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The value of trans-scrotal ultrasonography at bull breeding soundness evaluation (BBSE): The relationship between testicular parenchymal pixel intensity and semen quality

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ABSTRACT

Bull breeding soundness evaluation (BBSE) is commonly undertaken to identify bulls that are potentially unfit for use as breeding sires. Various studies worldwide have found that approximately 20% of the bulls fail their routine prebreeding BBSE and are therefore considered subfertile. Multiple articles describe the use of testicular ultrasound as a noninvasive aid in the identification of specific testicular and epididymal lesions. Two previous studies have hypothesized a correlation between ultrasonographic testicular parenchymal pixel intensity (PI) and semen quality; however to date, no published studies have specifically examined this link. The aim of this study, therefore, was to assess the relationship between testicular parenchymal PI (measured using trans-scrotal ultrasonography) and semen quality (measured at BBSE), and the usefulness of testicular ultrasonography as an aid in predicting future fertility in bulls, in particular those that are deemed subfertile at the first examination. A total of 162 bulls from 35 farms in the South East of Scotland were submitted to routine BBSE and testicular ultrasonography between March and May 2014, and March and May 2015. Thirty-three animals failed their initial examination (BBSE1) due to poor semen quality, and were re-examined (BBSE2) 6 to 8 weeks later. Computer-aided image analysis and gross visual lesion scoring were performed on all ultrasonograms, and results were compared to semen quality at BBSE1 and BBSE2. The PI measurements were practical and repeatable in a field setting, and although the results of this study did not highlight any biological correlation between semen quality at BBSE1 or BBSE2 and testicular PI, it did identify that gross visual lesion scoring of testicular images is comparable to computer analysis of PI ($P < 0.001$) in identifying animals suffering from gross testicular fibrosis.

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1. Introduction

Beef suckler cow enterprises heavily rely on natural service sires to achieve pregnancy in their females, and bulls are also often used to 'sweep up' following a period of artificial insemination in both dairy and beef herds [1]. Bull breeding soundness evaluation (BBSE) is commonly undertaken to identify bulls that are potentially unfit for use as breeding sires, and thus to avoid poor herd reproductive performance and economic losses [2]. Few male animals are truly infertile; however, it is accepted that approximately 20 to 40% of bulls examined as part of routine screening fail their BBSE and are therefore considered subfertile [3]. However, collection and assessment of semen collected via electro-ejaculation (EEJ) may not always be a true representation of the quantity and quality of semen produced by a bull throughout a breeding season [4]. This can lead to difficulties in decision making on farm,

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and potential misclassification of bulls as unfit for purpose based on the results of a single BBSE conducted using semen collected via EEJ.

Measurement of testicular weight (and a proxy for this; testicular circumference) should be undertaken as part of all BBSE [5] and is widely accepted as a predictor of sperm output [6]. However, this measurement involves a gross measurement of the scrotal exterior circumference and does not account for potential (non-palpable) pathology or lesions of the testicular tissue that may affect fertility [7]. Multiple articles describe the use of testicular ultrasound as a noninvasive aid in the identification of specific testicular and epididymal gross lesions [7–12]. However, few studies have examined the correlation between ultrasonographic testicular parenchymal pixel intensity (PI) and semen quality [7]. Those that have show little correlation between the two measurements at the time of testing [13]. Three articles have proposed a link between parenchymal PI and future fertility [13–15]. However, the results across these studies were not consistent, nor always conducted on sexually active animals. The aim of this field study was to assess the relationship between testicular parenchymal PI (measured using trans-scrotal ultrasonography) and semen quality (measured at BBSE), and thereby assess the usefulness of testicular ultrasonography as an aid in predicting the future fertility of sexually mature bulls in clinical veterinary practice.

2. Materials and methods

2.1. Farm and bull selection

This field study was conducted in the South East of Scotland using bulls belonging to clients of a single first opinion farm animal veterinary practice and approved by the Royal (Dick) School of Veterinary Studies Veterinary Ethical Review Committee (VERC Ref:29–14). The veterinary practice routinely performs 150 to 200 BBSEs per year across approximately 40 beef suckler enterprises. BBSEs of all bulls enrolled in the study were undertaken as part of the routine examination of animals approximately 8 weeks in advance of the breeding season (BBSE1). Animals that failed BBSE1 and were classified as subfertile due to poor semen quality were re-examined 6 to 8 weeks later (BBSE2), which allowed for one spermatic cycle to be completed between both evaluations. This enabled assessment of persistent or transient subfertility, and therefore decision making by the veterinarian and farmer on whether a bull was deemed suitable as a breeding sire or not. Although BBSE does not guarantee fertility, it provides producers confidence that they are greatly reducing the risk of using bulls that will fail to achieve normal fertility levels due to physical or semen quality problems [16].

2.2. BBSE

All BBSEs were performed on farm by trained and experienced examiners following British Cattle Veterinarian Association guidelines [16]. A 4-stage BBSE was performed at each examination and involved a general physical examination, examination of the external reproductive tract (including scrotal circumference measurement using a Reliabull measuring tape), examination of the internal reproductive tract, and collection and examination of a semen sample collected via EEJ. If a sample of poor quality was collected on first EEJ, a second and final semen sample was collected by EEJ after a 20-minute rest period. Gross motility was assessed using a bright field microscope at $\times$ 10 magnification, and the percentage of progressively motile spermatozoa was estimated using phase contrast microscopy at $\times$ 40 magnification. Sperm morphology was assessed using eosin-nigrosin stained semen smears at $\times$ 100 magnification. Percentage of normal spermatozoa, detached heads, proximal cytoplasmic droplets, head defects, coiled tails, distal mid piece reflex, coiled principal piece, white blood cells, "other" and total abnormal spermatazoa were calculated by counting a total of 200 spermatozoa per slide. Bulls were classified as subfertile due to poor semen quality if the ejaculate contained less than 60% progressively motile spermatozoa and/or less than 70% morphologically normal spermatozoa [16].

2.3. Testicular ultrasound and pixel intensity (PI)

A B-mode ultrasound scanner equipped with a 4.5- to 8-MHz linear array transducer (Easi-Scan; BCF Technology, Strathclyde, Scotland) was used to image the testes of each bull submitted for BBSE before EEJ was carried out. The same equipment was used for every examination and the settings for focus, gain, brightness, and contrast standardized at the machine median settings. All images were taken by the same examiner (MT). The testicles were prepared before each examination using disposable paper towels so that they were clean and dry. A conductive ultrasound gel was used as a coupling material between the scrotum and transducer, and pressure applied until minor scrotal skin indentation occurred. The ultrasound transducer was held vertically (parallel to the long axis of the testes) on the caudal surface of the scrotum. The image was aligned until the mediastinum of the testes was clear and apparent. The image was then frozen and stored. This process was repeated with the ultrasound transducer in the horizontal plane (at the widest part of the testicle) and both views were repeated for the other testicle. Each ultrasound examination therefore comprised of four images from each bull (Fig. 1A, B).

Computer analysis of each image was undertaken using image analysis software (Image J, U. S. National Institutes of Health, MD, USA [17]). The examiner was blinded to the bulls and testicular ultrasonographic images by anonymous numbering of the images. Testicular PI of images in the vertical plane was determined by drawing six circles 10 mm in diameter in the parenchyma of the testicle within 10 mm of the mediastinum of the testicle (three medially and three laterally to the mediastinum testes) where the parenchyma appeared homogenous. The same method was used for images in the horizontal plane using four circles 10 mm in diameter (2 cranially and two caudally to the mediastinum testes; Fig. 1C, D). PI within the drawn areas was measured according to shade on a 1 to 255 gray-scale (1 corresponding to black and 255 corresponding to white). A macro was established to calculate the mean, mode, minimum, maximum, and standard deviation (a proxy for

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