# Sure, Fathers Give Birth, Too!

Postnatal paternal folate deficiency increases congenital disabilities through H3K4me3 histone methylation changes in sperm and embryos.

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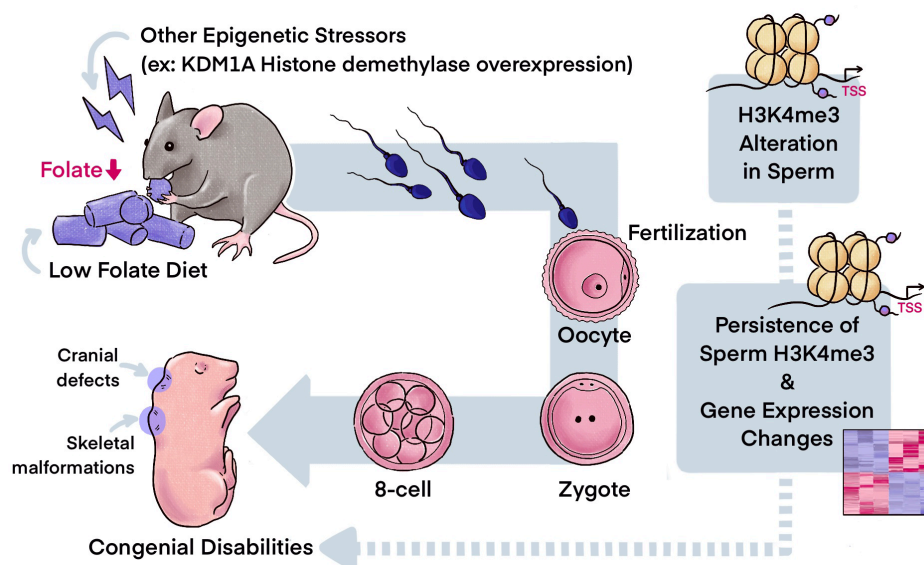
Folate-deficient postnatal diet can change H3K4me3 patterns in sperm that are not erased after fertilization, but persist throughout preimplantation embryos. H3K4me3 alterations are associated with genes implicated in congenital disabilities, such as skeletal and cranial malformations. For example, male mice fed with a low-folate diet produce offsprings showing a spectrum of congenital skeletal malformations. FD diet and overexpression of KDM1A histone demethylase additively generate negative pregnancy outcomes, indicating that multiple epigenetic stressors influence sperm quality.

父生我身母育吾身. An old Chinese proverb says that "my father gave birth to my body and my mother reared." The phrase may sound puzzling because we all know who physically bears a child. However, recent studies on paternal contribution to offspring fitness indicate that the abovementioned proverb makes sense and is gaining attention by emerging science.

A father's preconception health draws attention due to its crucial effects on babies' overall fitness. Fathers having physical or mental chronic illnesses have greater odds of having preterm or low-weight babies and even pregnancy loss (Kasman, 2021). In addition, lifestyle habits such as smoking, drinking, and unhealthy diet have negatively impacted sperm quality (Cescon et al., 2020; Schagdarsurengin and Steger, 2016). Impaired sperm epigenetically record memories of damages on their chromatin and transfer them to the offspring through generations (Anway et al., 2005). Lismer et al. (2021) showed that a folate-deficient diet interfering with adequate methyl supply changes H3K4me3 histone methylation in sperm. Their reanalysis of a previously published dataset revealed that the paternal epigenetic marks are not much erased by chromatin reprogramming after fertilization, but persist in preimplantation embryos (Zhang et al., 2016). Changes in H3K4me3 patterns are linked to dysregulated gene expression in early development, leading to congenital disabilities in the offspring.

First, Lismer et al. (2021) fed wild-type (WT) or KDM1A histone demethylase transgenic (TG) male mice with folate-sufficient (FS) or folate-deficient (FD) diet after weaning, establishing four experimental groups: FS WT, FD WT, FS TG, and FD TG. Later, when these mice mature, they bred these male mice with WT female mice fed with a regular chow diet to examine pregnancy outcomes, H3K4me3 histone methylation in sperm and eight-cell embryos, and transcriptome of early embryos. The FD diet increases variability in pregnancy loss at both preimplantation and postimplantation stages. Offspring sired by FD, TG, or FD TG males showed a spectrum of congenital skeletal malformations, such as crooked breastbone and bent ribs. The FD diet aggravates congenital disabilities in offsprings produced by TG males. Therefore, postnatal paternal folate deficiency is associated with pregnancy loss and skeletal deformation, similar to a lifetime paternal folate deficiency beginning in utero (Lambrot et al., 2013). There is an additional effect of methyl donor deprivation on over-demethylation of histones.

Next, Lismer et al. (2021) performed principal component analysis (PCA) of the H3K4me3-marked regions. They found that folate deficiency significantly changes H3K4me3 enrichment in sperm. Chromatin regions with increased H3K4me3 are predominantly located near transcriptional start sites (TSSs), whereas those with decreased H3K4me3 are usually far from the TSSs. Promoters with altered H3K4me3 are enriched in genes involved in preimplantation and postimplantation embryogenesis, organ development, and chromatin remodeling. These results show that developmental genes are highly vulnerable to postnatal paternal folate deficiency, reflecting congenital disabilities, such as abnormal eyes and skull bones, in early embryos born to FD WT fathers. Paternal folate deficiency also worsens H3K4me3 alteration in TG

sperm, and FD TG fathers' embryos show severer defects. Therefore, FD diet and over-demethylation of histone cumulatively affect sperm H3K4me3.

PCA of eight-cell embryo transcriptome revealed evident clustering based on paternal diet and genotype. Many genes with altered expression in FD WT embryos are involved in the fundamental pathways of preimplantation development. In addition, H3K4me3 alterations in sperm were associated with changes in eight-cell embryo gene expression. Promoters marked with high H3K4me3 in sperm significantly corresponded to increased gene expressions in the embryo, and paternal H3K4me3 may instruct gene expression in the offspring at the preimplantation stage. Furthermore, additive effects of FD on TG sperm persist throughout the preimplantation stage of offspring development.

To determine whether H3K4me3 profiles in sperm are similar to those in embryos, they analyzed the previously published two-cell embryo ChIP-seq datasets. Interestingly, they observed that over two-thirds of regions with H3K4me3 in sperm were still enriched in the two-cell embryo. However, a previous study reported that H3K4me3 signatures in sperm are dynamically reprogrammed in an early embryo (Zhang et al., 2016). To understand the contradiction, they reanalyzed the dataset of ChIP-seq reads assigned to paternal alleles, considering low histone retainment in sperm and including reads without single nucleotide polymorphism (SNP). The reanalysis demonstrated that the paternal allele had retained significant H3K4me3 in preimplantation embryos. It was seen that the paternal H3K4me3 signatures persisted throughout development to the inner cell mass of the blastocyst. The transcription factor CTCF and Smc1, a subunit of CTCF's interaction partner cohesion, facilitate chromatin interactions at the promoter and distal regulatory sites (Phillips and Corces, 2009). They are likely to play a critical role in retaining sperm H3K4me3 and gene expression in the embryo because signals for CTCF, Smc1, and H3K4me3 in sperm overlap with H3K4me3-enriched regions in one-cell embryos.

Finally, Lismer et al. (2021) compared H3K4me3 enrichment in eight-cell embryos to that in sperm, oocytes, and two-cell embryos using previously published datasets (Jung et al., 2017; Liu et al., 2016; Zhang et al., 2016). They found that the narrow H3K4me3 regions in sperm partially overlapped with broad H3K4me3 domains in the oocyte, but these regions largely overlapped with H3K4me3 domains in the two- and eight-cell embryos. They also found partial retention of the sperm-specific aberrant H3K4me3 in the preimplantation embryo. Regions with altered H3K4me3 in eight-cell embryos were linked to the genes expressed in eight-cell embryos that significantly overlap genes with deregulated expression in FD embryos.

The findings of Lismer et al. (2021) demonstrate that histone methylation epigenomic programming is not limited to in utero events of global reprogramming during primordial germ cell migration but can be affected by a postnatal paternal diet that impacts heritable sperm epigenome (Lambrot et al., 2013). The authors' careful analysis considering extremely low histone retainment in sperm chromatin and the lack of SNPs in some ChIP-seq reads revealed that paternally inherited H3K4me3 alterations near promoters in FD sperm

largely persist through the preimplantation embryonic stage. Thus, the H3K4me3 alterations that persisted may result in the deregulation of gene expression during development. Furthermore, altered H3K4me3 in sperm may be associated with gene expression profiles in later stages of development because some promoters with altered H3K4me3 in FD sperm drive genes implicated in later developmental processes, such as those involved in organogenesis. How some epigenetic changes in sperm escape reprogramming and persist throughout embryogenesis is a pressing question.

Other factors such as noncoding RNA, DNA methylation, DNA-binding proteins, and other histone modifications may act independently or in concert with sperm H3K4me3 to mediate epigenetic inheritance through the paternal germline and many other histone modifications that colocalize with H3K4me3 in sperm (Chen et al., 2016; Erkek et al., 2013; Jung et al., 2017; Jung et al., 2020; Kim, 2019; Lismer et al., 2020; Ly et al., 2017; Sharma et al., 2016). Paternal exposure to multiple stressors may cause enrichment imbalances among multiple histone modifications in different combinations, an absolute influencer on offspring development.

It is easy to understand that pregnant moms are responsible for their babies in their tummies. We nod at a pregnant woman sipping healthy-fancy mineral water whose husband is free to lift a bottle of ice-cold beer in hot summer. Paternal contribution to babies' health is not kept in mind at large. Taegyo, a traditional Korean prenatal education, is an equal opportunity activity instructing both a mother-to-be and a father-to-be on prenatal activities (Noh and Yeom, 2017). Indeed, both parents should start thinking about what to eat and how to live before deciding to conceive, reminding themselves that fertility is a team sport.

CONFLICT OF INTEREST
The author has no potential conflicts of interest to disclose.

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