130 consent of the owners was obtained. All patients underwent grey-scale ultrasound of the scrotum  
131 with a 12 MHz linear transducer, prior to contrast ultrasound. Thoracic radiography and complete  
132 abdominal ultrasound (data not shown) was performed if there was a suspicion of neoplasia. All  
133 dogs were sedated with medetomidine (10 µg/kg IM) and butorphanol (0.2 mg/kg IM) prior to  
134 contrast harmonic -ultrasound examination, in order to avoid patient movements and to achieve a  
135 better image quality.

136 Contrast ultrasound equipment included two systems with coded harmonic capabilities (CnTI  
137 Esaote Megas Esatune®, Esaote, Genova, Italy and CnTI Mylab 30 Gold®, Esaote, Genova, Italy)  
138 and a linear probe with a receive frequency of 5 MHz. The mechanical index was always lower than  
139 0.15, which corresponded to an acoustic pressure lower than 45 kPa, to minimize microbubbles  
140 destruction. –A single focal zone was placed at the level of the mediastinum testis and the overall  
141 gain and time-gain compensation were set so that only a very low background signal from the  
142 testicular capsule and mediastinum testis was maintained to have an anatomical reference. All the  
143 testes were examined in the longitudinal plane. A bolus of 0.03 ml/kg of prepared solution (~~5~~  
144 ~~mg/ml~~) of sulphur hexafluoride microbubbles (5 mg/ml, SonoVue®, Bracco, Milan, Italy) was  
145 injected in the cephalic vein, followed by a flush of 5 ml of saline solution. A timer was activated at  
146 the start of the injection and perfusion of a single testis was visualized in real time for at least 90  
147 seconds. The entire procedure was eventually repeated approximately 5 minutes later for evaluation  
148 of the contralateral testis. All dogs underwent orchietomy, which was followed by histological  
149 examination performed at the Department of Veterinary Medical Science of the University of  
150 Parma.- The entire testes and epididymis were submitted to histology and standard colorations were  
151 performed. The reproductive status was not evaluated.

152 All the recorded videos were reviewed by one author (AV) and the enhancement patterns of the  
153 testes were subjectively described. The enhancement pattern was classified as homogeneous (no  
154 focal lesion detectable) or inhomogeneous (focal lesion detectable). If recorded as inhomogeneous,  
155 it was further classified as “hyperenhancing”, “isoenhancing”, “hypoenhancing”, by comparing the

156 brightness of the lesion to the surrounding testicular tissue after the injection of the contrast  
157 medium. A lesion was classified as hyperenhancing, if it was brighter than surrounding tissue either  
158 homogeneously or inhomogeneously or with rim enhancement or with prominent inner vessels. A  
159 lesion was considered isoenhancing when it was no more visible during contrast-ultrasound. A  
160 lesion was considered hypoenhancing when it was hypoechoic to the surrounding tissue.

161 Quantitative analysis was performed to support qualitative analysis of the enhancement. Time-  
162 intensity curves and colour-coded maps were reconstructed with a commercial software  
163 (QONTRAST®, Bracco, Milan, Italy), using the gamma variate bolus-corrected model. Peak  
164 intensity (PI, % of the ~~s~~Signal ~~i~~Intensity) and regional blood flow (RBF, the ratio between a value  
165 proportional to the area under the curve and the mean transit time) were considered. For  
166 inhomogeneous lesions, two regions of interest (ROI) were drawn, one including the area of the  
167 lesion and the other including surrounding tissue. Attention was paid to draw ROIs equal in  
168 dimension and in depth (Leinonen et al 2011).

169 Enhancement patterns of neoplastic, non-neoplastic and non-pathologic testes were compared using  
170 Fisher's exact test.

171 For quantitative analysis, data were normally distributed (Shapiro-Wilk test). Perfusion parameters  
172 of inhomogeneous lesions were compared to the surrounding tissue with Student's t test. Neoplastic  
173 lesions, non-neoplastic lesions and non-pathologic testes perfusion data were compared with  
174 ANOVA test, and subsequently with Games-Howell post-hoc test. Statistical data processing was  
175 performed using a commercial software package (Microsoft Excel version 97 SR-1: Microsoft  
176 Corporation, Redmond, Washington, USA) and WinPepi v. 11.28 (Abramson JH, WinPepi (PEPI-  
177 for-Windows, freeware computer programs for epidemiologists. Epidemiologic Perspectives &  
178 Innovation 2004; 1:6. Freeware available from <http://www.brixtonhealth.com/pepi4windows.html>).  
179 Values were considered significant when  $P < 0.05$ .

180

181 RESULTS

182

183 The technique was reliably performed in all cases, yielding ~~images of good quality~~consistent  
184 results. No adverse effects were noted in any animal during the procedure. Serum chemistry profile  
185 and complete blood cell count were normal in all patients.

186 No histopathological abnormalities were found in the 14 testes examine from the 7 healthy dogs. In  
187 these testes, subcapsular arteries, followed by intra-parenchymal arteries could be visualised during  
188 the wash-in phase (Fig 1-C). After a few seconds, a homogeneous moderate enhancement of the  
189 parenchyma was observed, with parenchymal vessels still distinguishable (Figure 1-D). After the  
190 peak phase, a rapid homogeneous decrease of echogenicity was detected. After 90 seconds only few  
191 microbubbles were visible in the testicular parenchyma.

192 Sixty pathologic testes were ~~considered~~found in the 42 dogs. Twenty-four patients had unilateral  
193 lesions, whilst 18 were bilateral. Among the 60 pathologic testes, 40 lesions were neoplastic (24  
194 interstitial cell tumours, 9 seminomas, 7 Sertoli cell tumours). The remaining 20 lesions were non-  
195 neoplastic (16 testicular degenerations, 2 chronic necrotizing orchitis, 1 testicular atrophy, 1  
196 interstitial cell hyperplasia). No signs of metastasis were found outside of the testes in dogs with  
197 primary testicular neoplasia.

198 Among the interstitial cell tumours, ~~11~~14 were classified as solid and 13 as angiomatous. Solid  
199 interstitial cell tumours were either hypo or hyperechoic nodules when examined with B-mode  
200 ultrasound. Angiomatous interstitial cell tumours had a similar appearance but two cases showed up  
201 as cystic-like nodular lesions. With contrast-ultrasound, 21 of the testes with interstitial cell tumour  
202 were inhomogeneous, with the focal lesions showing an hypere~~n~~hancing pattern (13  
203 homogeneous, 5 heterogenous, 3 with rim enhancement) and 3 were inhomogeneous with the focal  
204 lesions showing an hypoenhancing pattern (Figure 2).

205 Among the seminomas, 7 were diffuse while 2 were intratubular type. With B-mode ultrasound  
206 diffuse seminomas were hypoechoic solid nodules with thin hyperechoic striations. The  
207 enhancement pattern of diffuse seminomas was peculiar; all of the testes were inhomogeneous with



208 the focal lesion showing an hypo-isoechoic background and several prominent inner vessels, which  
209 were still distinguishable in the wash-out phase (Figure 3). Intratubular seminomas were not  
210 detected with B-mode ultrasound. With contrast ultrasound the enhancement was homogeneous.

211 Sertoli cell tumours were all histologically classified as solid type. They appeared as nodules with  
212 different echogenicity, in two cases they had hypoechoic cystic-like cavities. With contrast-  
213 ultrasound, the testes with Sertoli cell tumour were all inhomogeneous with the focal lesions  
214 showing an hyperenhancing pattern (3 homogeneous, 3 heterogeneous and 1 with rim  
215 enhancement) (Fig 4).

216 Overall, neoplastic lesions were better visualized in the wash-in phase and tended to maintain the  
217 pattern during peak and wash-out.

218 Degenerated testes had a normal or increased echogenicity, normal or reduced dimensions and in  
219 two cases several parenchymal hyperechoic foci were present, which histologically corresponded to  
220 small areas of fibrosis. Among the dogs with testicular degeneration, 4 dogs had bilateral  
221 involvement, whilst 5 had a tumour in the contralateral testis (4 interstitial cell tumours and one  
222 seminoma). Two dogs had monolateral involvement and one dog had interstitial cell hyperplasia in  
223 the contralateral testis. None of the dogs had signs of feminization. Testosterone/oestrogen blood  
224 levels however were not assayed. With contrast-ultrasound, all of the degenerated testes had  
225 homogeneous pattern with an enhancement subjectively lower than non-pathologic tissue.

226 Testicular atrophy was manifest as a small testis that was inhomogeneous with B-mode in a dog  
227 with a contralateral sertolioma. With contrast ultrasound a very faint homogeneous enhancement  
228 was detected.

229 The testes with chronic necrotizing orchitis were characterized by reduced dimensions and  
230 echogenicity. With contrast-ultrasound both of them had a scant homogeneous enhancement  
231 (Figure 5).

232 The interstitial cell hyperplasia appeared as an ill-defined small nodule, isoechoic to the  
233 surrounding parenchyma. With contrast-ultrasound, it was isoenhancing to the surrounding tissue  
234 and for this reason it was not visible.

235 ~~Overall, neoplastic lesions were better visualized in the wash-in phase and tended to maintain the~~  
236 ~~pattern during peak and wash-out.~~

237 Examination of the subjective findings to establish their diagnostic value showed that testes which  
238 were inhomogeneous with a hyperenhancing lesion were significantly associated with neoplasia  
239 (sensitivity: 87.5%, CI 95% 72.5-95.3%; specificity: 100%, CI 95% 87.3-100%; positive predictive  
240 value: 100% CI 95% 87.6-100%; negative predictive value: 87.1%, CI 95% 77.7-95.1%). Among  
241 the neoplasms, lesions that showed persistent inner vessels with a hypo-isoechoic background were  
242 significantly associated with seminomas (sensitivity: 77.8%, CI 95% 40.2-96%; specificity: 100%,  
243 CI 95% 86.2-100%; positive predictive value: 100% CI 95% 56-100%; negative predictive value  
244 93.9%, CI 95% 72.3-98.9%), while interstitial and Sertoli cell tumours showed a similar  
245 enhancement pattern.

246 Perfusion parameters of neoplastic lesions and their surrounding tissue are presented in table 1.  
247 Perfusion parameters of non-pathologic tissue, neoplastic and non-neoplastic lesions are presented  
248 in table 2. PI and RBF were higher in neoplastic lesions when compared to the surrounding tissue  
249 ( $P < 0.001$ ). Comparing neoplastic lesions, non-neoplastic lesions and non-pathologic testes, there  
250 were statistically significant differences between group means, for both PI and RBF values, as  
251 determined by ANOVA test ( $P < 0.001$ ). Neoplastic lesions had a significantly higher PI and RBF  
252 than non-pathologic ( $P < 0.05$ ) and non-neoplastic testes ( $P < 0.01$ ). Non-neoplastic lesions had a  
253 lower PI and RBF than non-pathologic testes ( $P < 0.05$ ) and neoplastic lesions ( $P < 0.01$ ).

254

255 DISCUSSION

256

257 ~~Testicular tumours are frequent in dogs but there are relatively few reports of their ultrasonographic~~  
258 ~~features (England 1995, Johnston et al 1991, Pugh and Konde 1991). In general there are no broad~~  
259 ~~ultrasonographic features that appeared to be characteristic of a particular type of testicular lesion~~  
260 ~~(Johnston et al 1991, Pugh and Konde 1991).~~

261 To our knowledge, this is the first study to describe the ultrasonographic features of normal and  
262 chronic pathologic testes in dogs using contrast-enhanced ultrasound. ~~This diagnostic technique can~~  
263 ~~give more information on lesions and tissues vascularisation when compared to colour Doppler~~  
264 ~~(Haers and Saunders 2009). This diagnostic technique is relatively easy to perform for a B-mode~~  
265 ~~expert ultrasonographer, but because many factors influence the degree of contrast enhancement,~~  
266 ~~such as different media, imaging units, injection protocol, dosage, mechanical index, site of the~~  
267 ~~focal zone, a special attention must to be paid in setting the machine (O'Brien et al 2004). Although~~  
268 this was a multicentre study ~~the same medium, dosage, injection protocol, imaging units, and the~~  
269 ~~same setting of the machine were used, in order to minimize operator-dependent variability.~~

270 ~~Sulphur hexafluoride is a safe contrast agent, side effects described in humans and dogs are rare,~~  
271 ~~usually minor, and include headache, nausea, pain at the injection site, altered taste, sensation of~~  
272 ~~heat (humans) and vomiting (dogs) (Jackobsen et al 2005, Dolan et al 2009, Seiler et al 2013).~~  
273 ~~Contraindications in humans include ischemic cardiomyopathy, severe pulmonary hypertension,~~  
274 ~~severe systemic hypertension and right-to-left cardiac shunts (Jackobsen et al 2005).~~

275 In the ~~non-pathologic normal~~ testes the contrast medium flow had a rapid wash-in and wash-out,  
276 with a short peak phase and a moderate enhancement. ~~The enhancement pattern was different~~  
277 ~~differences~~ to other organs ~~such as liver and spleen and~~ may relate to the smaller total blood volume  
278 of the testis, compared with the liver and spleen and to differences of the blood supply and vascular  
279 anatomy (Lock et al 2011). The liver is supplied by a dual system, hepatic artery and portal vein,  
280 and hepatic and splenic microcirculation is characterized by the presence of large sinusoids, ~~-in~~  
281 ~~which the transit of microbubbles is very slow, resulting in a persistent enhancement~~ (Nyman et al  
282 2005, Ohlerth et al 2007). ~~In the testes the flow of microbubbles is rapid, since no sinusoids are~~

283 ~~present in their parenchyma. In fact, t~~Testes are supplied by testicular arteries, which are branches  
284 of the abdominal aorta and enter the tunica albuginea to form capsular arteries. ~~The capsular arteries~~  
285 ~~have centripetal branches that enter the parenchyma and flow toward the mediastinum. As they~~  
286 ~~approach the mediastinum, they arborize into recurrent rami that branch back in the opposite~~  
287 ~~direction.~~The veins exit the mediastinum and empty into the pampiniform plexus, which drains into  
288 the ~~isps~~ilateral testicular veins (Horstmann et al 1992).

289  
290 A limitation of the present study was that the reproductive status of the animals included in the  
291 healthy group has not been evaluated. Further studies are needed on normal dogs with a  
292 documented reproductive status in order to better characterise the perfusion pattern of normal testes  
293 with contrast ultrasound. Another limitation was that dogs were sedated. It is important to recognise  
294 that vascular status can affect perfusion dynamics, and comparisons of perfusion parameters can  
295 only be made for animals subject to the same sedative or anaesthetic regimen. ~~Recently, alpha 2-~~  
296 ~~adrenergic agonist dexmedetomidine, similar to medetomidine used in this study, has been proved~~  
297 ~~to reduce organ blood flow in dogs and therefore to influence perfusion parameters of contrast-~~  
298 ~~enhanced ultrasound. Peak intensity was lower in kidneys in dogs sedated with dexmedetomidine.~~  
299 ~~Arrival time and time to peak were significantly higher in liver, spleen, kidneys and intestine in~~  
300 ~~dogs sedated with dexmedetomidine (Restitutti et al 2013). Further studies on testicular contrast~~  
301 ~~ultrasound are needed in dogs with different sedation protocols, as well as in~~ conscious non-sedated  
302 ~~dogs, including a larger number of subjects.~~

303 ~~-Testicular tumours are frequent in dogs but there are relatively few reports of their~~  
304 ~~ultrasonographic features (Johnston et al 1991, Pugh and Konde 1991, England 1995). In general,~~  
305 ~~there are no reported ultrasonographic features that appeared to be characteristic of a particular type~~  
306 ~~of testicular lesion, when B-mode ultrasound is considered (Johnston et al 1991, Pugh and Konde~~  
307 ~~1991).~~

308

309 In humans, testicular tumours are generally described as being hypervascular with colour Doppler,  
310 but this feature can also be identified in cases of acute orchitis (Horstmann et al 1992). Recent  
311 reports have described the use of contrast-enhanced ultrasonography of the human testes ([Caretta et](#)  
312 [al 2010](#), Bertolotto et al 2011, [Valentino et al 2011](#), [Hedayati et al 2012](#), ~~Caretta et al 2010~~).  
313 ~~Contrast ultrasound was thought to be useful in assisting the diagnosis of testicular masses, and in~~  
314 ~~cases of acute scrotal pain, varicocele, testicular trauma and acute segmental infarction (Bertolotto~~  
315 ~~et al 2011, Valentino et al 2011, Caretta et al 2010, Hedayati et al 2012).~~ In a further study, hyper-  
316 ~~enhancement in the early wash in phase showed a sensitivity of 88.4% and a positive predictive~~  
317 ~~value of 97.4% for neoplastic testicular lesions (Lock et al 2011).~~ In the cases of neoplasia, contrast  
318 ultrasound demonstrated a slight or strong enhancement of the lesion in the early wash-in, which  
319 became hypoechoic later in the wash-out phase, with a sensitivity of 88.4% and a positive  
320 predictive value of 97.4% (Lock et al 2011).

321 In the present study, most testicular tumours were hyperenhancing to the surrounding tissue, when  
322 examined with contrast ultrasound. Contrary to contrast dynamics observed in the liver, there was  
323 no specific phase associated with different patterns of lesional contrast-enhancement over time.  
324 Thus a lesion with early hyper-enhancement tended to maintain that pattern during the entire  
325 examination, but best visualization was provided during the wash-in, similarly to humans (Lock et  
326 al 2011, Valentino et al 2011). Hyperenhancement was either homogeneous, with rim enhancement,  
327 or with prominent inner vessels and had a sensitivity of 87.5% and a positive predictive value of  
328 100% for neoplasia, similar to values found in the human literature (Lock et al 2011, Valentino et al  
329 2011). In this study, prominent and persistent inner vessels within a hypoechoic background lesion  
330 were peculiar features of diffuse seminomas, which are not reported in humans. Human seminomas  
331 are described as hyper-enhanced focal lesions with rapid wash-out and are not distinguishable from  
332 other solid neoplasms (Lock et al 2011, Valentino et al 2011). The reason ~~for~~ this difference in  
333 findings between species is unknown, although there is a correspondence in the description of grey-  
334 scale ultrasonographic features ([Caldwell et al 1980](#), Lock et al 2011, ~~Caldwell et al 1980~~).

335 Interestingly, intratubular-type seminomas could not be imaged with contrast ultrasound, due to  
336 their very small dimensions, and this could represent a limitation of this diagnostic technique.

337 However, a larger number of seminomas would be needed to further assess and confirm these  
338 particular ultrasonographic features. Interstitial cell and Sertoli cell tumours could not be  
339 differentiated with contrast ultrasound since most of them appeared as hyper-enhanced lesions,  
340 which were well visualized in the early wash-in phase.

341 It is known that testicular cytology is a powerful and minimally invasive diagnostic tool to assess  
342 testicular pathology (~~Dahlbom et al 1997~~, Santos et al 2010). Another advantage of cContrast  
343 ultrasound is to support testicular cytology/histology by indicating the proper sampling site, if a  
344 fine-needle-aspiration or a biopsy is requested. The definition of enhancing and subsequently of  
345 viable tumour regions is better characterized with contrast-ultrasound, resulting in increased  
346 accuracy of percutaneous biopsy (Gelb et al 2010, Sparchez et al 2011).~~and allowing avoiding~~  
347 ~~hypovascular/necrotic tissue~~.

348 Most of benign lesions such as testicular degeneration or atrophy and chronic orchitis appeared as  
349 diffuse lesions, homogeneously hypo-enhancing when compared to non-pathologic testes. In men,  
350 benign lesions such as necrosis, atrophy, ectasia of rete testis, hematoma, epidermoid cysts and  
351 torsion are described as hypo- or non-enhancing lesions (Lock et al 2011, Valentino et al 2011,  
352 Patel et al 2012). Testicular degeneration and atrophy is commonly described in dogs as a change  
353 secondary to testicular neoplasia in the contralateral testis where it is caused by an excess of sexual  
354 hormones secreted by the tumour, but it is also recognised as an age-related change (Peters and van  
355 Slujis 1996).

356 The descriptive assessments of testicular enhancement pattern for different lesion types were  
357 confirmed by quantitative perfusion analysis, which may help to better visualize the lesion  
358 vascularization, especially when the colour-coded maps are considered.

359 In conclusion, contrast ultrasound appears to be a feasible diagnostic tool in the assessment of  
360 testicular perfusion in the dog and in particular may allow the documentation of focal testicular

361 lesions, with some limitations due to the cost of contrast medium, the need for dedicated ultrasound  
362 equipment and time required to perform the examination. This technology, using second-  
363 generation contrast medium, may provide an additional tool to facilitate in vivo classification of  
364 testicular lesions. Finally, hypervascularisation appears to be an important feature in the diagnosis  
365 of malignancy.

366

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