Animal Reproduction Science 172 (2016) 26–31

Contents lists available at ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

Sperm DNA integrity in frozen-thawed semen from Italian Mediterranean Buffalo bulls and its relationship to in vivo fertility

Rosanna Serafini[a,*], Charles C. Love[b], Angelo Coletta[c], Gaetano Mari[d], Beatrice Mislei[d], Chiara Caso[c], Rossella Di Palo[a]

[a] Department of Veterinary Medicine and Animal Production, University of Naples "Federico II", Naples, Italy
[b] Department of Large Animal Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, TX, USA
[c] Associazione Nazionale Allevatori Specie Bufalina (ANASB), 81100, Caserta, Italy
[d] AUB-National Institute of Artificial Insemination, University of Bologna, Italy

ARTICLE INFO

Article history:
Received 10 February 2016
Received in revised form 21 June 2016
Accepted 30 June 2016
Available online 30 June 2016

Keywords:
Buffalo sperm
DNA assays
Fertility

ABSTRACT

The relationship among sperm attributes of DNA integrity, sperm motility, morphology, viability, acrosome integrity and in vivo fertility of frozen-thawed Italian Mediterranean Buffalo (IMB) sperm has not been reported. Straws of frozen-thawed semen samples from three bulls were examined. Sperm DNA assays (i.e., neutral Comet assay, Sperm Bos Halomax-SBH and Sperm Chromatin Structure Assay-SCSA) were not correlated to each other ($P > 0.05$). Many neutral Comet assay measures were correlated to total sperm motility-TMOT (% head-H-DNA, r = 0.74; Olive moment, r = −0.76; $P < 0.05$) and coiled tails (r-values ranged from% H-DNA, r = −0.80 to tail length, r = −0.71; $P < 0.05$). The COMP-α_t was negatively correlated to viable acrosome intact (VAI) sperm, and distal droplets ($r = -0.60$ and −0.61; $P < 0.05$), whereas Mean-α_t and Mode-α_t were positively correlated to bent mid-pieces ($r = 0.63$ and 0.61; $P < 0.05$). The SBH assay was positively correlated to non-viable acrosome damaged (NVAD) sperm ($r = 0.60$; $P < 0.05$) and negatively correlated to viable acrosome damaged (VAD) sperm ($r = -0.63$; $P < 0.05$). The overall pregnancy rate (PR-at 30 and 45 d post artificial insemination-AI) and the calving rate were 57%, 55% and 45%, respectively. Among sperm features analyzed the area under the Receiver Operating Characteristic (ROC) Curve was significant ($P < 0.05$) for TMOT, NVAD, Standard Deviation-α_t (SD-α_t) and neutral comet measures (Olive tail moment and tail moment, % H- DNA and tail area) in estimating pregnancy.

1. Introduction

The Italian Mediterranean Buffalo (*Bubalus bubalis*, IMB) is a national breed of approximately 380,000 animals, of which 94,769 are registered to the Studbook of the National Breeders Association of the Buffalo (ANASB). IMB is the only buffalo breed in the world that has undergone a breeding selection program since 2001, and progeny tests have been carried out to select males (i.e., 5–6 bulls per year) of high genetic merit, which are sent to the bull centers (BC) for semen collection, evaluation and freezing. Previous researchers have evaluated quality in Murrah (Vale, 1994; Pant et al., 2003) and swamp buffalo (Koonjaenak

* Corresponding author at: Department of Large Animal Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, 4475 TAMU, 77843-4475, TX, USA.
E-mail address: rosanna.serafini@gmail.com (R. Serafini).

http://dx.doi.org/10.1016/j.anireprosci.2016.06.010
0378-4320/

et al., 2007a,b); but research of the IMB bulls is limited (Galli et al., 1993; Sansone et al., 2000; Presicce et al., 2003; Boccia et al., 2010). There are currently no recommendations for the evaluation of sperm quality in the IMB bulls and generally decisions about reproductive parameters (i.e., testes size and sperm quality) are based on information from Bos taurus and Bos indicus bulls (Chenoweth et al., 1992).

The number of IMB offspring resulting from frozen-thawed sperm is low (i.e., only 6% of the registered IMB cows are bred with frozen-thawed semen; ANASB, 2014) due to the predominance of use of natural mating. However, the use of frozen semen from selected sires has the potential to improve the genetic value of the IMB population and interest in frozen IMB semen is currently increasing for both on-farm insemination, international shipment and sex-sorting. Currently, methods to evaluate the sperm quality of IMB bulls of high genetic merit are needed to identify techniques which maximize the fertility of frozen-thawed IMB bull sperm.

The aims of this study were to determine, in IMB bull sperm, the relationship among 1) three measures of DNA quality: the neutral Comet assay, the Sperm Chromatin Structure Assay (SCSA) and the Sperm Bos Halomax (SBH); 2) three measures of DNA quality and sperm motility, morphology, viability; and 3) sperm DNA quality, motility, morphology, viability, and fertility.

2. Materials and methods

2.1. Sampling

This study used frozen/thawed semen from three ejaculates from each of three IMB bulls (aged 2–3 years) under progeny test housed at Centro Tori Chiacchierini (Perugia, Italy), where semen was regularly collected, analyzed and processed.

Straws from each IMB bull were transferred and stored at the Laboratory of Biotechnology applied to Animal Production (Department of Veterinary Medicine and Animal Production, Naples, Italy).

Two straws from each ejaculate were used for analysis. Analyses included total sperm motility (TMOT), sperm viability and acrosome integrity, sperm morphology, SCSA, neutral Comet assay and SBH.

2.2. Semen processing

Semen was thawed in a water bath at 37 °C for 30′ and TMOT was visually evaluated by a phase-contrast microscope (Nikon SMZ 1500, Plan Apo WD70, Nikon, Japan), 200 x magnification, equipped with a warm stage set at 37 °C, by the same operator.

Immediately post-thaw, semen was centrifuged with 2 mL of Dulbecco's phosphate buffered saline (DPBS) at 400*g* for 10 min to remove freezing extender components. Following centrifugation, the supernatant was discarded and the sperm pellet concentration was determined by a hemocytometer. An aliquot was diluted to a concentration of 1 million/mL in DPBS for further analysis with the Comet assay.

2.3. Sperm morphology

Prior to and immediately post-centrifugation semen was diluted with buffered formol saline in a 1.5 mL Eppendorf tube and evaluated by differential-interference contrast microscopy (Nikon Diaphot 300, Nikon Inc., Melville, NY, USA; 1000× magnification). A total of 100 sperm per sample was evaluated and all abnormalities identified on each sperm were recorded, as reported by Kenney et al. (1983). The following sperm morphological features were identified: normal, abnormal heads (mis-shaped heads, micro- and macrocephalic sperm, and large nuclear vacuoles), abnormal acrosomes, detached heads, proximal cytoplasmic droplets, distal cytoplasmic droplets, swollen or irregular midpieces, bent or coiled midpieces/tails, and premature germ cells.

2.4. Sperm acrosome intactness and viability (*Pisum sativum agglutinin-PSA/propidium iodide-PI*)

Samples were diluted to a final concentration of 0.05 mg/mL fluorescein isothiocyanate (FITC)-conjugated agglutinin derived from *Pisum sativum* (FITC-PSA) staining (Sigma-Aldrich, Milan, Italy) and 12 μM PI (Sigma-Aldrich, Milan, Italy) in DPBS, incubated for 10 min at room temperature and immediately evaluated by flow cytometry (BD FACSCalibur Flow Cytometry System, BD Biosciences, San Jose, CA, USA), as described by Love et al. (2012). A total of 5000 cells were analyzed and sperm was classified as non-viable acrosome intact (NVAI), non-viable acrosome damaged (NVAD), viable acrosome intact (VAI) and viable acrosome damaged (VAD).

2.5. Sperm DNA assays

Unless otherwise stated, the SCSA, the neutral Comet assay and the SBH were performed as previously described by Serafini et al. (2015a). SCSA was performed by flow cytometry (BD FACSCalibur Flow Cytometry System, BD Biosciences, San Jose, CA, USA) and measures included Mean-α_t, Standard Deviation-α_t (SD-α_t), COMP-α_t and Mode-α_t.

For the neutral Comet assay, electrophoresis was performed in a horizontal unit (Single Cell Gel Electrophoresis system, Scie-Plas Ltd, Cambridge, UK) at 0.7 V/cm for 30 min, at room temperature in the dark. For each sample 2 slides (2 wells/slide) were prepared and 50 sperm/slide were counted (25 sperm/well). The samples, stained with propidium iodide (PI), were evaluated by fluorescent microscopy (Leica microscope model DMRA2, equipped with an objective HC Plan APO, magnification 20 x/0, 70, ∞/0.17/c). Images acquisition was performed using a software program (Leica QFluoro, Wetzlar, Germany) and microscope images were evaluated with software (CometScore Version 1.5 TriTek Corp, Sumerduck, VA, USA). Comet measurements included the following parameters, grouped as dimension measures (i.e., comet length, tail length, comet height, comet area, head diameter, head area, tail area), intensity measures (comet intensity, comet mean intensity, head intensity, head mean intensity, tail inten-