Original article

Digital image analysis of testicular and prostatic ultrasonographic echogencity and heterogeneity in dogs and the relation to semen quality.

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Abstract

A semi-automated ultrasonographic method was developed to measure echogenicity and heterogeneity of the testes and prostate gland and relationships of these measures with semen quality were assessed in 43 fertile dogs. The relationship between animal age and body weight upon the volume of the testes, epididymal tail volume and prostate volume were also established.

Mean testicular echogenicity was negatively correlated with the percentage of morphologically normal live spermatozoa (more echogenic testes were associated with fewer normal sperm) but not with any other semen quality measure. Mean testicular heterogeneity was positively correlated with the total spermatozoal output (more heterogenous testes, being those with anechoic parenchyma and prominent echogenic stippling, were associated with greater sperm output) but not with any other semen quality measure. There was no relationship between either mean prostatic echogenicity or mean prostatic heterogeneity and any semen quality measure.

There was no relationship between age and any testicular or prostatic parameter; however bodyweight was significantly correlated with total testicular volume, total epididymal tail volume and total prostatic volume.

Testicular and prostatic ultrasongraphic echogenicity and heterogeneity can be objectively assessed using digital image analysis and testicular echogenicity and heterogeneity may be useful adjunct measurements in a breeding soundness examination.

Keywords: canine; testes; prostate; semen quality; ultrasound

Introduction

Understanding reproductive function and fertility in the male is an essential element of breeding management in dogs. Commonly, reproductive potential is assessed by conducting a breeding soundness examination involving, amongst other things, clinical examination, ultrasound examination and semen collection and evaluation (Memon, 2007).

B-mode real-time ultrasonography allows the accurate assessment of the size, shape, position, margination and internal architecture of the testes (England, 1991; Eilts et al., 1993; Paltiel et al., 2002; Gouletsou et al., 2008; Souza et al., 2014) and prostate gland (Blum et al., 1985; Juniewicz et al., 1989; England, 1991; Eilts et al., 1993; Ruel et al., 1998; Paltiel et al., 2002; Gouletsou et al., 2008; Freitas et al., 2013; Freitas et al., 2015). Ultrasonography also provides a valuable tool in assessing reproductive pathology (Cartee and Rowles, 1983; Feeney et al., 1987; Pugh and Konde, 1991; Cooney et al., 1992; England, 1995; Keenan, 1998; Nautrup and Tobias, 2001; Hecht, 2008).

In clinical practice, ultrasound images are subjectively assessed and described in terms of their image texture; principally echogenicity and heterogeneity. A small number of studies have proposed a relationship between grossly detectable lesions within the testes and semen quality (England, 1991; Vencato et al., 2014). Objective analysis of echogenicity from measurements of pixel intensity is however possible using digital image analysis (Ivancic and Mai, 2008). This allows measurement of the characteristics of the tissue (Pierson and Adams, 1995; Cardilli et al., 2010) and enables detection of changes in echogenicity which may not be detected by the human eye (Rivers et al., 1996; Arteaga et al., 2005). Quantitative ultrasound measurement of ultrasonographic homogeneity/heterogeneity has also been previously assessed by calculation of the standard deviation of pixel intensity (Hershkovitz et al., 2010), and for testes ultrasound pixel heterogeneity has been directly correlated with tissue biochemical composition (Omer et al., 2012; Ahmadi et al., 2013). There are however only a few reports demonstrating a relationship between quantitative measurement of either testes echogenicity or heterogeneity and semen quality (Arteaga et al., 2005; Ahmadi et al., 2012). Kastelic and Brito (2012) proposed that the primary clinical use of ultrasonography was for grossly detectable lesions since quantitative pixel analysis was not predictive of semen quality in bulls. To date no quantitative ultrasonographic studies of the testes or prostate gland of the dog appear to have been published.

The aim of this study was to measure testicular and prostatic ultrasonographic echogenicity and heterogeneity using digital image analysis, and investigate the relationships between these measures and semen quality in a group of known fertile dogs.

Materials and Methods

Study animals

Forty-three stud dogs (21 Labrador Retrievers, 12 Golden Retrievers, 6 German Shepherds, 1 Border Collie, 1 Flat Coated Retriever, 1 Irish Water Spaniel and 1 Standard Poodle) with a mean weight of 35.5 ± 5.8 kg (range 20.6 to 54.1 kg) aged between 1.1 and 9.3 years (mean 4.2 ± 2.0 years) were examined. Dogs were selected on the basis that they met the following inclusion criteria; (1) clinically healthy, (2) over one year of age, (3) reproductively intact males with no previous scrotal or prostatic surgery or exogenous hormone treatment, (4) proven fertility within the previous 6 months, and (5) having not ejaculated within the previous 48 hours.

Ultrasonographic measurements

Ultrasound examinations of the testes, prostate and epididymal tail were undertaken once on each dog by the second and third authors respectively using a real time B-mode ultrasound machine (Pandion 300s, Pie Data UK Ltd., Crawley, UK) with a 10MHz (testes) and 7.5MHz (prostate) mechanical-sector transducer. All machine settings including focal depth and gain settings were established at the first examination according to best image quality and remained unaltered for all remaining examinations which were performed over a 4-week period.

The testes were imaged in the sagittal, transverse and dorsal planes and the prostate in the sagittal and transverse planes. Length, width and height of the testis and tail of the epididymis and of each lobe of the prostate were measured using the electronic callipers of the machine.

Testicular volume was calculated using the formula: volume = l x w x h x 0.71 where l = sagittal diameter; w = transverse diameter and h = dorsal diameter (Hsieh et al., 2009; Gouletsou et al., 2008). Epididymal tail volume and the volume of each lobe of the prostate were calculated using the formula for an ellipse: volume = l x w x h x 0.523 where l = length in a cranio-caudal direction (dorsal plane) w = sagittal diameter in a latero-medial direction (dorsal plane) and h = sagittal diameter (sagittal plane).

Measurement of echogenicity and homogeneity

Frozen digital images of the right and left testes in sagittal cross-section and of the prostate in the transverse plane were acquired onto a Laptop computer (Ergo Computing UK Ltd., Nottingham, UK) using video creation hardware (Dazzle Video Creator, Pinnacle Systems GmbH, Mountain View, California) and capture software (www.virtualdub.org). Images underwent semi-automated analysis using a macro developed with ImageJ software (National Institutes of Health, Bethesda, Maryland; http:rsb.info.nih.gov/ij/) to recover values for mean pixel intensity in the sampling window. Using this method the echogenicity of anechoic urine in the bladder and hepatic parenchyma of four normal 2 to 4 year old dogs were 6.7% and 78.0% respectively; higher percentage values representing structures that were more echogenic.

To measure echogenicity within the testes and prostate gland, two selected reference points (one in the near field and one in the far field) were selected on the hyperechoic capsule of the testes or the prostatic capsule (these being selected as the most echogenic structures identifiable). The computer macro then randomly placed nine sampling regions of interest (each 2.0 mm^2) over the testicular parenchyma (avoiding the central mediastinum) (Figure 1), or three sampling regions within each lobe of the prostate gland. Within each region of interest the mean pixel intensity (PI) was measured. Of the two reference points the highest measurement of mean PI (most echogenic) was used to calculate the echogenicity of the comparative region of interest as a percentage of the highest mean pixel intensity using the following formula:

Percentage echogenicity = (Mean PI of capsule / Mean PI of testicular or prostatic parenchyma) x 100. This methodology therefore related all echogenicity measurements to a standard echogenic structure that would be consistent between dogs.

The mean of the nine echogenicity values for testicular echogenicity and the mean of six echogenicity values for prostate echogenicity were reported as mean echogenicity for that organ.

Heterogeneity of testicular and prostatic echogenicity was calculated as the standard deviation of the mean echogenicity of the regions of interest for each organ. Using this method low values (low variation between regions of interest) represented more homogenous tissues, whilst high values for heterogeneity (high variation between the regions of interest) represented tissues that were less homogenous. These measurements were at the tissue level rather than reporting gross changes that would be observable by eye at the organ level.

Semen evaluation

Semen was collected by digital manipulation in the absence of a teaser bitch (England, 1999a) immediately after the ultrasound examination. First and second fractions were collected and the combined volume recorded. Since the total duration of sexual excitement could vary between dogs, thus affecting the total volume of the third (prostatic) fraction produced, prostatic fluid was collected over a defined time of 1-minute immediately following collection of the second fraction. Sperm motility (straight line velocity [VSL]) was objectively measured using computer image analysis (Hobson Tracking systems Ltd, Sheffield, UK) as previously described (Smith and England, 2001). The percentage of fast forward progressively motile sperm [PPFM]) was determined as described by England (1999b). The percentage of morphologically normal live sperm (NLS) was assessed after examining 100 nigrosin-eosin stained spermatozoa at x 1000 magnification, sperm concentration was measured using a haemocytometer, and total spermatozoal output (TSO) was calculated as previously described (England, 1999a).

Data analysis

Measurements taken from the right and left testes, and the right and left prostatic lobes were compared using tests of difference. These measurements were then combined to provide values for total testicular volume (TTV), total epididymal tail volume (TEV) and total prostatic volume (TPV) volume.

Mean testicular echogenicity (MTE), mean testicular heterogeneity (MTH), mean prostatic echogenicity (MPE) and mean prostatic heterogeneity (MPH) were calculated for each dog.

Linear regression was used to determine whether age was related to the four ultrasound parameters (MTE, MTH, MPE and MPH). Relationships between MTE, MTH, MPE and MPH and semen quality were initially investigated using Spearman's rank correlation tests. Potential relationships were further investigated using linear regression. Relationships between age, bodyweight and

TTV, TEV and TPV and were investigated using linear regression with age and bodyweight as explanatory variables.

Statistical analysis was performed using XLStat software (Addinsoft, USA). Values were considered significant when $P<0.05$.

Results

There were no differences between measurements of the left compared with right testes, or left compared with right prostatic lobes.

There was substantial variation between the ultrasonographic measurements for the 43 dogs (Table 1). Mean straight line velocity (VSL) was 16.7 ± 1.2 mm/s (range 8 to 39 mm/s), the mean percentage of fast forward progressively motile sperm was 88 ± 1.71% (range 35 to 95%), the mean percentage of morphologically normal live sperm (NLS) was 76.0 ± 1.1 % (range 57 to 88%), mean sperm concentration was 463.9 x 10^6 ± 50.7 x 10^6 (range 95 to 1650 x 10^6), and mean total spermatozoal output (TSO) was 863.5 x 10^6 ± 74.1 x 10^6 (range 180 to 1785 x 10^6).

Age was not related to MTE (r^2 = 0.007, P = 0.583), MTH (r^2 = 0.005, P = 0.656), MPE (r^2 = 0.033, P = 0.240) or MPH (r^2 = 0.012, P = 0.483). Mean testicular echogenicity was negatively correlated with the percentage morphologically normal live sperm (r = -0.455, P = 0.003, r^2 = 0.165, P = 0.009); more echogenic testes were associated with fewer normal sperm (Figure 2). There were no relationships between MTE and any other semen quality measure. Mean testicular heterogeneity was positively correlated with total spermatozoal output (r

= 0.416, P = 0.007, r^2 = 0.220, P = 0.002); more heterogenous testes at the tissue level were associated with greater sperm output (Figure 3). There were no relationships between MTH and any other semen quality measure. There were no relationships between mean prostatic echogenicity or mean prostatic heterogeneity and any measure of semen quality.

There was no relationship between age and any of the ultrasonographic measurements of testes, epididymis or prostate volume. Bodyweight was significantly related to TTV (r^2 = 0.27, P <0.001), TEV (r^2 = 0.25, P = 0.001) and TPV (r^2 = 0.21, P = 0.002) (Figures 4a, 4b and 4c).

Discussion

This study assessed testicular and prostatic parenchymal echogenicity by measurement of pixel intensity in various regions of interest compared with an anatomically consistent echogenic reference point. This method has advantages over simple measurement of pixel intensity performed in the work of other authors (Pierson and Adams, 1995; Arteaga et al., 2005; Ivancic and Mai, 2008; Cardilli et al., 2010), where tissue echogenicity could be influenced by sound attenuation caused by subcutaneous tissue and tissue depth. This study also measured heterogeneity by examining the standard deviation of the mean pixel intensity of the regions of interest. A refinement of the methodology would be to sample a greater number of regions of a smaller area and perhaps consider statistical evaluation of the range of values for pixel intensity rather than to calculate the mean value, which by its nature tends to smooth the data.

Interestingly, within this population of recently fertile dogs there was substantial variation in semen quality and in many of the testicular and prostatic ultrasound measurements. Comparison between ultrasound parameters and semen quality showed that there was a significant negative correlation between mean testicular echogenicity and the percentage of morphologically normal live spermatozoa. Testes that were more echogenic were associated with fewer normal sperm in the ejaculate. Ahmandi et al. (2012) also studied fertile males but were~~as~~ unable to demonstrate a significant relationship between testicular echogenicity and semen quality, although their study included only six animals. We are uncertain of the histological variations that were present in our population of dogs that resulted in increased testicular echogenicity and were associated with poorer morphology, but not total sperm output. Presumably, in these dogs Sertoli cell numbers were normal, but subtle microstructural changes were present that affected sperm morphology. Induction of testicular pathology by scrotal insulation has been shown to cause changes in testicular echogenicity and associated changes in sperm morphology (Artega et al., 2005). That study found that hypoechoic testes were associated with poor morphology. We propose that either increased or decreased testicular echogenicity may reflect ultrastructural changes within the testes that are associated with altered sperm morphology.

Commented [RM1]: Gary – we have said at the start of the discussion that we think their method could have led to echogenicity being affected by tissue depth etc, could that explain anything?

The present study also demonstrated that mean testicular heterogeneity was positively correlated with the total spermatozoal output; less heterogeneous testes were associated with reduced sperm output. It is important to recognise that heterogeneity was measured as the variation in pixel intensity within small sampling windows, such that testes which had an almost anechoic parenchyma with prominent echogenic stippling were recorded as high heterogeneity. Presumably, focal regions of increased homogeneity (reduced variation between the anechoic parenchyma and the echogenic stippling) reflects a reduced density of fluid-containing seminiferous tubules accounting for reduced total sperm output but not changes in sperm morphology. Finding a relationship between testicular parenchymal heterogeneity and semen quality has only previously been reported in a small study in rams, where an inverse correlation was found between heterogeneity measurements and the percentage of sperm with normal morphology and progressive motility in samples collected 60 days after the ultrasound examination (Ahmadi et al., 2012). The present study found no relationship between sperm morphology or several objective measures of motility, but we collected and evaluated semen immediately after the ultrasound examination unlike the study of Ahmadi et al. (2012).

An important clarification for clinical use of quantitative measurement of pixel intensity and calculation of the variation of pixel intensity is that these are measures at the level of the parenchyma, and are not a gross or overall assessment at the level of the organ which is a very different concept. Indeed, testes that have an overall heterogenous appearance characterised by irregular and diffuse echogenic structures within the parenchyma are often associated with low sperm output (Vencato et al., 2014).

No relationships were found between echogenicity and heterogeneity of the prostate gland or any other measures of the prostate gland and semen quality. These findings are not surprising since the prostate gland solely contributes fluid to the first and third fractions of the ejaculate (England et al., 1990), although the volumes of these fluids did not relate to any measurement of the prostate in the present study, unlike previous observations by Wheaton et al. (1979) who found that seminal volume was correlated to prostatic size. This may relate to the collection of a mixed first and second fraction and the time-restricted collection of third fraction in the present study, since ejaculation may have been completed within 1 minute in some dogs but not others.

Examination of the data collected from the left and right testes, and the left and right lobes of the prostate gland found no differences in any of the ultrasonographic measurements, similar to the findings of other authors (Pugh et al., 1990; England, 1991; Villaverde et al., 2014) but in contrast to the work of Souza et al. (2012). In this study, ultrasonographic measurements of the left testis were higher than the right testis in two different breeds. However, a small number of dogs were used, in contrast with the present study, and a larger number of animals could demonstrate different results. Total testicular volume, total epididymal tail volume and total prostatic volume were positively correlated ~~to~~ with bodyweight, similar to the findings previously reported in other studies (Amann, 1986; Woodall and Johnstone, 1988; Ruel et al., 1998; Atalan et al., 1999a,b).

This study intentionally included dogs from a large age range and found no relationship between age and echogenicity or heterogeneity results from the testes or prostate, suggesting that age was not a contributing factor to the differences in ultrasonic appearance observed. No relationship was found between age of the dog and total testicular volume, total epididymal tail volume and total prostatic volume, unlike the work of Mantziaras et al. (2014) who found a tendency for testes volume to increase until approximately 6 years of age and then to decrease. In contrast with the present study, prostatic volume has also been found to be related to age by other authors (Ruel et al., 1998; Atalan et al., 1999a,b; Lowseth et al., 1990; Mantziaras et al., 2014). It is possible that the inclusion criteria of the present study, requiring the dog to have been recently fertile, may have eliminated some older dogs with testicular disease or prostatic enlargement which may have been included in other studies. In particular, previous work demonstration a link between age and prostatic size has frequently included dogs with prostate pathology (Brendler et al., 1983; Kay et al., 1989; Nielsen et al., 1990).

Conclusion

Testicular and prostatic echogenicity and heterogeneity can be objectively measured by means of a semi-automated method using conventional ultrasound equipment. Relationships were apparent between mean testicular echogenicity and mean testicular heterogeneity with semen quality, such that superior semen samples was observed in testes that were less echogenic and had greater heterogeneity at the tissue level; corresponding to testes with an anechoic parenchyma with prominent echogenic stippling.

It is feasible that objective measurement of testicular echogenicity and heterogeneity may be useful adjunct measurements in a breeding soundness examination.

Conflict of interest statement

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

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Legends to Figures

Figure 1.

Software image representing the objective measurement of pixel intensisty. Black rectangle and white rectangle are the near and far field echogenic reference points whilst the coloured squares represent the nine sampling regions over the parenchyma.

Figure 2.

Relationship between mean testicular echogenicity and percentage morphologically normal living sperm (NLS) for 43 dogs. Solid line shows regression analysis with 95% confidence limits.

Figure 3.

Relationship between mean testicular heterogeneity and total sperm output (TSO) for 43 dogs. Solid line shows regression analysis with 95% confidence limits.

Figure 4.

Relationship between body weight and (a) total testicular volume, (b) total epididymal tail volume and (c) total prostatic volume for 43 dogs. Solid line shows regression analysis with 95% confidence limits.