Chapter 14
Do chromatin dynamics in spermatogenesis have implications for fertility and epigenetic inheritance?

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Male infertility is intertwined with environmental exposures including obesity, toxicants, and micronutrient deficiency. Remarkably the paternal environment also impacts the health of future offspring, potentially in a transgenerational manner. Evidence suggests that a connecting molecular link between environmental factors, male infertility and offspring health is the sperm epigenome. Alterations in metabolism associated with diet and obesity, or toxicant exposures can alter the sperm epigenome and in turn gene expression in spermatogenesis and the next generation leading to infertility and disease. However, we have a poor understanding at the molecular and genomic levels of how this occurs. The epigenome refers to the biochemical content associated with DNA and includes chromatin, organized as nucleosomes in which a section of DNA is wrapped around a core comprised of two of each histones H2A, H2B, H3 and H4. Each histone carries post-translational modifications such as phosphorylation, acetylation and methylation; these in turn influence the degree of chromatin compaction (e.g. euchromatin vs heterochromatin) and gene expression. While DNA can be thought of as storing genetic information, epigenetic processes interpret the genome, dictate spatio-temporal features of gene expression, are critical for cell differentiation, and are connected to human disease including male infertility. In general, the term epigenome includes three known layers of biochemical information: 1) the chromatin and specific patterns of post-translational modifications to histones; 2) DNA methylation which occurs at the 5-position of cytosine residues within CpG dinucleotides (Chapter 13); and 3) noncoding-RNA (Chapter 15). This chapter will focus on chromatin components of the sperm epigenome and its dynamic assembly during spermatogenesis and ability to impact fertility and disease intergenerationally.

Spermatogenesis is a highly complex cell differentiation process including proliferative mitosis and meiosis, and includes a massive remodeling of the chromatin in the haploid phase of spermiogenesis (Chapter 9). Unlike somatic cell development, the chromatin architecture of the male germline is highly varied as cells progress through differentiation. This highly specialized chromatin is specifically adapted to accommodate meiotic recombination, X chromosome inactivation, demarcation of imprinted genes, and the safe packaging of the genome and epigenome for delivery at fertilization. Disruption of the proper establishment and reprogramming of chromatin in spermatogenesis can have dire consequences for fertility and the health of subsequent generations (Fig. 1).

Unique to spermatogenesis is the incorporation of histone variants including those that are testis-specific (H1t, H2A.X., H2A.Z., H3.3, H2AL-1/2, HILS H3t, TH2A, TH2B). For example, beginning in the spermatogonia, the histone variant H3t gradually replaces the canonical histone H3. In meiosis, there is sequential incorporation of histone variants such as H1t, macroH2A.X, H2A.Z, and H3.3. In spermatocytes, the majority of histones H2A and H2B are replaced by the co-expressed germline-specific TH2A and TH2B. Interestingly, the combinations of H3.3 with macroH2A in spermatocytes and H3.3 with H2A.Z in round spermatids confer either more stable or unstable chromatin domains, respectively. The sex chromosomes are also enriched for variants and temporarily accumulate H2A.Z, which is subsequently replaced by H2A.B.3 at X-linked genes that escape sex chromosome inactivation. Localization of H2A.B.3 at the exon-intron boundaries of active genes, likely regulates RNA splicing events. Sex chromosomes also incorporate the replication-independent histone H3.3, which is encoded by genes *H3f3a* and *H3f3b*, both producing identical H3.3 but functioning at different stages of spermatogenesis.

A global reorganization in chromatin packaging occurs in spermiogenesis where most histone proteins are first replaced by the temporary transition proteins. Transition proteins (TP1 and TP2) are small basic proteins with overlapping functions and are important for chromatin condensation and DNA integrity. The incorporation of transition proteins onto chromatin is facilitated by the replacement of TH2A with variant H2A.L.2, promoting the opening of H2A.L.2-contaning nucleosomes. Their assembly on the genome ultimately permits the final eviction of histones and the incorporation of protamines. This histone-to-protamine exchange

begins in round spermatids by the weakening of interactions between histones and the DNA as a consequence of histone hyperacetylation. The high content in positively charged amino acids of protamines promotes compaction of the negatively charged paternal genomic DNA into supercoiled toroids. Compared to somatic cell heterochromatin, sperm DNA is ten-fold more compact due to this protamine facilitated organization.

Interestingly, 1% of sperm histones are retained in mice and about 10-15% in men. Retained histones are conserved across species from mice to men and are found at the regulatory regions of promoters implicated in spermatogenesis, sperm function, embryo development, metabolism and routine cellular processes. Histones are preferentially retained at high CpG enriched genomic regions that are predominantly DNA hypomethylated in the sperm of mice and men. Many of the retained canonical histones present in mature spermatozoa are enriched at key promoters and enhancers of developmental genes.

When interest in paternal epigenetic inheritance was emerging, it was unclear whether retained histones in sperm served specific functions and whether specific histones and their modifications were transmitted at fertilization. This changed when it came to be known that sperm histones were found to mark genes implicated in embryo development and suggested they may serve beyond gene regulation in spermatogenesis. This role of sperm transmitted histones has been demonstrated in mice. In a foundational study, it was shown that disrupting histone H3 lysine 4 dimethylation (H3K4me2) at transcriptional start sites in sperm had drastic consequences on the health of the next generations. Heterozygous transgenic male mice overexpressing the histone demethylase KDM1A in developing sperm were reproductively compromised and gave rise to offspring with severe developmental defects. These findings on the epigenetic landscape of mature spermatozoa and its evolutionary conservation, suggest sperm packages paternal information that is transmitted to the next generation and be instructive for developmental processes.

Environmentally-altered sperm chromatin has been linked to epigenetic inheritance through the paternal germline. Male mice fed a folate deficient or a high-fat diet have differentially enriched H3K4me3 in sperm at genes involved in developmental and metabolic processes that correspond to birth defects and metabolic dysfunction in their offspring, respectively. Regions with aberrant H3K4me3 in folate deficient sperm were retained in the pre-

implantation embryo and associated with alterations in embryonic gene expression. These studies demonstrated that diet-induced sperm H3K4me3 alterations are transmitted to the embryo and involved in environmentally-induced intergenerational phenotypes. Elucidating how other histone modifications are sensitive to environmental stress in sperm, escape epigenetic reprogramming in the embryo, and alter tissue gene expression and function will be critical to further understand the role of chromatin in paternal epigenetic inheritance.

In conclusion, there is strong evidence from mouse models that the unique chromatin composition in sperm is essential for spermatogenesis, can be responsive to the environment and can in turn alter embryonic gene expression, development and offspring health. Studies are needed to determine whether similar epigenetic mechanisms occur in men as this will open novel routes for the prevention and diagnosis of infertility, and the prevention of paternal disease transmission across generations.

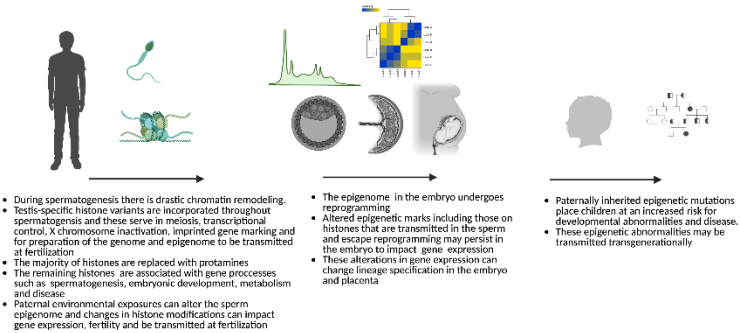

Figure 1. Spermatogenesis is characterized by unique chromatin remodeling that is implicated in fertility and intergenerational disease transmission.

Suggested reading

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