Theriogenology 79 (2013) 946–952

Contents lists available at SciVerse ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Sperm chromatin in beef bulls in tropical environments

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ARTICLE INFO

Article history:
Received 1 April 2012
Received in revised form 19 December 2012
Accepted 19 January 2013

Keywords:
Bull
Sperm chromatin
Semen parameter
Sperm chromatin structure assay

ABSTRACT

Sperm chromatin status was assessed in 565 Zebu and Zebu crossbred beef bulls in extensive tropical environments using the sperm chromatin structure assay (SCSA). The SCSA involved exposure of sperm to acid hydrolysis for 0.5 or 5.0 minutes, followed by flow cytometry to ascertain relative amounts of double-stranded (normal) and single-stranded (denatured) DNA, which was used to generate a DNA fragmentation index (%DFI). With conventional SCSA (0.5-minute SCSA), 513 bulls (91%) had <15 %DFI, 24 bulls (4%) had 15 to 27 %DFI, and 28 bulls (5%) had >27 %DFI. In 5.0-minute SCSA, 432 bulls (76%) had <15 %DFI, 68 bulls (12%) had 15 to 27 %DFI and 65 bulls (12%) had >27 %DFI. For most bulls, the SCSA was repeatable on two to four occasions; however, because most bulls had <15 %DFI, repeatability of the SCSA will need to be determined in a larger number of bulls in the 15 to 27 %DFI and >27 %DFI categories. The %DFI was negatively correlated with several bull semen parameters and the strongest negative correlation was with normal sperm. There was a strong positive correlation between %DFI and sperm head abnormalities. Based on these findings, most Zebu beef bulls in extensive tropical environments had relatively stable sperm chromatin. Based on the apparent negative correlations with conventional semen parameters, we inferred that the SCSA measured a unique feature of sperm quality, which has also been suggested for other species. Further studies on the relationships between sperm chromatin stability and fertility are required in beef bulls before chromatin status can be used as an additional predictor of the siring capacity of individual bulls in extensive multiple-sire herds.

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1. Introduction

Factors which contribute to the capacity of beef bulls to sire calves in large multiple-sire mating groups in extensive environments remain poorly understood. In a study on semen quality and siring capacity in beef bulls, sperm morphology accounted for 35% tp 57% of the variation between animals [1]. Furthermore, 14% of bulls sired approximately 30% of calves, and in some mating groups, 58% of bulls individually sired only approximately 10% of calves [1]. The bulls in the study by Holroyd et al. [1] had been subjected to a breeding soundness evaluation and the findings therefore revealed that many bulls do not achieve their expected contribution to mating. The findings also highlighted the general lack of indices that can be used as predictors of the siring capacity of beef bulls in extensive multiple-sire herds.

Sperm chromatin structure and stability has emerged as a semen parameter that is linked to fertility in men [2,3]. One assay for chromatin stability is the sperm chromatin structure assay (SCSA), which measures the degree of DNA denaturation from double- to single-stranded when sperm DNA is exposed to brief acid hydrolysis [4,5]. The SCSA generates a DNA fragmentation index (%DFI); thresholds

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0093-691X/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.theriogenology.2013.01.012

have been set for men who are fertile (<15 %DFI; stable chromatin), moderately fertile (15–27 %DFI; moderately stable chromatin), and infertile (>27 %DFI; unstable chromatin) [2,5]. A notable feature of the SCSA in men is the apparent lack of a relationship with conventional semen parameters, which led to the conclusion that the SCSA was an independent measure of sperm quality [6]. In an early study in beef bulls, sperm chromatin structure as judged by the SCSA, was correlated with the outcome after heterospermic insemination [7].

Infertility in men with >27 %DFI is often manifested as a higher incidence of early embryonic mortality [2]. In tropical beef herds, calving periods are typically extended even when heifers and cows are well managed for fertility and bulls are subjected to a breeding soundness evaluation. Perhaps extended calving periods are caused, at least in part, by early embryonic mortality before a pregnancy is established. Furthermore, sperm chromatin status could be a contributing factor to early embryonic mortality in cattle. Studies on sperm chromatin stability and fertility in bulls have yielded conflicting findings. Relationships between chromatin stability and field fertility of AI bulls were reported in some studies [8–11], but no relationship was found in another study of AI bulls [12]. As already noted, one study in bulls reported a relationship between chromatin stability and heterospermic insemination outcome which was not as well predicted by conventional parameters used to assess semen [7]. In a further study, chromatin stability was related to sperm nuclear morphology, which in turn was related to fertility [13]. All studies on sperm chromatin in bulls have used a relatively small number of animals.

The emergence of molecular gene markers in beef cattle, and trend toward the use of reduced numbers of bulls of high genetic merit in extensive multiple-sire herds, have emphasized the need to develop indices to reliably predict the siring capacity of individual bulls. The aims in the present study were to: (1) ascertain the prevalence of sperm chromatin instability in bulls in extensive tropical environments; and (2) determine the relationships between sperm chromatin status and conventional semen parameters. Sperm chromatin status was determined for 565 Zebu and Zebu crossbred beef bulls.

2. Materials and methods

2.1. Semen collection and storage

Semen was obtained by two veterinarians experienced in standard electroejaculation procedures [14]. Shortly after collection, an aliquot (1–2 mL) of undiluted semen was placed in a cryovial (Nunc or Greiner Bio-one; Cellstar) and snap frozen in liquid nitrogen. Samples were stored in liquid nitrogen for transport to the laboratory and then remained in liquid nitrogen or were transferred to −80 °C until required for the SCSA. It would not be expected that storage in liquid nitrogen or at −80 °C would influence the SCSA.

2.2. Sperm chromatin structure assay

Sperm chromatin stability was assessed using the SCSA as described by Evenson and Jost [4]. An aliquot (5–10 μL) of semen was placed in a 1.5-mL centrifuge tube (Quality Scientific Plastics) and diluted in TNE buffer (0.01 M TRIS-HCl, 0.15 M NaCl, 1 mM EDTA; pH 7.4) to obtain a sperm concentration of 1 to 2 $\times$ 10^6 sperm per mL. An aliquot (60 μL) of diluted semen was then placed in a 1.2-mL microtiter tube (Quality Scientific Plastics). The sample was mixed with 120 μL of a pH 1.2 detergent solution containing 0.15 M NaCl, 0.08 N HCl, and 0.1% Triton X-100. After acid hydrolysis, 360 μL of staining solution (6 μg/mL acridine orange, 0.2 M Na_2HPO_4, 0.1 M citric acid monohydrate, 1 mM disodium EDTA, and 0.15 M NaCl, pH 6.0) were added and the sample placed in an ice slurry for at least 3 minutes before assessment on a flow cytometer.

Chromatin status was ascertained using a FACSCalibur Flow Cytometer (Becton Dickinson, Sydney, Australia) equipped with an argon ion laser (488 nm) and interfaced with a computer. For instrument calibration and setup, a reference semen sample was collected from a bull external to this study and prepared as described by Evenson and Jost [4]. The reference sample was repeated after every five test bull samples to ensure instrument settings remained stable between samples and sample days. Each sample was measured twice at a flow rate of <300 sperm per second and results represent the average value of the two measurements. A total of 5000 sperm were evaluated for each replicate sample. Off-line analysis of the flow cytometric data was carried out using CellQuest software (v3.1f; Becton Dickinson). Sperm populations were divided into four significant statistical regions, drawn using CellQuest software (v3.1f; Becton Dickinson). These regions were: (1) the main population of cells; (2) the %DFI; (3) cellular debris; and (4) sperm with high DNA stainability [5]. The %DFI represents the proportion of sperm in a sample with denatured chromatin and is considered the most important SCSA variable related to fertility [4]. The SCSA does not provide information on the extent of DNA denaturation for individual sperm.

2.3. Experiment 1: Acid hydrolysis kinetic experiment

To determine the relationship between duration of acid hydrolysis (pH 1.2) and apparent sperm chromatin instability, a time-course study was conducted using semen obtained by ejaculation from Zebu crossbred (Droughtmaster) beef bulls (2–5 years old; N = 5). After acid hydrolysis for 0.5, 2, 5, 10, 20, 30, and 60 minutes, 360 μL of staining solution was added and the samples placed in ice for 3 minutes before assessment using flow cytometry, as described in section 2.2.

2.4. Experiment 2: Prevalence of sperm chromatin instability in beef bulls

Semen was obtained by electroejaculation from bulls (N = 565) at 14 locations in tropical northern and central Queensland, Australia. The bulls were undergoing a breeding soundness evaluation and there had not been any previous culling or selection of bulls. This was considered important, so as not to bias the results if there had been some selection for fertility indices. The ejaculation history of bulls before semen collection was not known.

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