IJAS Iranian Journal of Applied Animal Science

Spermatozoa Molecules in Relation to Bulls Fertility

Review Article

S.A. Lone[1*], R. Sinha[1], A. Rahim[1], B.A. Ganaie[1], A. Singh[1] and N. Shah[1]

[1] Artificial Breeding Research Station, ICAR-National Dairy Research Institute, Karnal, 132001, Haryana, India

Received on: 29 Nov 2016
Revised on: 27 Mar 2017
Accepted on: 31 Mar 2017
Online Published on: Sep 2017

*Correspondence E-mail: drloneshabir@gmail.com

Online version is available on: www.ijas.ir

ABSTRACT

Bull fertility may be defined as the process by which spermatozoa fertilize and activate the ovum and then support embryonic development. Bull fertility is a complex trait having relatively low heritability and plays a vital role for efficient production and reproduction of bovine. Various mechanisms involved in regulating bull fertility associated phenotype and reliable biomarkers are poorly defined. Primary spermatozoa physiology indicators namely sperm molecules and epigenetic factors may play a vital role in predicting sperm physiology and fertility. Spermatozoa and their fingerprints or patterns can play a pivotal role in prediction of fertility in bovine. No reliable tests exist despite genomic selection for evaluating quality of semen and fertility of bull. This review focuses on various molecules related to bull fertility viz., sperm RNAs, seminal plasma proteins, sperm proteins and sperm epigenome. In combination with single-nucleotide polymorphism (SNP), microarrays or sperm molecules and epigenome markers can be used to determine sperm quality and to predict bull fertility.

KEY WORDS sperm epigenome markers, sperm proteins, sperm RNAs.

INTRODUCTION

Spermatocytogenesis is a process by which spermatogonial stem cells undergo mitotic and meiotic divisions to form haploid spermatids. In spermiogenesis, major changes take place within nucleus of spermatid to form head by chromatin structure rearrangement and tightly packing of the DNA (Ward and Coffey, 1991). The packaging of DNA involves the replacement of somatic cell histones by arginine and cysteine rich protamines. The latter two amino acids play a vital role in formation of inter and intra-molecular covalent disulfate bonds between protamines. Two types of protamines have been identified in mammals, namely protamine 1 (PRM1) and protamine 2 (PRM2). The former has been detected in all species including bull spermatozoa, while the latter has been found only in some mammals like human and mouse (Oliva, 2006; Balhorn, 2007). The most important factor which affects reproductive efficiency and economic sustainability of cattle production is fertility. During last 50 years, despite the great improvement in milk production through intensive genetic selection, the efficiency of reproduction has been reduced in dairy cattle due to decreased fertility (Royal *et al.* 2000; Lucy, 2001). Although artificial insemination (AI) is a very successful assisted reproductive technique for cattle, challenges associated with infertility are still faced by AI industry and dairy farmers. Sperm fertility significantly varies among bulls. Fertility variation is regulated by compensable and non-compensable factors, limits efficient reproduction of cattle. Compensable sperm defects related to viability, motility and acrosomal integrity in good fertility bulls can be achieved by increasing sperm concentration in semen deposited in the cow's reproductive tract. It may not be possible to ever demonstrate adequate zygotic, embryonic or fetal development for bulls affected with non-compensable defects (Peddinti *et al.* 2008; Blaschek *et al.* 2011).

Over decades, researchers have attempted to identify semen quality parameters that help to predict sire fertility. But till today, there is no single laboratory assay that can predict the highest fertility bulls or their semen prior to breeding (Rodríguez-Martínez, 2003; Gillan *et al.* 2008). AI breeding is the only current and accurate approach for measuring bull fertility. Genomic selection of sires and dams is a recent trend on which dairy cattle breeding relies upon. About 90% of sires in North America selected by their genomic superiority have reduced generation interval compared to traditional progeny testing programs. The various spermatozoa molecules associated with bull fertility have been discussed below:

Sperm ribonucleic acids (RNA)

The termination of male genome transcription during mid-spermiogenesis leads to transcriptionally inert mature spermatozoa. In bull spermatozoa, diverse populations of messenger RNAs (mRNA) have been detected (Gilbert *et al.* 2007). Sperms lack 18S and 28S ribosomes due to absence of translational activity in contrast to somatic cells and embryos (Kaya and Memili, 2016).

Around 3000 different transcripts have been detected in the mature spermatozoa of normal fertile men using DNA Microarray. All these transcripts have been proven to be transcription results during spermatogenesis (Miller *et al.* 2005). DNA microarrays have enabled the presence of mRNAs in bull sperm (Feugang *et al.* 2010). The detected transcripts includes protamine 1 (PRM1), Casein beta (CSN2) and Amelogenein X/Y (AMELX and AMELY). The proteins of these transcripts are involved in chromatin condensation, transport and signal transduction, respectively. Many mRNAs have been detected in Holstein bull sperm using the next generation sequencing. Among all these mRNAs, HMGB4 was abundantly found in addition to PRM1 (Card *et al.* 2013; Feugang *et al.* 2010).

Using microarray and suppression-subtractive hybridization methods, higher levels of transcripts in high fertile bulls have been demonstrated, whose proteins participate in metabolism, glycosylation, signal transduction, translation and protein degradation in sperm cells (Lalancette *et al.* 2008). Different populations of transcripts result from various sperm RNA extraction methods (Bissonnette *et al.* 2009). Fresh and frozen-thawed Holstein bull sperm had a different panel of five genes in transcript (Chen *et al.* 2014).

Spermatozoa have got an abundant quantity of small non-coding RNAs (sncRNA) including microRNAs (miRNA), which regulate gene expression by degrading mRNA targets or interfering with the translational machinery. sncRNAs are also richly present in human spermatozoa (Krawetz *et al.* 2011). In bull sperm, miRNAs are associated with bull fertility (Govindaraju *et al.* 2012a). Although the total significance and functions of sperm sncRNAs are not understood, they may provide some information regarding spermatogenesis or their functioning during early embryonic development.

Seminal plasma and sperm proteins

Sperm metabolism is regulated by various soluble and structural proteins that influence bull's fertility (Killian *et al.* 1993; Naaby-Hansen *et al.* 1997; Cancel *et al.* 1997; Cooper, 1998; Gerena *et al.* 2000). Some proteins can be positively rather than negatively associated with fertility. Cryopreservation leads to alteration in some sperm proteins that influence the metabolism and fertility of semen (Roncoletta *et al.* 2006).

Certain studies demonstrated the use of protein markers as a method of bull fertility and semen freezing qualities differentiation. From a particular bull, semen fertility may be altered by the presence or absence of particular proteins in the sperm membrane. The functions of some seminal proteins such as osteopontin (OPN), prostaglandin D synthase (PGDS), bovine seminal plasma proteins (BSPs), fertility associated antigen (FAA) and acidic seminal fluid protein (aSFP) have been reported previously (Cancel *et al.* 1997; Manjunath *et al.* 2002). In the female genital tract, bovine seminal plasma proteins (BSP) might serve as docking for high density lipoproteins (HDL) (Roncoletta *et al.* 2006). It is suggested that BSP proteins may be involved in sperm membrane stabilization and prevention of premature acrosomal reaction but mediate acrosomal reaction in latter steps by participating in membrane modifications (Desnoyers and Manjunath, 1992; Manjunath *et al.* 1994; Manjunath *et al.* 2002; Desnoyers *et al.* 1994; Therien *et al.* 1997; Therien *et al.* 1998; Therien *et al.* 1999). Roncoletta *et al.* (2006) revealed that in fresh sperm membrane protein extract, concentration as well as the distribution of BSP proteins, represented 45% of the total protein fraction (spots SM244, SM245 and SM246). They found SM244 spot, characterized as 14.5 kDa protein, its amount was 2.5 times greater in the least fertility group bulls.

Higher seminal BSP levels lead to higher removal of cholesterol and phospholipids from the sperm membrane resulting in destabilization which in turn reduces sperm resistance to freezing and increases susceptibility to cold shock (Manjunath *et al.* 2002). Acid seminal plasma protein (aSFP) (SM239 spot-15 kDa) is secreted from ampulla and epididymis, but not by the testicles (Einspanier *et al.* 1991; Wempe *et al.* 1992). Interaction of this protein with the cattle sperm membrane was reported by Schoneck *et al.* (1996).

Spermatozoa need to perform certain functions like adequate motility, hyperactivation, acrosomal reaction, zona

penetration and support of embryogenesis. These functions make spermatozoa capable of fertilization, egg activation and support of embryonic development. In order to support motility, sperm proteins are essential for providing energy, zona binding, acrosomal reaction and nuclear functions (Govindaraju *et al.* 2012b; Ashrafzadeh *et al.* 2013). Protamine 1 (PRM1) and histones are the nuclear proteins present in the bull sperm. PRM1 includes the majority of nuclear proteins and bull spermatozoa processes diverse histones including testis specific (TH2B), H3 and H4 (Oliveira *et al.* 2013). The histone makeup and their exact amounts and functions in the bull sperm are still unclear. Recent studies demonstrated the association between levels of PRM1 and bull fertility (Dogan *et al.* 2015). PRM1 and histones have essential roles in sperm nuclear structures formation and function. Health of the sperm DNA and zygotic genome activation are influenced by variations in compactness of the nuclear genome. Nuclear proteins, PRM1 and histones involved in shaping chromatin structure and post-translational modifications of these nuclear proteins are involved in influencing physiology of spermatozoa. PRM1 levels in sperm are correlated significantly with bull fertility (Dogan *et al.* 2015). Alteration in the PRM1/histone ratio may lead to changes in sperm nuclear shape which might be an underlying mechanism regulating fertility of bulls.

Energy related proteins viz., phosphoglycerate mutase 2 and isocitrate dehydrogenase are up or down regulated in asthenozoospermia in men, respectively (Zhao *et al.* 2007).

During egg activation and embryonic development, sperm phospholipase C (PLC) Zeta and Post acrosomal WW domain binding protein (PAWP) play an important role by acting as sperm factors (Aarabi *et al.* 2014).

Park *et al.* (2012) reported that spermatozoa from high fertile bulls had higher levels of proteins enolase 1 (ENO1), ATP synthase, p53 protein 2, alpha-2-HS-glycoprotein and glutathione peroxide, while ropporin-1, voltage dependent anion channel 2 (VDAC2) and ubiquinol-cytochrome-c reductase complex coreprotein 2 (UQCRC2) were higher in low fertile bull spermatozoa. Soggiu *et al.* (2013) reported that proteins alpha-enolase, isocitrate dehydrogenase and triosephosphate isomerase are associated with fertility.

Defective spermatozoa having ubiquitin protein on their surface, which is negatively correlated with fertility (Sutovsky *et al.* 2015).

However, Rodríguez-Lozano *et al.* (2014) revealed that there is no correlation between ubiquitin and fertilizing capacity of bull spermatozoa. Using three different proteomic approaches (SDS-PAGE, MALDI-MS and LC-MS/MS), several fertility related proteins have been identified in rooster spermatozoa and seminal plasma (Labas *et al.* 2015). Various proteomic methods, such two dimensional polyacrylamide gel electrophoresis (2D-PAGE) or two dimensional differential gel electrophoresis (2D-PAGE) or gel-free (mass spectrometry) and other reductionist methods, such as immunoblotting (Western Blotting or immunocytochemistry) and flowcytometry, can be used to study sperm proteins (Kaya and Memili, 2016). Using these methods, it is possible to compute associations between cellular levels or spatiotemporal locations of specific sperm proteins and bull fertility phenotypes. Thus the knowledge of reliable fertility phenotypes, protein markers associated with high semen fertility, and application of sophisticated analytic techniques are central to marker development.

Sperm epigenome

Epigenetics is defined as the changes occurring in the gene expression or physiological output without altering the DNA sequence. DNA methylation and chromatin structure changes play a key role in influencing sperm fertility. Methylation of DNAs cytosine nucleotides interferes with binding of transcription factors to DNA, which leads to suppression of gene expression (Kaya and Memili, 2016). In men altered DNA methylation in genes, HSPA1L and HSPA1B are associated with reduced fertility (Jenkins *et al.* 2015). In DNA of buffalo sperm, differential methylated regions have been identified by Verma *et al.* (2014). A combined system has been reported for analyses of both DNA methylome and transcriptome in bovine sperm and early embryos (Shojaei Saadi *et al.* 2014). Association between bull fertility and DNA methylation in specific regions in sperm needs to be elucidated (Kaya and Memili, 2016). Bisulfide sequencing, DNA methylation microarrays or CpG island/promoter microarrays can be used to study methylation profiles of the DNA. With the help of these methods, it is possible to pinpoint a panel of DNA methylation markers to evaluate semen quality and predict bull fertility.

CONCLUSION

Bull fertility is essential in regulating reproductive efficiency in bovine. Sperm molecules and epigenetic factors are primary indicators of sperm physiology that aid in understanding sperm biology and prediction of sperm fertility. Although role of many molecules in spermatozoa of domestic animals is still to be discovered, the fingerprints or patterns of these molecules can help in predicting bull fertility.

ACKNOWLEDGEMENT

The authors are highly thankful to in Charge Artificial Breeding Research Center, ICAR-National Dairy Research

Institute, Karnal, Haryana, India for providing necessary articles and instructions for preparation of this manuscript.

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