UCONN | College of Agriculture, Health and Natural Resources
Animal Science

Whole-genome bisulfite sequencing of bovine gametes and *in vivo*-produced pre-implantation embryos

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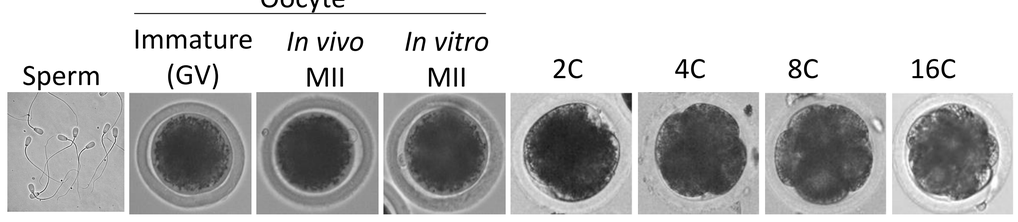
Abstract

Dynamic changes in DNA methylation are crucial in the process of early mammalian embryogenesis. Global DNA methylation studies in the bovine, however, remain mostly at the immunostaining level. We adopted the whole genome bisulfite sequencing (WGBS) method to characterize stage-specific genome-wide DNA methylation in bovine sperm, immature oocytes, oocytes matured *in vivo* and *in vitro*, as well as *in vivo* developed embryos at the 2-, 4-, 8- and 16-cell stages. We found that the major wave of genome-wide DNA demethylation was complete by the 8-cell stage when *de novo* methylation became prominent. Sperm and oocytes were differentially methylated in numerous regions (DMRs), largely intergenic, suggesting that these noncoding regions may play important roles in gamete specification. DMRs were also identified between *in vivo* and *in vitro* matured oocytes, demonstrating environmental effects on epigenetic modifications. Moreover, virtually no (less than 1.5%) DNA methylation was found in mitochondrial DNA. Finally, by using RNA-seq data generated from embryos at the same developmental stages, we revealed a weak inverse correlation between gene expression and promoter methylation. These data provide insights into the critical features of the methylome of bovine embryos, and serve as an important reference for embryos produced by assisted reproduction, such as *in vitro* fertilization and cloning, and a model for the epigenetic dynamics that occur in human early embryos.

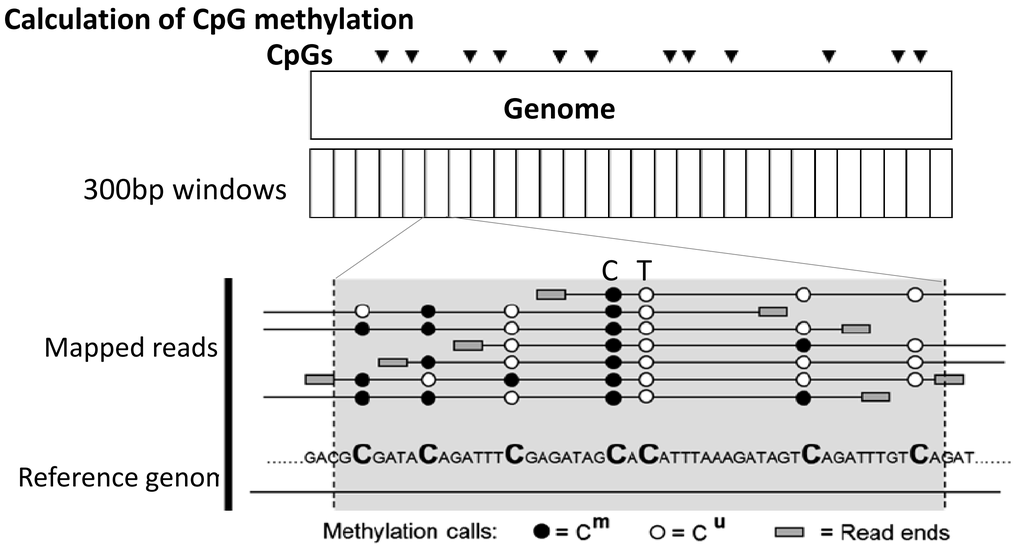
Materials and Methods

Pools of 20 sperm were included from a bull with proven fertility. Single oocytes (n=6) and *in vivo* developed embryos (n=4, per stage) were collected from Holstein cows (n=10). WGBS libraries were prepared and sequenced using the Illumina HiSeq 4000 platform. Sequencing reads were filtered and aligned to the bovine reference genome (UMD3.1.1) using Bismark. A 300-bp tile-based method was applied to bin the genome into consecutive windows to facilitate comparison across samples. The DNA methylation level was calculated as the fraction of read counts of the total number of cytosines (methylated) in the total read counts of reported cytosines and thymines (methylated and unmethylated), when more than three CpG sites were revealed in a tile. Gamete-specific differentially methylated regions (DMRs) were identified when DNA methylation levels were greater than 75% in one type of gamete and less than 25% in the other with FDR-corrected Fisher's exact test (P<0.05).

Materials



Calculation of CpG methylation



300-bp windows of the genome

$$\text{Average CpG me-level} = \frac{C^m}{C^m + C^u} = \frac{C}{C+T}$$

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Results

I. Global methylome dynamics during embryonic development

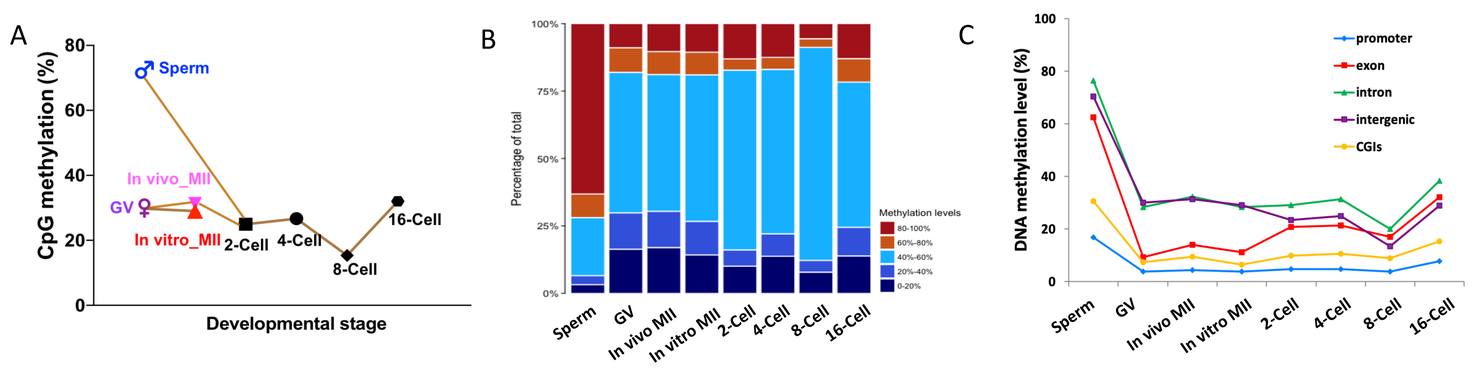

Figure 1. A. Line chart of averaged levels of CpG methylation across stages. B. Stack bar plot of the percentages of tiles with high (80%-100%), intermediate (60%-80%; 40%-60%, 20-40%), and low (0-20%) methylation levels. C. Line chart of the average DNA methylation levels of annotated genomic features across stages. GV: germinal vesicle oocytes; MII: matured oocytes.

II. DNA methylation changes in consecutive stages

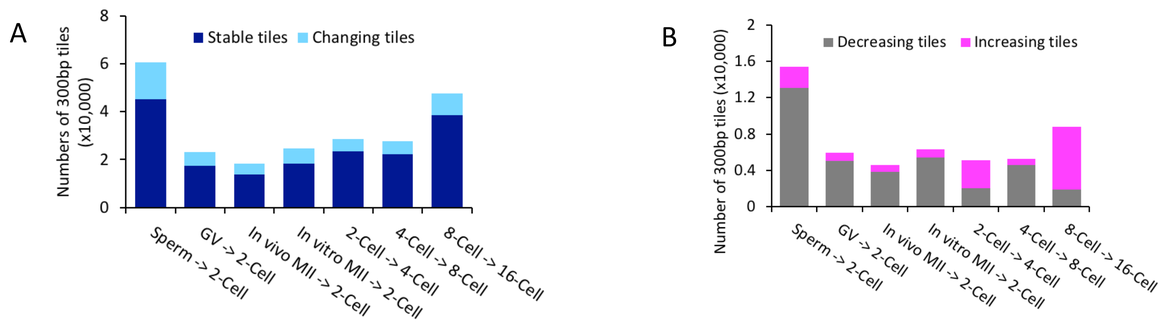

Figure 2. A. Histograms of the numbers of stable (dark blue) and changing (sky blue) tiles between consecutive stages. B. Decreasing (gray) and increasing (pink) tiles between consecutive stages.

III. Differentially methylated regions (DMRs) in gametes

A

Stages	No. of DMRs	No. of genes
Sperm vs. GV	4,654	543
Sperm vs. *In vivo* MII	2,653	354
Sperm vs. *In vitro* MII	6,211	668
GV vs. *In vivo* MII	755	77
GV vs. *In vitro* MII	936	93
In vivo MII vs. *In vitro* MII	801	68

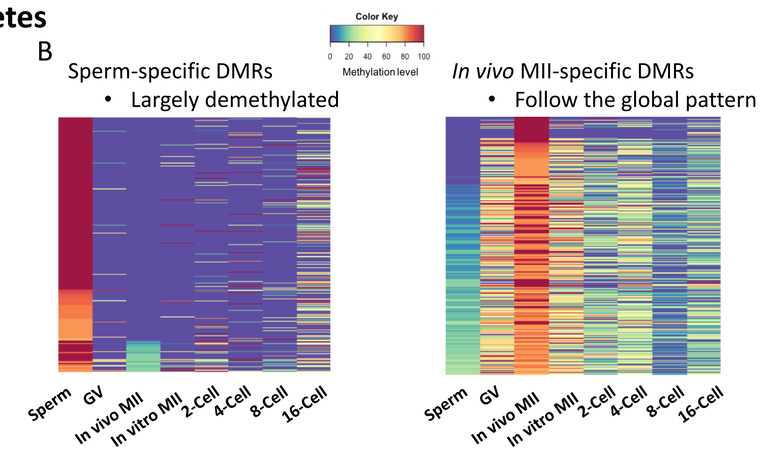

Figure 3. A. The numbers of DMRs and corresponding genes between gametes of different types. B. Gamete-specific DMRs dynamics during embryonic development.

IV. DNA methylation of X chromosome and imprinted genes

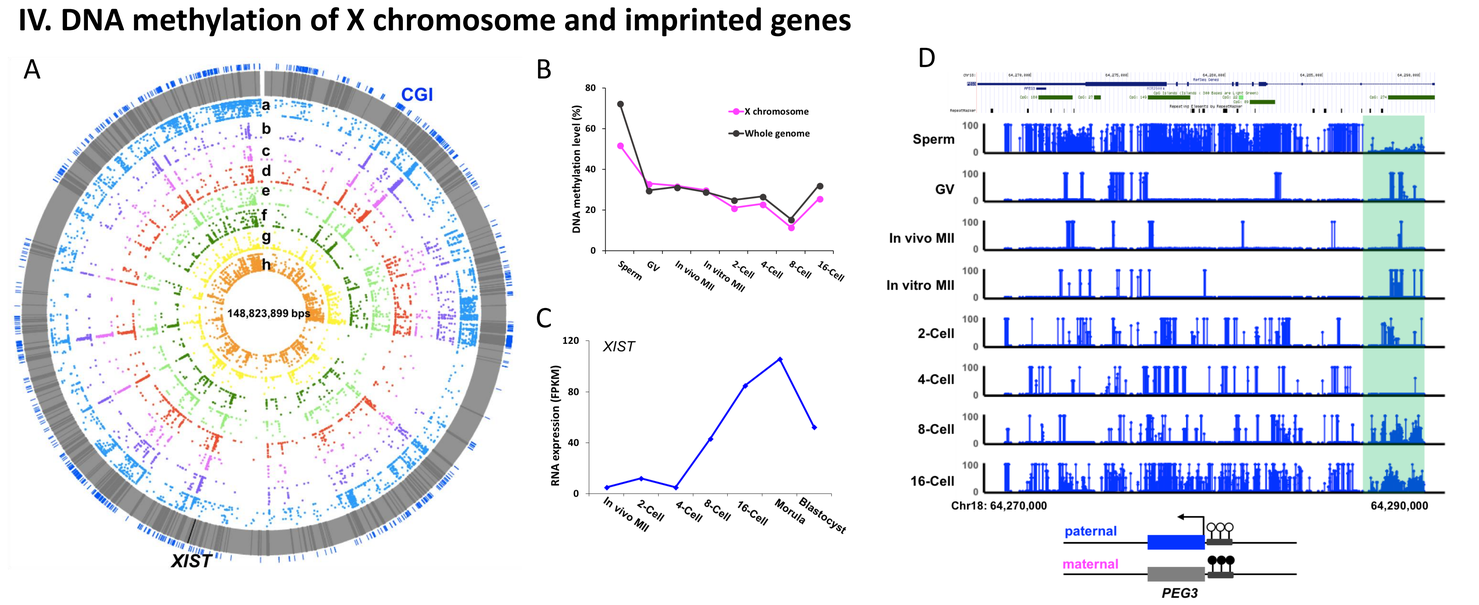

Figure 4. A. Circos plot visualization of the X chromosome DNA methylation. Genes are denoted by gray lines, *Xist* gene by a black line, and CGIs by blue lines. a. sperm, b. GV, c. *in vivo* MII, d. *in vitro* MII, e. 2-cell, f. 4-cell, g. 8-cell, h. 16-cell. B. Line plot showing methylation dynamics of the X chromosome followed the global pattern of methylation changes. C. Line chart of fragment per kilobase million (FPKM) expressions levels of *XIST* in bovine early embryos. D. Methylation pattern of the imprinted control region (ICR) of the paternally expressed *PEG3* .

Conclusions

Global demethylation during bovine embryo cleavage up to 8-cell stage and *de novo* methylation at 16-cell stage, refining the current knowledge on bovine embryo DNA methylation dynamics and provide valuable resources for future studies