A descriptive analysis of bull sperm methylome using reduced representation bisulphite sequencing (RRBS).

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Application In the context of genomic selection, more information on the epigenetic features transferred to the embryo alongside the paternal genetic heritage is necessary in order to improve semen quality control procedures as well as to guarantee semen fertility and proper embryo development.

Introduction Spermatozoa have a remarkable epigenome in line with their degree of specialization, their unique nature and different requirements for successful fertilization. Accordingly, the incorrect establishment of DNA methylation patterns during male germ cell differentiation has been associated with male infertility in mouse and Human (1). While bull semen is widely used in artificial insemination, the literature describing DNA methylation in bovine spermatozoa is still scarce. Yet domestication, the creation of highly specialized breeds and decades of genetic improvement have shaped the bovine genome. This undoubtedly has had a profound impact on the methylome, since DNA methylation is directly affected by the CpG content of the genome and its alteration by DNA polymorphism. The purpose of this study was therefore to characterize the bull sperm methylome relative to bovine somatic cells.

Material and methods Genomic DNA from Holstein sperm, fibroblasts and monocytes (collected from two individuals for each cell type) was extracted according to standard procedures, in the presence of 50 mM dithiothreitol for sperm. RRBS libraries were prepared from 200 ng of MspI-digested genomic DNA and sequenced on an Illumina HiSeq2500 sequencer to produce 75 bp paired-end reads (Integragen). Sequences were analyzed using an integrated pipeline combining homemade scripts together with external tools (2). High quality reads were aligned on the bovine reference genome (UMD 3.1 assembly) or on an artificial genome containing the consensus sequence of each bovine repeat (Repbase database) using Bismark (3). Only CpGs covered by 5 to 500 uniquely mapped reads for each sample were retained for subsequent analyses. For each pair of cell types, differentially methylated CpGs (DMCs) were identified using methylKit (4). A CpG was considered as a DMC when the associated q-value was weaker than 0.001 and the methylation difference between two conditions was at least 25%. DMCs were then annotated relative to gene features, CpG islands and repetitive elements, and genes containing DMCs subjected to GO enrichment analysis. Validations were carried out by means of bisulphite-pyrosequencing.

Results Bull sperm was compared with bovine fibroblasts and monocytes. The samples were clearly clustered according to the cell type using both hierarchical clustering and principal component analysis. Interestingly, the distance between sperm and other cell types was more important than the distance between monocytes and fibroblasts, highlighting the methylation specificities of germinal cells compared to somatic cells. DMCs were next identified for each pairwise comparison. A subset of 174,103 sperm-specific DMCs was isolated, as being differential in both sperm vs. monocyte and sperm vs. fibroblast comparisons, but not in that involving monocytes vs. fibroblasts. Interestingly, 79% of these sperm-specific DMCs were hypomethylated in sperm. Consistent with previous studies in other species, these hypomethylated sperm-specific DMCs were enriched for genes relevant to the germline differentiation program and sperm functions. The most remarkable observation was a dramatic enrichment for repeats (24.5% vs. 13.2% in background), and particularly for satellites (64.7% vs. 17.8%). We suspected that only a small fraction of the repetitive elements was represented in the uniquely mapped reads, and hypothesized that more information on the repeats hypomethylated in sperm could be extracted from the ambiguous reads. We therefore built an artificial genome containing one copy of each bovine repeat and aligned the totality of the reads on this artificial genome. The hypomethylation of sperm was clear in satellites and also in rDNA repeats encoding ribosomal RNAs. Hypomethylation of the most abundant satellite in the bovine genome, BTSAT4, was confirmed by bisulphite-pyrosequencing.

Conclusion These results highlight the hypomethylation of bull spermatozoa when compared with somatic cells. The genes hypomethylated in bull sperm are conserved across species, which may denote an important role in germline differentiation. In addition, the sperm-specific hypomethylation especially targets bovine repetitive elements. Whether hypomethylation of repetitive elements is of functional significance and contributes to the establishment of normal spermatogenesis needs to be ascertained.

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