Animal Reproduction Science 148 (2014) 1–17

Contents lists available at ScienceDirect

Animal Reproduction Science

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

Seminal plasma proteome of electroejaculated *Bos indicus* bulls

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ARTICLE INFO

Article history:
Received 26 August 2013
Received in revised form 16 April 2014
Accepted 30 April 2014
Available online 9 May 2014

Keywords:
Proteome
Electrophoresis
Sperm
Bos indicus
Seminal plasma

ABSTRACT

The present study describes the seminal plasma proteome of *Bos indicus* bulls. Fifty-six, 24-month old Australian Brahman sires were evaluated and subjected to electroejaculation. Seminal plasma proteins were separated by 2-D SDS-PAGE and identified by mass spectrometry. The percentage of progressively motile and morphologically normal sperm of the bulls were 70.4 ± 2.3 and 64 ± 3.2%, respectively. A total of 108 spots were identified in the 2-D maps, corresponding to 46 proteins. Binder of sperm proteins accounted for 55.8% of all spots detected in the maps and spermadhesins comprised the second most abundant constituents. Other proteins of the *Bos indicus* seminal plasma include clusterin, albumin, transferrin, metalloproteinase inhibitor 2, osteopontin, epididymal secretory protein E1, apolipoprotein A-1, heat shock 70 kDa protein, glutathione peroxidase 3, cathelicidins, alpha-enolase, tripeptidyl-peptidase 1, zinc-alpha-2-glycoprotein, plasma serine protease inhibitor, beta 2-microglobulin, proteasome subunit beta type-4, actin, cathepsins, nucleobinding-1, protein S100-A9, hemoglobin subunit alpha, cadherin-1, angiogenin-1, fibrinogen alpha and beta chain, ephirin-A1, protein DJ-1, serpin A3-7, alpha-2-macroglobulin, annexin A1, complement factor B, polymeric immunoglobulin receptor, seminal ribonuclease, ribonuclease-4, prostaglandin-H2 D-isomerase, platelet-activating factor acetylhydrolase, and phosphoglycerate kinase 1. In conclusion, this work uniquely portrays the *Bos indicus* seminal fluid proteome, based on samples from a large set of animals representing the Brahman cattle of the tropical Northern Australia. Based on putative biochemical attributes, seminal proteins act during sperm maturation, protection, capacitation and fertilization.

1. Introduction

Seminal plasma is a complex mixture of secretions from the testes, epididymis and accessory sex glands providing a medium that supports sperm during ejaculation, migration through the female reproductive tract and

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http://dx.doi.org/10.1016/j.anireprosci.2014.04.016
0378-4320/

fertilization. To date, studies of the bovine seminal plasma proteome have primarily been done in *Bos taurus* cattle, where diverse classes of proteins and their physiological roles were described (Killian et al., 1993; Mortarino et al., 1998; Kelly et al., 2006; Druart et al., 2013). Based on such studies and from others, it has been found that seminal plasma proteins can protect the sperm against oxidative stress during epididymal transit and storage, and against immune responses in the female reproductive tract after ejaculation (Yanagimachi, 1994; Kraus et al., 2005). It has been also demonstrated that epididymal and accessory sex gland fluids provide a system which supports sperm motility through the provision of specific enzymes and substrate effectors (Fink et al., 1989). During sperm transit in the female reproductive tract, specific seminal plasma proteins bind to the sperm membrane and play important roles in capacitation and sperm interactions with the oviductal epithelium (Therien et al., 1998, 1999; Gwathmey et al., 2006). In addition, proteins from seminal plasma have the capacity to interact with both sperm and oocyte, participating in the acrosome reaction, fertilization and initial embryonic development (Goncalves et al., 2007, 2008).

The divergence that gave rise to the two domestic cattle subspecies (*Bos taurus* and *Bos indicus*) likely occurred about 330,000 years ago (Achilli et al., 2008). The *Bos indicus*, originally confined to the Asian Continent was brought in the beginning on the 20th century to countries such as Australia, United States and Brazil because it was thought that attributes of those animals would fit into the climate and pasture conditions of certain regions of those countries. Today, the *Bos indicus* subspecies dominate the tropical areas of these countries and is the most predominant type of beef cattle. The Brahman, a typical *Bos indicus* breed, is one of the most common cattle breeds in Northern Australia, due to its tick resistance, and heat and drought tolerance (Bortolussi et al., 2005). Despite the economic importance of these animals and uniqueness of their tropically adjusted behavior and physiology, knowledge of their reproductive biology is still limited, especially in males. For instance, to date only eleven proteins have been identified in the seminal plasma of *Bos indicus* bulls (Assumpcao et al., 2005) and a single study conducted by (Jobim et al., 2004) has reported statistical associations between seminal plasma proteins and quality parameters of frozen-thawed semen samples from Nelore (*Bos indicus*). Thus, the objective of the present study was to describe the seminal plasma proteome of *Bos indicus* bulls.

2. Materials and methods

2.1. Animals, sample collection and semen analysis

The present study used 56 animals selected from a subset of a cohort of Brahman (*Bos indicus*) bulls bred for a large genetic study about the relationships between meat quality, production and fertility traits, conducted as part of the Cooperative Research Centre for Beef Genetic Technologies program (Johnston et al., 2009; Corbet et al., 2013).

A bull breeding soundness examination was conducted when bulls were at the average age of 24 months, and included measurements of live weight and scrotal circumference, and collection of a sample of semen by electroejaculation (Burns et al., 2013). Semen samples were assessed immediately post-collection by phase contrast microscopy (×400) using a warm stage set at 37 °C to determine the percentage of sperm with forward progressive motility. An aliquot of the semen sample was diluted into a 0.2% glutaraldehyde in phosphate buffered saline for assessment of sperm morphology. The morphology of 100 sperm cells was determined by examining a thin coverslip preparation of semen using phase contrast microscopy with a differential interference contrast objective (×1000). The percentage of morphological normal sperm (PNS) was determined by the same accredited technician (Fordyce et al., 2006). The remaining semen sample was centrifuged at 700 × *g* for 10 min (4 °C) to separate seminal plasma from spermatozoa, and each fraction was then transferred into cryo tubes and snap frozen in liquid nitrogen. Semen collection procedures were approved in by the J M Rendel Laboratory Animal Experimental Ethics Committee (CSIRO, Queensland), as approvals RH198/04 and RH219/06.

2.2. Determination of protein concentration

Seminal plasma samples were thawed and centrifuged at 10,000 × *g*, 60 min, 4 °C. One aliquot of the supernatant was used for determination of protein concentrations (Bradford, 1976), and the remaining aliquots were stored at −80 °C. Briefly, an analytical calibration curve was obtained from standard solutions of bovine serum albumin (Sigma-Aldrich, AU). Samples were measured in triplicates, after dilution at a ratio of 1:80 (v/v) in milli-Q water (Millipore, USA). Diluted samples (100 μL) were mixed with 2.5 mL of Bradford's reagent and read after 10 min at 595 nm (Ultraspec III, SG Lifesciences, USA). Samples from the 56 bulls included in the present study had protein concentrations of at least 12 mg/mL.

2.3. Two-dimensional gel electrophoresis

From each bull, a sample of seminal plasma containing 750 μg of total protein was mixed with a re-hydration buffer (7 M urea, 2 M thiourea, 65 mM DTT [dithiothreitol], 0.5%, v/v free ampholytes [IPG buffer, pH 3–11], 0.5%, w/v CHAPS [3-3-cholamidopropyl dimethylammonio-1-propanesulfonate] and trace amounts of bromophenol blue) sufficient to make up 450 μL of protein solution. Then, each sample was loaded in the reswelling tray, overlaid with a 24 cm IPG (immobilized pH gradient) strip (pH 3–11, linear; GE Lifesciences, USA), and allowed to rehydrate for 20 h. Isoelectric focusing was carried out in Ettan™ IPGphor 2™ apparatus (GE Lifesciences, USA) at 20 °C, according to the following program: 100 V for 2 h, 250 V for 2 h, 1000 V for 1 h, 5000 V for 2 h gradient, 5000 V for 8 h and a 30 V hold. After focusing, the IPG strips were incubated for 15 min in equilibration buffer I (6 M urea, 50 mM Tris–HCl pH 8.8, 29.3%, v/v glycerol, 2%, w/v SDS, 1%, w/v DTT) and re-equilibrated for an additional 15 min in buffer II (equilibration buffer I, containing 2.5%, w/v iodoacetamide instead of DTT). After equilibration, the IPG strips were fixed with agarose (5% in SDS-PAGE running buffer)