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Genome-wide profiling of sperm DNA methylation in relation to buffalo (*Bubalus bubalis*) bull fertility

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ABSTRACT

The DNA methylation pattern in spermatozoa of buffalo bulls of different fertility status was investigated. Spermatozoa isolated DNA from two groups of buffalo bulls (n = 5), selected based on their artificial insemination–generated conception rate data followed by IVF efficiency, were studied for global methylation changes using a custom-designed 180 K buffalo (*Bubalus bubalis*) CpG island/promoter microarray. A total of 96 individual genes with another 55 genes covered under CpG islands were found differentially methylated in sperm of high-fertile and subfertile buffalo bulls. Important genes associated with biological processes, cellular components, and functions were identified to be differentially methylated in buffalo bulls with differential fertility status. The identified differentially methylated genes were found to be involved in germ cell development, spermatogenesis, capacitation, and embryonic development. The observations hint that methylation defects of sperm DNA may play a crucial role in determining the fertility of breeding bulls. This growing field of sperm epigenetics will be of great benefit in understanding the graded fertility conditions of breeding bulls in commercial livestock production system.

1. Introduction

All sexually reproducing species generate their offspring by successful fusion between male and female gametes. In contrary to the previous perception that the male gamete works only as a delivery agent for the parental haploid genome to the presumptive embryo [1], recent findings suggest that the epigenetic and RNA carriage of sperm delivered to the oocyte also contributes immensely for the successful fertilization and orderly development of preimplantation embryo [2–4]. A number of acquired sperm defects such as epigenetic anomalies, aneuploidy, Y chromosome microdeletions, and nonspecific DNA strand breaks have been described to have their implications on male fertility [5–8]. Earlier works reporting candidate gene sequencing [8] and genome-wide association study in oligozoospermic and azoospermic men [9] have revealed that single-gene polymorphisms are not likely to be the cause of most cases of male infertility, and similar to other complex diseases, epigenetic alterations may have a vital contribution to infertility [10]. Abnormal epigenetic programing of the germ line is proposed as a possible mechanism for compromised spermatogenesis. During germ cell maturation and gametogenesis, the germ cells undergo extensive epigenetic reprograming, widespread erasure of somatic cell–like patterns of DNA methylation, and establishment of sex-specific patterns by *de novo* DNA methylation [11]. Several studies conducted in human indicated that DNA methylation is altered, at least in imprinted genes in oligozoospermic subjects and subjects with improper

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histone-to-protamine ratio [12–14]. Abnormal embryo phenotypes have also been associated with disrupted methylation status of imprinted genes [15]. Both human and animal studies indicated that abnormal sperm DNA methylation patterns associated with subfertility include aberrant methylation of both imprinted [8,12–14] and non–imprinted genes [16–18].

Suboptimum fertility in breeding sires undergoing natural service, or artificial insemination (AI) is considered a major factor responsible for poor herd fertility where traditional veterinary medicine approaches have been reported to be of only limited impact [19]. In this context, the standard laboratory assessment of semen qualities, such as, sperm number, viability, motility, morphology, and semen volume, are found to be poor predictors of fertility, demonstrating remarkable intra- and interindividual variations [20,21]. A more objective molecular approach is required to look into the issues related to fertility problems.

DNA methylation is the stable, covalent addition of a methyl group to cytosines in enriched CG regions of the genome, described as CpG islands, that can represent response to environmental cues or exposures that may modify gene expression [22]. Several methods exist to detect changes in the DNA methylation pattern. Most of these techniques use bisulfite treatment of DNA to uncover its methylation status. The most commonly used assays are methylation-specific PCR [23] and combined bisulfite restriction analysis [24]. But these methods examine only a limited number of cytosines and a single gene per assay. In contrast, sequencing of bisulfite-deaminated and PCR-amplified DNA produces results for every CpGs in the region of interest [25]. However, the read length following these methods is limited and the process is both costly and time-consuming. As an alternative, oligonucleotide microarrays represent a powerful tool for inexpensively generating a large volume of data in a single experiment, while working at a single-base resolution [26]. The methyl DNA immunoprecipitation adds further advantage to the microarray assays by enriching the samples for regions of methylated DNA [27]. The present study was planned to investigate the pattern of sperm DNA methylation in sperm of buffalo bulls with differential fertility status using a high-density buffalo-specific oligonucleotide methylation array.

2. Materials and methods

All chemicals used were from Sigma–Aldrich (St. Louis, MO, USA), unless otherwise indicated.

2.1. Selection of bulls

Thirty-eight murrah buffalo bulls of age 3 to 5 years having more than 50 insemination records over a period of 12 years were considered for this classification (Fig. 1). Ten buffalo bulls, five each in high-fertility (HF) and subfertility (SF) groups were selected based on their conception rate data. These bulls were used under a multiherd progeny testing program in India. All of these bulls were reared under uniform feeding and management schedule and were inducted into progeny testing program after evaluation of their breeding soundness, semen quality, and freezability parameters, namely semen volume, sperm count, viability, progressive motility, post-thaw motility, and viability. Frozen semen samples (0.25 mL straws) from these bulls were assigned randomly for breeding buffalo cows in three different herds maintained with uniform feeding and management schedule. The bottom five bulls (SF group) had a conception rate in the range of 31% to 36%, whereas the top five bulls (HF group) were in the range of 54% to 58%.

To validate the classification of bulls in their respective fertility groups, cryopreserved semen samples from these 10 bulls were subjected for IVF using optimized *in vitro* oocytes maturation and IVF procedure [28]. *In vitro* culture (IVC) of presumptive zygotes was done in modified Charles Rosenkrans 2 amino acid–based IVC medium supplemented with 1% (vol/vol) minimal essential media and nonessential amino acids, 2% (vol/vol) minimal essential media and essential amino acids, 0.14 mg/mL glutamine, 1.5 mM glucose, 0.36 mg/mL sodium lactate, 0.036 mg/mL sodium pyruvate, and 8 mg/mL BSA. *In vitro* culture medium was replaced after 72 hours with replacement medium (IVC medium with 10% fetal bovine serum). A second media replacement was done on the fifth day of culture. Embryos were cultured up to 7 days, and different cleavage (2, 4, 8, and 16 morula and blastocyst) stages were recorded at 36, 48, 60, 84, 120, and 168 hours postinsemination, respectively. All experiments were repeated four times. Based on the conception rate data and its further validation

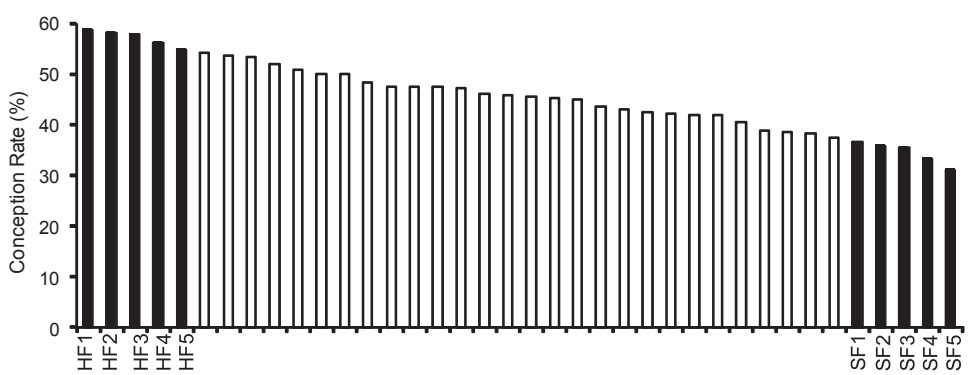

Fig. 1. Conception rates (CRs) of 38 buffalo bulls screened based on AI data. (Top) Five bulls were designated as high fertile (HF), and (bottom) five designated as subfertile (SF).