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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 ultrasonographic 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.

1. 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

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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, 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.

2. Materials and methods

2.1. 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 h.

2.2. 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 10 MHz (testes) and 7.5 MHz (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 = $l \times w \times h \times 0.71$ where l = sagittal diameter; w = transverse diameter and h = dorsal diameter (Gouletsou et al., 2008; Hsieh et al., 2009). Epididymal tail volume and the volume of each lobe of the prostate were calculated using the formula for an ellipse: volume = $l \times w \times h \times 0.523$ 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).

2.3. 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) (Fig. 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) × 100. This