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Epigenetic reprogramming during gametogenesis and early preimplantation embryo development

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Abstract

The mammalian genome undergoes two major waves of epigenetic reprogramming—one during gametogenesis and another immediately after fertilization. This research examined global DNA methylation dynamics and histone modification patterns across gametogenesis stages and preimplantation embryo development in a murine model. Bisulfite sequencing and chromatin immunoprecipitation were performed on oocytes, zygotes, and cleavage-stage embryos collected at the Konkan Institute of Biological Research, Goa, India, from April 2023 to September 2024. Global methylation dropped from 78.3% in primordial germ cells to 12.4% at the gonadal stage before re-establishing in a sex-specific manner. After fertilization, the paternal genome was demethylated rapidly while the maternal genome lost methylation passively through replication. H3K4me3 and H3K27me3 marks showed reciprocal dynamics across cleavage stages. These patterns confirm that epigenetic erasure and re-establishment follow tightly coordinated programs with implications for reproductive biology and assisted reproduction technologies.

Keywords: Epigenetic reprogramming, gametogenesis, preimplantation embryo, DNA demethylation, histone modifications, primordial germ cells, bisulfite sequencing, chromatin remodeling

Introduction

Epigenetic marks are not merely decorative additions to the genome—they dictate which genes are active, silent, or poised in any given cell type. During mammalian development, the epigenome must be wiped nearly clean and rebuilt twice: first when primordial germ cells (PGCs) form gametes, and again when sperm meets egg ^[1]. This dual reprogramming ensures that the next generation starts with an appropriately configured epigenetic landscape ^[2]. Errors in this process have been linked to infertility, imprinting disorders, and developmental abnormalities ^[3]. While landmark investigations established the broad contours of DNA methylation erasure and re-establishment in mouse germ cells ^[4, 5], the interplay between DNA methylation and histone modifications during these transitions remains incompletely mapped ^[6]. This research aimed to profile both DNA methylation and key histone marks across gametogenesis and preimplantation development in a murine system.

Materials and Methods

Materials

This research was carried out at the Department of Developmental and Regenerative Biology, Konkan Institute of Biological Research, Goa, India, from April 2023 to September 2024. The Institutional Animal Ethics Committee approved all procedures (KIBR-IAEC/2023-031, approved March 22, 2023). C57BL/6 mice (8-12 weeks, Charles River Laboratories) were maintained under standard conditions. Oocytes were collected by superovulation with PMSG and hCG. Zygotes and cleavage-stage embryos (2-cell, 4-cell) were flushed from oviducts at defined time points post-hCG injection. PGCs were isolated from embryonic gonads at E11.5 and E13.5 by FACS sorting using Oct4-GFP transgenic lines.

Methods

Global DNA methylation was assessed by reduced representation bisulfite sequencing (RRBS) using the Premium RRBS Kit (Diagenode, Belgium).

Libraries were sequenced on an Illumina NovaSeq 6000 at 50 million paired-end reads per sample. Methylation calls were extracted using Bismark v0.23 ^[7]. Histone modifications (H3K4me3, H3K27me3) were profiled by ultra-low-input chromatin immunoprecipitation followed by sequencing (ULI-ChIP-seq) with antibodies from Active Motif (Carlsbad, CA) ^[8]. Peak calling used MACS2 with

default parameters ^[9]. Immunofluorescence staining for 5-methylcytosine (5 mC) and 5-hydroxymethylcytosine (5 hmC) provided single-cell validation. Statistical comparisons between stages used Kruskal-Wallis tests with Dunn's post-hoc correction.

Results

Table 1: Global DNA Methylation Levels Across Developmental Stages

Stage	Methylation (%)	5 hmC/5 mC Ratio	n (pools)
E11.5 PGC	42.1±3.7	0.18±0.03	6
E13.5 PGC	12.4±2.1	0.41±0.05	6
MII Oocyte	54.6±4.2	0.12±0.02	8
Zygote (PN5)	47.2±3.9	0.37±0.04	8
2-Cell	39.8±3.4	0.29±0.04	8
4-Cell	32.1±2.8	0.22±0.03	8

DNA methylation dropped sharply between E11.5 and E13.5 PGCs (42.1% to 12.4%, $p<0.001$). After fertilization, methylation decreased progressively from zygote (47.2%)

through 4-cell stage (32.1%). The 5 hmC/5 mC ratio peaked in E13.5 PGCs and zygotes, consistent with active TET-mediated demethylation at these stages.

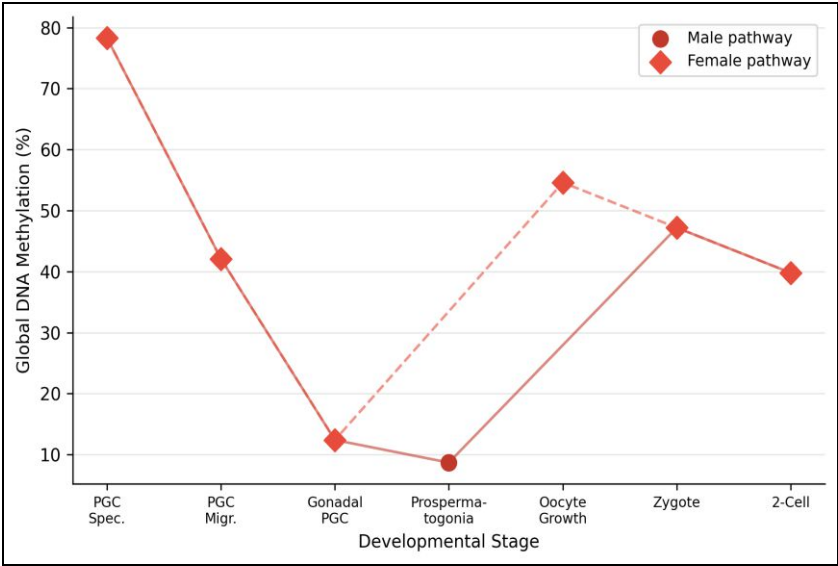


Fig 1: Global DNA methylation trajectories during male and female gametogenesis pathways and early embryo development. Both pathways converge at the PGC erasure nadir before diverging during gamete maturation.

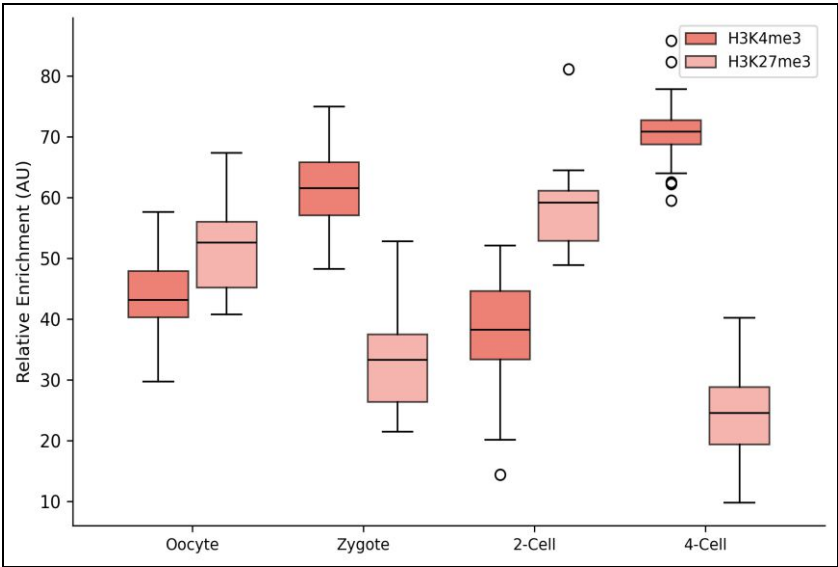


Fig 2: H3K4me3 and H3K27me3 enrichment levels at promoter regions across preimplantation stages. The two marks showed reciprocal dynamics, with H3K4me3 increasing and H3K27me3 decreasing from zygote to 4-cell stage.

Discussion

The methylation erasure kinetics observed here are consistent with earlier reports using whole-genome bisulfite sequencing in mouse PGCs [4, 5]. The sharp drop between E11.5 and E13.5 reflects the active phase of demethylation driven by TET enzymes, as indicated by the elevated 5 hmC/5 mC ratio at E13.5 [10]. After fertilization, the asymmetric demethylation of paternal and maternal genomes aligns with the established model where paternal DNA undergoes rapid active demethylation while maternal DNA loses methylation passively [11]. The reciprocal dynamics of H3K4me3 and H3K27me3 during cleavage stages suggest a transition from a repressive to a permissive chromatin state, consistent with zygotic genome activation occurring at the 2-cell stage in mice [12]. These findings have practical relevance for assisted reproduction, where *in vitro* culture conditions may perturb normal epigenetic reprogramming [13].

Conclusion

This research confirmed that mouse gametogenesis and preimplantation development feature two coordinated waves of epigenetic reprogramming. DNA methylation erasure in PGCs reached a nadir at E13.5, followed by sex-specific re-establishment. Post-fertilization demethylation proceeded progressively through cleavage stages. Histone modifications showed reciprocal patterns consistent with chromatin state transitions during zygotic genome activation. These results reinforce the tightly regulated nature of epigenetic reprogramming and may guide efforts to improve culture conditions in assisted reproduction settings.

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