Animal Reproduction Science 177 (2017) 25–34

Contents lists available at ScienceDirect

Animal Reproduction Science

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

Profiling of sperm gene transcripts in crossbred (*Bos taurus x Bos indicus*) bulls

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ARTICLE INFO

Article history:
Received 23 April 2016
Received in revised form
20 November 2016
Accepted 2 December 2016
Available online 5 December 2016

Keywords:
Crossbred bulls
Gene expression
Microarray
Sperm motility
Transcriptome

ABSTRACT

Crossbred cattle in some sectors of the world have a significant role in enhancing milk production thereby enhancing the per capita milk availability as a human food source. However, there are certain constraints associated with crossbred animals, such as disease susceptibility, increased reproductive problems, repeat breeding and poor seminal quality. The semen of crossbred bulls has a poor freezing capacity, increased cryo-damage, poor mass cell motility, greater percentages of dead/abnormal sperm and poor initial and post-freeze cell motility. The rejection rate of crossbred bulls for cryostorage of semen has been reported to be as great as 50% as a result of unacceptable semen quality. The identification of superior bulls using molecular technologies is needed which necessitates identification of the genes having a role in sperm function. The present study was, therefore, conducted to gain information on identification and expression of genes having a role in sperm motility in crossbred bulls. The gene transcripts in bulls with sperm of superior and inferior quality were profiled in Vrindavani crossbred cattle by microarray analyses and the results were verified by real time-quantitative PCR. Microarray analyses revealed 19,454 genes which were differentially expressed. At a two-fold cut off, 305 genes were differentially ($P < 0.01$) expressed with 160 genes upregulated and 145 genes down regulated. Some of the upregulated candidate genes were further validated by RT-qPCR. These genes had a four to 16 fold upregulation in sperm with inferior motility as compared to sperm of crossbred bulls with superior motility.

1. Introduction

Conception requires transport of sperm, initially in the male and subsequently female reproductive tract. To assess the fertilizing ability of males/bulls/sires, the semen ejaculate is evaluated for various physical variables such as volume, color, consistency, concentration, percent live sperm, percent sperm abnormalities, acrosomal integrity, mass sperm motility, initial progressive sperm motility and post thaw sperm motility, before initiation of a breeding program. Among the different seminal variables, sperm motility is foremost in determining a bulls' fertilizing capacity. However, mere visual evaluation of semen sam-

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http://dx.doi.org/10.1016/j.anireprosci.2016.12.003
0378-4320/

ples may not be sufficient to assess the fertility potential of bulls. Numerous *in vitro* analyses methods have been proposed which determine the fertilizing capacity of ejaculates (Rodriguez-Martinez, 2006; Rodriguez-Martinez and Barth 2007). The sperm motility is controlled by many genes that have a role in flagellar structure, energy metabolism, mitochondrial functions and ion exchange channels. With the rapid progress made in genomics, the profiling of mRNA transcripts in sperm could be a rapid technology for the assessment of semen's fertilizing potential.

Crossbred (*Bos taurus* x *Bos indicus*) cattle, have a major role in the White Revolution in India that relates to increased milk production thereby enhancing the per capita milk availability. However, there are certain constraints associated with crossbred animals such as greater disease susceptibility, increased reproductive problems, repeat breeding and poor seminal quality (Venkatasubramanian et al., 2003; Dhanju et al., 2006; Martin et al., 2012). The semen of crossbred bulls has a compromised freezing capacity, increased cryoinjuries, poor mass sperm motility, a greater percentage of dead/abnormal sperm and less initial and post-freeze cell motility (Dhanju et al., 2006; Ghosh et al., 2007). The rejection rate of crossbred bulls for cryopreservation of semen has been reported to be as great as 50%, owing to their unacceptable semen quality (Mathew et al., 1982; Sahni and Mohan, 1988; Rao and Rao, 1991; Kumar, 2006; Ghosh et al., 2007). The crossbred cattle population in India is 39.732 million (Livestock Census, 2012) indicating more crossbred bulls need to be used that have superior quality semen to satisfy the need for increasing the crossbred cattle population. This identification of bulls that have greater semen cryopreservation capacity using molecular techniques is needed and identification of the genes that have a role in sperm function could help to address this need. The present study was, therefore, conducted to gain information on identification and expression of genes having a role in sperm motility of crossbred bulls.

2. Materials and methods

2.1. Animals

The present investigations were conducted on bulls (*Bos taurus* x *Bos indicus*) of composite breeding which were developed and given the name, Vrindavani, at the Indian Veterinary Research Institute, Izatnagar, India. The Vrindavani cattle are the crosses of Holstein Frisian, Brown Swiss, Jersey (exotic breed) with Hariana (indigenous breed). The exotic inheritance level of these crosses is between 50% and 62.5%. These bulls were maintained at the Germplasm Centre (GPC) of the institute.

2.2. Semen collection and evaluation

Semen was collected from the bulls during the early morning hours, using an artificial vagina maintained at 42–45 °C. Immediately after collection, each ejaculate was kept in a water bath (Memmert, Minitube International, Germany) and maintained at 37 °C and subjected to volume and mass sperm motility evaluation. For motility evaluations, 10× and 40× magnifications occurred using a phase contrast microscope fitted with a thermostatically controlled warm stage at 37 °C (Motic B1 Series, Motic Asia, Hongkong).

Mass sperm motility (MM) of the semen was recorded by placing a small drop of freshly collected neat semen on clean grease free, pre-warmed glass slide at 37 °C and the sample was examined without cover slip under low power magnification (10X). The mass motility of sperm was graded on a 0–5 scale based on the presence of waves and swirls in the sample (Salisbury et al., 1978). After diluting the fresh semen with Tris egg yolk glycerol extender at 37 °C, initial progressive motility of sperm was assessed subjectively (scale = 0% to 100%), by placing a drop of diluted semen on a glass slide covered with a cover slip and viewing fields under high magnification (40×). The motility was recorded as the percentage of progressively motile sperm and it was designated as initial progressive motility (IPM). Post thaw motility of sperm (PTM) of frozen semen was assessed after thawing the semen at 37 °C for 30 s and placing a drop of semen on a slide and covering it with a cover slip at 37 °C with high magnification (40X) and recording the motility as a percentage of progressively motile sperm. These seminal variables were estimated to identify normal (good) and motility impaired (poorly motile) sperm producing bulls. The 55% IPM and 35% PTM values were considered as the threshold/criterion for classifying the semen as freezable (good) or non-freezable (poor). The semen samples which had a value above the threshold were considered as good/freezable whereas below the threshold, were designated as poor/non-freezable semen. Based on semen variables, the two most desirable and two worst bulls were shortlisted for subsequent microarray analyses. Semen samples for RNA isolation from four shortlisted/selected bulls were collected in sterile and DEPC-treated eppendorf tubes and transported to laboratory at 4 °C in an icebox containing ice packs.

2.3. Sperm purification

To ensure RNA isolation exclusively from normal sperm, it was imperative to discard the somatic cells, immature/damaged sperm and other contaminants from the semen. To achieve this, the semen samples were purified with BoviPure™ (Nidacon International, Gothenburg, Sweden). Semen samples were layered gently on three volumes of pre-warmed (37 °C) Bovipure™ in a 15 mL centrifuge tubes. These were then centrifuged at 1500*g* for 30 min at room temperature (18–24 °C). The supernatant was discarded and the sperm pellet obtained at the bottom of the tube was topped up with 8 mL 1X PBS (Ambion, USA). Similar centrifugations were again conducted on the sperm pellet. Another wash with 1 mL 1x PBS was performed and the sperm pellet was suspended in 0.5 mL 1 X PBS and mixed with 0.5 mL RNA Later (Ambion, USA) and stored at −80 °C until the isolation of RNA.