USE OF CONTRAST-ENHANCED ULTRASONOGRAPHY IN CHRONIC PATHOLOGIC CANINE TESTES

Antonella Voltaa, Sabrina Manfredia, Massimo Vignolib, Marco Russoc, Gary C. W. Englandd,
Federica Rossie, Enrico Bigliardia, Francesco Di Iannia, Enrico Parmigiani, Carla Bresciani,
Giacomo Gnudii*

adi Department of Veterinary Medical Sciences, University of Parma, Via del Taglio 10 43100 Parma, Italy;
bPrivate Practice Associazione Veterinaria PetCare, Via Belvedere 33 - 40043 Marzabotto (BO), Italy;
cDepartment of Veterinary Clinical Sciences, University “Federico II”, Corso Umberto I, 40 80138 Naples, Italy;
dSchool of Veterinary Medicine and Science, University of Nottingham, Sutton Bonington Campus, Sutton Bonington, Leicestershire, LE12 5RD, United Kingdom
ePrivate Practice Clinica Veterinaria dell’Orologio, Via Gramsci 1/4 40037 Sasso Marconi (BO), Italy

*Corresponding Author: Giacomo Gnudi. E-mail: giacomo.gnudi@unipr.it
Address: Via del Taglio 10, 43100 Parma, Italy
Phone +39 0521032789, Fax +39 0521032791

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ABSTRACT

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

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

INTRODUCTION

Testicular disease is common in the dog and diagnostic B-mode ultrasound is frequently used for breeding soundness examination and also for the diagnosis of testicular abnormalities (England 1991). Whilst B-mode ultrasound is useful for detecting parenchymal lesions as well as measuring
testicular volume (England 1991, England 1995), for many organ systems it has limited ability to
differentiate malignant from benign disease; conditions which frequently have different
vascularisation and might be differentiated using contrast-enhanced ultrasound assessment of
vascular perfusion.

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

In men, ultrasonography is used extensively for the evaluation of intrascrotal lesions, and the most
important goal of this diagnostic technique is the differentiation of malignant scrotal masses
2011).

In dogs, B-mode ultrasound and colour and power Doppler ultrasonography have been described
for normal testes (Pugh and Konde 1990, Pugh and Konde 1991, Pugh and Konde 1990, Gunzel-
Apel et al 2001). There have been useful B-mode descriptions of testicular tumours and other
scrotal abnormalities including hydrocele, testicular atrophy, inguinal hernia and infectious orchitis
neoplasms are described as lesions with different echogenicity, without a single characteristic
1995). Although no specific features have been reported associated with particular tumour types,
three Sertoli cell tumours in undescended testes were reported to have a similar appearance, being
hypoechoic with large anechoic areas (Pugh and Konde 1991).
Recently, contrast-enhanced ultrasound with second-generation contrast media has been introduced to clinical practice in human and veterinary medicine. The modern contrast agents are blood pool agents and do not leave the intravascular space. They comprise phospholipid-coated microbubbles containing gases of high molecular weight and low solubility in water, which provide better resistance to pressure and prolonged persistence in blood (Nyman et al 2005). The microbubbles have a non-linear response (rhythmic size changes of the bubble are not equivalent), when insonated with acoustic frequencies from 1 MHz to above 3 MHz and have low acoustic pressure (low Mechanical Index, MI). The non-linear response generates fundamental and harmonic components. The harmonic component strongly increases the backscattered signal, compared with the signal received from the bubbles in the fundamental frequency. Low MI imaging has the advantage of being minimally destructive to the bubbles and allows real-time imaging of the vascularity to the level of capillaries (O’Brien et al 2004).

This new technology has proved to be a significant development for ultrasonographic examination because of the ability to image the microvasculature of tissues and organs in real time. Contrast ultrasound in dogs is particularly useful for the detection and characterisation of lesions of the liver, kidneys, spleen, and prostate gland (O’Brien et al 2004, Nyman et al 2005, Ohlert et al 2007, Rossi et al 2008, Haers and Saunders 2009, Haers et al 2010, Nyman et al 2005, O’Brien et al 2004, Ohlert et al 2007, Rossi et al 2008, Vignoli et al 2011). In humans there are few reports describing the use of contrast ultrasound in testicular lesions, but it has been shown that the technique may facilitate detection of changes in testicular microcirculation in cases of varicocele and segmental infarction Bertolotto et al 2011, Caretta et al 2010). Contrast ultrasound is more accurate than grey-scale and Doppler ultrasound for confirmation of diagnosis in acute scrotal disease, particularly infarction, trauma and torsion, as well as for detection of changes of microcirculation in cases of varicocele and for identifying testicular masses (Caretta et al 2010, Bertolotto et al 2011, Valentino et al 2011, Lock et al 2011).
Commonly neoplastic testicular masses have increased vascularization and this feature may be important in the diagnosis of neoplasia (Lock et al. 2011).

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

MATERIALS AND METHODS

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

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

Seven clinically healthy adult dogs that were to be castrated by owner’s request (age ranged between 2 and 4 years, body weight ranged between 6 and 37 kg), and 42 adult intact male dogs with scrotal anomalies (age ranged between 1.5 and 12 years and body weight between 5 and 45 kg) were enrolled in this multicentric study over a four-year period (2009-2012). Prior to the ultrasonographic evaluation, a complete general physical examination, serum chemistry profile and complete blood cell count were performed. Reproductive status was not evaluated. Informed
consent of the owners was obtained. All patients underwent grey-scale ultrasound of the scrotum with a 12 MHz linear transducer prior to contrast ultrasound. Thoracic radiography and complete abdominal ultrasound (data not shown) was performed if there was a suspicion of neoplasia. All dogs were sedated with medetomidine (10 μg/kg IM) and butorphanol (0.2 mg/kg IM) prior to contrast harmonic ultrasound examination, in order to avoid patient movements and to achieve a better image quality.

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

All the recorded videos were reviewed by one author (AV) and the enhancement patterns of the testes were subjectively described. The enhancement pattern was classified as homogeneous (no focal lesion detectable) or inhomogeneous (focal lesion detectable). If recorded as inhomogeneous, it was further classified as “hyperenhancing”, “isoenhancing”, “hypoenhancing”, by comparing the
brightness of the lesion to the surrounding testicular tissue after the injection of the contrast medium. A lesion was classified as hyperenhancing, if it was brighter than surrounding tissue either homogeneously or inhomogeneously or with rim enhancement or with prominent inner vessels. A lesion was considered isoenhancing when it was no more visible during contrast-ultrasound. A lesion was considered hypoenhancing when it was hypoechoic to the surrounding tissue.

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

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

For quantitative analysis, data were normally distributed (Shapiro-Wilk test). Perfusion parameters of inhomogeneous lesions were compared to the surrounding tissue with Student’s t test. Neoplastic lesions, non-neoplastic lesions and non-pathologic testes perfusion data were compared with ANOVA test, and subsequently with Games-Howell post-hoc test. Statistical data processing was performed using a commercial software package (Microsoft Excel version 97 SR-1: Microsoft Corporation, Redmond, Washington, USA) and WinPepi v. 11.28 (Abramson JH, WinPepi (PEPI-for-Windows, freeware computer programs for epidemiologists. Epidemiologic Perspectives & Innovation 2004; 1:6. Freeware available from http://www.brixtonhealth.com/pepi4windows.html). Values were considered significant when P <0.05.
The technique was reliably performed in all cases, yielding images of good quality and consistent results. No adverse effects were noted in any animal during the procedure. Serum chemistry profile and complete blood cell count were normal in all patients.

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

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

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

Among the seminomas, 7 were diffuse while 2 were intratubular type. With B-mode ultrasound diffuse seminomas were hypoechoic solid nodules with thin hyperechoic striations. The enhancement pattern of diffuse seminomas was peculiar; all of the testes were inhomogeneous with
the focal lesion showing an hypo-isoechoic background and several prominent inner vessels, which were still distinguishable in the wash-out phase (Figure 3). Intratubular seminomas were not detected with B-mode ultrasound. With contrast ultrasound the enhancement was homogeneous. Sertoli cell tumours were all histologically classified as solid type. They appeared as nodules with different echogenicity, in two cases they had hypoechoic cystic-like cavities. With contrast-ultrasound, the testes with Sertoli cell tumour were all inhomogeneous with the focal lesions showing an hyperenhancing pattern (3 homogeneous, 3 heterogeneous and 1 with rim enhancement) (Fig 4).

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

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

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

The testes with chronic necrotizing orchitis were characterized by reduced dimensions and echogenicity. With contrast-ultrasound both of them had a scant homogeneous enhancement (Figure 5).
The interstitial cell hyperplasia appeared as an ill-defined small nodule, isoechoic to the surrounding parenchyma. With contrast-ultrasound, it was isoenhancing to the surrounding tissue and for this reason it was not visible.

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

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

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

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

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

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

In the non-pathologic normal testes the contrast medium flow had a rapid wash-in and wash-out, with a short peak phase and a moderate enhancement. The enhancement pattern was different to other organs such as liver and spleen and may relate to the smaller total blood volume of the testis, compared with the liver and spleen and to differences of the blood supply and vascular anatomy (Lock et al 2011). The liver is supplied by a dual system, hepatic artery and portal vein, and hepatic and splenic microcirculation is characterized by the presence of large sinusoids, in which the transit of microbubbles is very slow, resulting in a persistent enhancement (Nyman et al 2005, Ohlerth et al 2007). In the testes the flow of microbubbles is rapid, since no sinusoids are
Testes are supplied by testicular arteries, which are branches of the abdominal aorta and enter the tunica albuginea to form capsular arteries. The capsular arteries have centripetal branches that enter the parenchyma and flow toward the mediastinum. As they approach the mediastinum, they arborize into recurrent rami that branch back in the opposite direction. The veins exit the mediastinum and empty into the pampiniform plexus, which drains into the ipsilateral testicular veins (Horstmann et al 1992).

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

Testicular tumours are frequent in dogs but there are relatively few reports of their ultrasonographic features (Johnston et al 1991, Pugh and Konde 1991, England 1995). In general, there are no reported ultrasonographic features that appeared to be characteristic of a particular type of testicular lesion, when B-mode ultrasound is considered (Johnston et al 1991, Pugh and Konde 1991).
In humans, testicular tumours are generally described as being hypervascular with colour Doppler, but this feature can also be identified in cases of acute orchitis (Horstmann et al 1992). Recent reports have described the use of contrast-enhanced ultrasonography of the human testes (Caretta et al 2010, Bertolotto et al 2011, Valentino et al 2011, Hedayati et al 2012, Caretta et al 2010). Contrast ultrasound was thought to be useful in assisting the diagnosis of testicular masses, and in cases of acute scrotal pain, varicocele, testicular trauma and acute segmental infarction (Bertolotto et al 2011, Valentino et al 2011, Caretta et al 2010, Hedayati et al 2012). In a further study, hyperenhancement in the early wash-in phase showed a sensitivity of 88.4% and a positive predictive value of 97.4% for neoplastic testicular lesions (Lock et al 2011). In the cases of neoplasia, contrast ultrasound demonstrated a slight or strong enhancement of the lesion in the early wash-in, which became hypoechoic later in the wash-out phase, with a sensitivity of 88.4% and a positive predictive value of 97.4% (Lock et al 2011).

In the present study, most testicular tumours were hyperenhancing to the surrounding tissue, when examined with contrast ultrasound. Contrary to contrast dynamics observed in the liver, there was no specific phase associated with different patterns of lesional contrast-enhancement over time. Thus a lesion with early hyper-enhancement tended to maintain that pattern during the entire examination, but best visualization was provided during the wash-in, similarly to humans (Lock et al 2011, Valentino et al 2011). Hyperenhancement was either homogeneous, with rim enhancement, or with prominent inner vessels and had a sensitivity of 87.5% and a positive predictive value of 100% for neoplasia, similar to values found in the human literature (Lock et al 2011, Valentino et al 2011). In this study, prominent and persistent inner vessels within a hypoechoic background lesion were peculiar features of diffuse seminomas, which are not reported in humans. Human seminomas are described as hyper-enhanced focal lesions with rapid wash-out and are not distinguishable from other solid neoplasms (Lock et al 2011, Valentino et al 2011). The reason for this difference in findings between species is unknown, although there is a correspondence in the description of grey-scale ultrasonographic features (Caldwell et al 1980, Lock et al 2011, Caldwell et al 1980).
Interestingly, intratubular-type seminomas could not be imaged with contrast ultrasound, due to their very small dimensions, and this could represent a limitation of this diagnostic technique. However, a larger number of seminomas would be needed to further assess and confirm these particular ultrasonographic features. Interstitial cell and Sertoli cell tumours could not be differentiated with contrast ultrasound since most of them appeared as hyper-enhanced lesions, which were well visualized in the early wash-in phase.

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

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

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

In conclusion, contrast ultrasound appears to be a feasible diagnostic tool in the assessment of testicular perfusion in the dog and in particular may allow the documentation of focal testicular
lesions, with some limitations due to the cost of contrast medium, the need for dedicated ultrasound equipment and time required to perform the examination. This technology, using second-generation contrast medium, may provide an additional tool to facilitate in vivo classification of testicular lesions. Finally, hypervascularisation appears to be an important feature in the diagnosis of malignancy.

REFERENCES


