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reproduction

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#### ABSTRACT

This experiment was conducted to compare the accepted Field system of bull breeding soundness evaluation (BBSE) utilized by veterinarians in Canada with the centralized Swedish (Lab) system. To be classified as a satisfactory potential breeder, both systems require that bulls must have  $\geq$ 70% morphologically normal sperm; however, minimum progressively motile sperm ( $\geq$ 60% – Field;  $\geq$ 30% – Lab) and maximum sperm class abnormalities proportions differ (<20% – Field; <15% – Lab).

Semen was collected by transrectal massage (TM) from 54 sexually mature beef bulls. The percent progressively motile sperm was determined within minutes of collection and these data were shared. Sperm morphology was evaluated independently utilizing slide staining and microscopic assessment techniques typically used for the evaluation of bull semen within the respective countries. Mean progressively motile sperm was  $45\% (\pm 23.8\%)$ . The mean proportion of normal sperm recorded in the Field ( $83.6 \pm 12.1\%$ ) was greater than the Lab ( $71.1 \pm 18.2\%$ ) (P < 0.01) with more sperm head abnormalities recorded in the Lab ( $13.6 \pm 11.4$ ) than the Field ( $2.4 \pm 2.8$ ) (P < 0.01). The number of bulls exceeding the  $\geq 70\%$  morphologically normal sperm was 51 (94.4%) in the Field versus 36 (66.7%) in the Lab (kappa = 0.21). Only 13 bulls were classified as satisfactory potential breeders by both systems (kappa = 0.15).

Efforts to improve and standardize the evaluation of bull sperm morphology are needed, and the use of  $\geq$  30% progressively motile sperm threshold for TM-derived samples is recommended.

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#### 1. Introduction

\* Corresponding author. Tel.: +46 018672167; fax: +46 018673545. *E-mail addresses:* cwp128@mail.usask.ca (C.W. Palmer), Bull breeding soundness evaluations (BBSE) are considered to be very reliable for identifying fertile bulls as well as those that are subfertile (Barth, 2000). Bulls must be physically sound, achieve the minimum scrotal circumference and have good semen quality to be considered satisfactory breeders. A complete BBSE includes collection of semen and

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evaluation of sperm concentration, motility and morphology (Barth, 2000).

In Sweden, all sperm morphology evaluations of semen samples collected from bulls are traditionally performed at one semen reference laboratory by one or two technicians highly experienced in performing semen analyses. Based on studies of systematic investigations of sperm morphology and infertility in bulls Lagerlöf established the present sperm laboratory in the 1930s, one of the first in the world. The lab was then further developed by Bane who included assessment of wet-mount preparations of formol-saline fixed sperm. The procedures for the routine semen assessments in the laboratory are carefully defined and documented and the intra assay coefficient of variation have been found to be between 10 and 15%. However, in many other countries (e.g. Canada) semen evaluation is performed in the field by practicing veterinarians themselves (Palmer et al., 2005). To be able to perform a complete bull breeding soundness evaluation (BBSE) in the field (e.g. McGowan et al., 1995; Barth, 2000), Swedish practitioners might benefit from learning a semen evaluation method appropriate for field conditions. However, to our knowledge, there are no studies describing to what extent a semen evaluation protocol used in the field corresponds to a semen evaluation protocol used at our centralized semen evaluation laboratory. Quality control must be a consideration if we are to enable veterinarians to perform complete BBSEs in Sweden.

Collection of semen by an artificial vagina (AV) is commonly used in Swedish dairy and beef bulls at AI-stations, but is considered too difficult; time consuming and sometimes dangerous in untrained and potentially fractious beef bulls and is thus not amendable to routine on-farm use. Since electroejaculation (EEJ) is forbidden for animal welfare reasons on unanaesthetized animals in Sweden, an alternative method of semen collection applicable to field conditions is needed (Persson et al., 2007). Having the ability to provide a completed BBSE in the field in Sweden would be expeditious to both cattle producers and veterinarians and transrectal massage (TM) directed specifically towards the ampullary region of the ductus deferens has been shown to be very effective for producing semen emission and semen samples in the field (Palmer et al., 2004, 2005; Persson et al., 2006).

The objectives of this study were to compare evaluations of sperm morphology collected by TM completed under field conditions in Canada with evaluations of the same semen samples made in our reference laboratory in Sweden, and to compare the number of bulls classified as satisfactory potential breeders utilizing the percent normal sperm morphology and minimum percent motility thresholds in use by the respective countries.

#### 2. Materials and methods

#### 2.1. Animals and semen collection

Breeding soundness evaluations were performed on 54 range bulls in 3 community pastures in western Canada. These pastures utilize vast natural grasslands or rangelands where the cattle are handled infrequently; therefore, the bulls are commonly referred to as range bulls. The bulls varied in age from 1 to 9 years and included the following breeds: Angus (n = 29), Charolais (n = 15), Hereford (n = 7), Gelbvieh (n = 1), Corriente (n = 1), and Simmental (n = 1). Transrectal massage directed specifically at the ampullae (Palmer et al., 2004) was performed by two veterinarians trained in the use of this technique. Disposable plastic semen collection cones were attached to 10 mL plastic tubes to recover the semen sample. To prevent chilling of the sample, the tube and cone were immersed in a 500 mL water jacket with a 40 cm long handle. The water jacket temperature was maintained at 37 °C.

#### 2.2. Semen assessments in the field (Field)

Semen volume was determined in the graded collection tube and a semen sample was defined by a minimum of 1 mL of cloudy seminal fluid. Sperm concentration was estimated based on the density of the semen sample (Barth, 2000). Percent progressively motile sperm was determined with phase contrast microscopy at  $400 \times$  magnification after placing a coverslip over a 2–4 mm drop of semen on a warmed microscope slide.

Percent live sperm was determined by identifying sperm that did not take up eosin stain in an eosinnigrosin stained smear with bright field microscopy at 1000× magnification. Sperm morphology was determined by assessing the number of normal verses abnormal sperm per 100 counted cells in an eosin-nigrosin stained smear with bright field microscopy at  $1000 \times (Barth, 2000)$  and was performed by a single veterinarian trained in spermatology (C. Palmer). All Field evaluations including the assessment of sperm motility, percent live sperm and sperm morphology were performed chute-side within minutes of collection of the semen sample. The abnormal sperm were categorized and counted using a cell counter as follows: abnormal heads; abnormal midpieces; abnormal principal pieces; detached abnormal heads; detached normal heads; proximal droplets; other (not fitting one of the aforementioned categories). If a sperm cell had defects in more than one category both keys were pressed; therefore, at least 2 defects were recorded, but only a single cell was counted. The morphological abnormalities were recorded as a percentage of the total number of counted sperm. The standards for the Field evaluation were those used by the Western Canadian Association of Bovine Practitioners. Accordingly, that system recommended that a bull classified as a satisfactory potential breeder should have a minimum of 70% morphologically normal sperm. In addition, the number of sperm with nuclear abnormalities should not exceed 20%, and tail (including the midpiece and principal piece) or acrosomal abnormalities should not exceed 25%, respectively. Therefore, as an example, if a spermiogram had 75% morphologically normal sperm, but the remaining 25% of sperm had nuclear vacuoles then the bull would not be classified as a satisfactory potential breeder. Also, for a satisfactory classification, the percent progressively motile sperm should be  $\geq$  60% (Barth, 2000). All defects of the sperm head were considered to be nuclear defects except for loose (detached) heads and acrosome defects which were considered separately. Defects Download English Version:

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