Developmental Epigenetic Analysis of Sperm

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This is an update of Deepika Kubsad, Millissia Ben Maamar, Michael K. Skinner, Developmental Epigenetic Analysis of Sperm, Editor(s): Michael K. Skinner, Encyclopedia of Reproduction (Second Edition), Academic Press, 2018, Pages 239–244, ISBN 9780128151457, https://doi.org/10.1016/B978-0-12-801238-3.64864-X.

Abstract

The sperm contains a highly unique and specialized epigenetic landscape offering a great degree of interesting research opportunities. The mature sperm epigenome represents remnant marks used throughout spermatogenesis to generate sperm cells competent to perform their function, but also marks that appear to be useful beyond fertilization for embryo development. Because of its unique nature, the sperm epigenome and the marks which seem to reflect alterations or perturbations in spermatogenesis are thought to provide a predictive insight. Emerging data suggest that DNA methylation, non-coding RNAs and histone modifications in sperm can offer more predictive power than the traditional assessments of male infertility. This chapter will focus on the different epigenetic marks and their potential use in the context of male fertility.

Glossary

ART Assisted reproductive technologies.
DNA Deoxyribonucleic acid.
RNA Ribonucleic acid.
PGC Primordial germ cells.
DNMT DNA methyltransferases.
TET Ten-eleven translocation enzymes.
GWAS Genome wide association studies.
DAZL Deleted in azoospermia like.
MTHFR Methyleneetrahydrofolate reductase.

Key Points

- Developmental epigenetic analysis of sperm.

Introduction and Male Infertility

The definition of male infertility is the inability of a male to impregnate a fertile female. Infertility is a worldwide problem and according to Sharlip *et al.* affecting around 15% couples (Sharlip *et al.*, 2002). Furthermore, nearly half of human infertility cases

can be attributed to abnormal spermatogenesis (Kitamura *et al.*, 2015). Genetic causes for male infertility are related to a limited number of cases in which altered seminal parameters (motility, morphology and concentration) are directly associated with a genetic basis such as chromosome aberration or microdeletions at the Y chromosome (Jodar *et al.*, 2017). The intricacies to define a genetic origin of this disease have led some researchers to suspect that both genetic and environmental factors contribute. The complex pathology, including the difficulty of isolating molecular abnormalities associated with this diagnosis makes it challenging to determine its underlying cause. However, recent research indicates the presence of epigenetic factors which may provide further insights into the pathogenesis of male infertility (Jenkins *et al.*, 2017). The increased usage of human assisted reproductive technologies (ART) has created an enormous interest in identifying, diagnosing and potentially treating male infertility. In recent years the advancements in the field of ART have provided indirect evidence for the role of epigenetic mechanisms in male infertility (Kitamura *et al.*, 2015).

Epigenetics

Epigenetics is defined as "molecular factors and processes around DNA that alter genome activity (e.g., gene expression) independent of DNA sequence, and are mitotically stable" (Skinner, 2011). Nearly all the cells in multicellular organisms share an identical genotype, however, the diversity in cellular function is based on gene expression profiles that differ among individual cell types. Mechanisms such as DNA methylation, non-coding RNAs, chromatin remodeling and histone modification chemically and structurally alter the expression of genomic region (Tvrda *et al.*, 2015). These various epigenetic signatures are specific to each individual cell type and make up the cell epigenome. The epigenome is thought to reflect the organism developmental history and past environmental influences. The dynamic and tissue specific nature of the epigenome allow for flexibility in gene expression which can lead to changes in phenotypes in the organism. Throughout the process of mitosis, majority of the epigenetic landscape is replicated from one cell to another. During reproduction, some but not all of the epigenome is reset in the embryo (Murphy *et al.*, 2014). Although it was presumed DNA methylation was erased during early embryonic development to reset the epigenome (Surani, 2015), recent observations indicate lower density CpG sites ($>90\%$ epigenome) have an increase in methylation, while the high density sites ($<50\%$ epigenome) have DNA methylation erasure (Ben Maamar *et al.*, 2023). The retained epigenetic signatures after the erasure event allow the transfer of phenotypic traits on the subsequent generations (Ben Maamar *et al.*, 2021). Thus, the epigenetic modifications of the germline (sperm and egg) can have long-term consequences as the altered epigenome can be transmitted to the embryo and this process is referred to as epigenetic transgenerational Inheritance (Ben Maamar *et al.*, 2021; Anway *et al.*, 2005; Guerrero-Bosagna and Skinner, 2014).

Epigenetic transgenerational inheritance is a new field which studies the impact of non-genetic inheritance on epigenomes, specifically, transcriptomes of all subsequently derived somatic cells in the embryo. The transcriptome consists of the small fraction of the genome corresponding to genes and protein coding regions of the genome that have an influential role in the development of the embryo (Ben Maamar *et al.*, 2021; Guerrero-Bosagna and Skinner, 2014). This field of study provides a mechanism through which exposures in previous generations influence the phenotypes of subsequent generations through epigenetic inheritance and regulation. Germ cells are vehicles of inheritance that transmit genetic and epigenetic information from one generation to the next. Male and female mammalian germ cells inherently differ in their development, morphology and means of fertilization. Thus, it is not surprising that divergent epigenetic mechanisms accompany these cell types (Ben Maamar *et al.*, 2021; Murphy *et al.*, 2014).

DNA accessibility differs throughout an organism lifespan resulting in distinct sets of active genes varying at each developmental stage. Sperm cells are highly specialized. Their production occurs during spermatogenesis, a process involving extensive cellular, epigenetic and chromatin changes (Oliva and Castillo, 2011). Life cycle of the sperm begins at the embryonic stage as a specialized pool of somatic cells called primordial germ cells (PGC). PGC undergo an erasure of DNA methylation in high density CpG regions, increase in low density regions, and histone replacement event, essential for proper sperm and offspring development (Ben Maamar *et al.*, 2021; Murphy *et al.*, 2014). The replacement of the majority of histones with protamines helps promote nuclear compaction, increases sperm motility and insulates the genome against harmful molecules in the female reproductive tract (Jenkins *et al.*, 2017). After gonadal maturity, spermatogenesis ensues. Spermatogenesis takes place in the seminiferous tubules of the testes in three distinct phases: (i) an undifferentiated diploid male germ cell differentiates into two different types of spermatogonia. Spermatogonia Type A replicates through mitosis and maintains the spermatogonia population in the testes. Spermatogonia Type B can enter into meiosis and further differentiate into spermatozoa. (ii) Spermatogonia Type B increase in size turning into primary spermatocytes. Further mitotic divisions convert the diploid spermatocytes into haploid spermatids. (iii) The round spermatids continue to undergo nuclear and morphological changes to become mature spermatozoa. Spermatozoa continues to mature as it transits along the epididymis in order to acquire both motility and fertilization potential to reach full maturation (**Fig. 1**) (Jodar *et al.*, 2017). After the completion of sperm maturation and transition through the epididymus, they are then able to swim and fertilize an oocyte. During the complex process of fertilization, sperm travels through the female reproductive tract, penetrates the cumulus oophorus, binds and enters the zona pellucida and then the oocyte. At this stage, there is a second epigenetic reprogramming event which involves DNA methylation erasure in high CpG density regions and increase in low density CpG (Ben Maamar *et al.*, 2023), and histone reassembly following paternal DNA. The molecular reasons underlying the reassembly is to allow the condensed DNA to form normal DNA structure. After this stage, the zygote initiates development and continues to totipotent stem cell conversion (Murphy *et al.*, 2014).

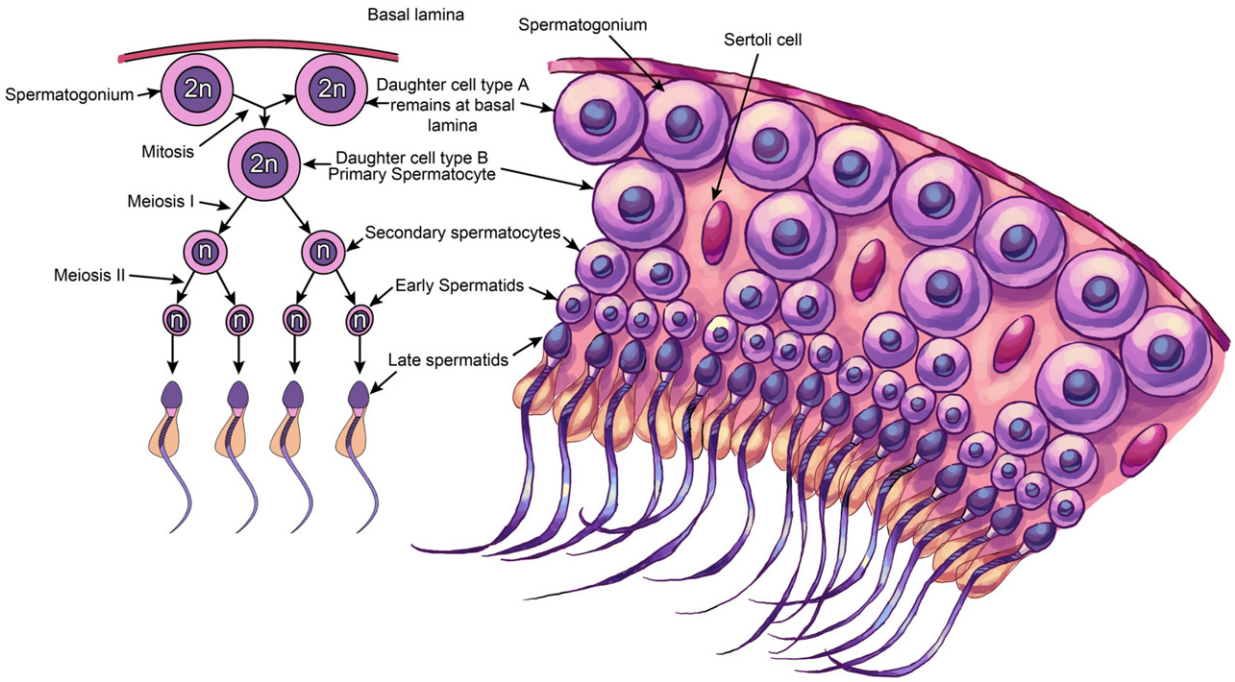

Fig. 1 The spermatogonia germ cells population lies on the basal lamina of the convoluted seminiferous tubules. Type A spermatogonia undergo mitosis: one of the daughter cells renew the stock of type A spermatogonia, the other becomes a type B spermatogonia. These divide and their daughter cells migrate towards the lumen. They differentiate themselves thereby into sperm cells up to the outer surface of the epithelium where the separation of the cytoplasm is not complete. Modified by Stephanie E. King from the reproductive system in *Human Anatomy & Physiology*, 9th edition. Published by Benjamin Cummings - An imprint of Addison Wesley Longman, Inc. 2012.

DNA Methylation

One of the most studied epigenetic marks is DNA methylation. This methylation occurs on the cysteine residues in the cytosine phosphate guanine (CpG) dinucleotides. Methylation near promoter regions prevent the expression of that gene (Gunes and Kulac, 2013). The process of initiation and the maintenance of these methylation marks is controlled by a family of proteins, the DNMT (DNA methyltransferases) (Portela and Esteller, 2010).

Throughout the male germ cell development, there are two instances during which epigenetic reprogramming takes place: during primordial germ cell development and post fertilization in the zygote. The first epigenetic reprogramming event is initiated when the PGCs begin to proliferate and migrate to the genital ridges where the future gonads will reside. During the primordial germ cell reprogramming event, the erasure of CpG region epigenetic marks which upon sex determination and differentiation of gonadal somatic cell lineages, inserts new epigenetic marks to promote sex specific imprinting patterns and germ cell function. The demethylation event occurs asynchronously across the genome suggesting the presence of regulatory mechanism to control the pace and location of DNA demethylation (Murphy *et al.*, 2014). However, during this programming low density CpG sites have an increase in DNA methylation that is retained during embryonic development (Ben Maamar *et al.*, 2023). Although the high CpG density portion of the genome undergoes a rapid demethylation event, the majority of the genome retains methylation (Ben Maamar *et al.*, 2023). The majority of the resistant loci consists of long terminal repeat transposable elements along with long and short interspersed elements. It is predicted that the methylation prevents the activation from these retroviral elements thus protecting the germline from their activation (Murphy *et al.*, 2014).

The second reprogramming event occurs after fertilization when paternal DNA is demethylated and the methylation patterns are reset in the developing embryo (**Fig. 2**). After fertilization, high density CpG DNA is demethylated and methylated in the developing embryo resulting in a reset of parental germlines. During this process, epigenetic marks specific to germ cells are reset for early embryonic pluripotency. This active removal of methylation is coordinated by Ten-eleven translocation enzymes (TET). The demethylation event of the zygote takes place prior to the first cell division and is thought to occur through active hydroxy-mediated demethylation. This reset is not complete thus some methylation marks are maintained in the developing embryo (**Fig. 2**). Recently, researchers have used genome wide association studies (GWAS) to identify specific loci that escape the reprogramming event. The maintenance of certain methylation patterns in the embryo are known as imprinted genes. These loci have become the foundation for identifying pathways that are biochemically regulated by epigenetics (Murphy *et al.*, 2014). These GWAS studies have also indicated that epigenetic marks on the sperm not only facilitate its own development, but also the development of the embryo (Murphy *et al.*, 2014). In the developing embryo, regulatory processes such as genomic imprinting and X chromosome inactivation and environmental altered transgenerational epigenetic sites are continued to be influenced by

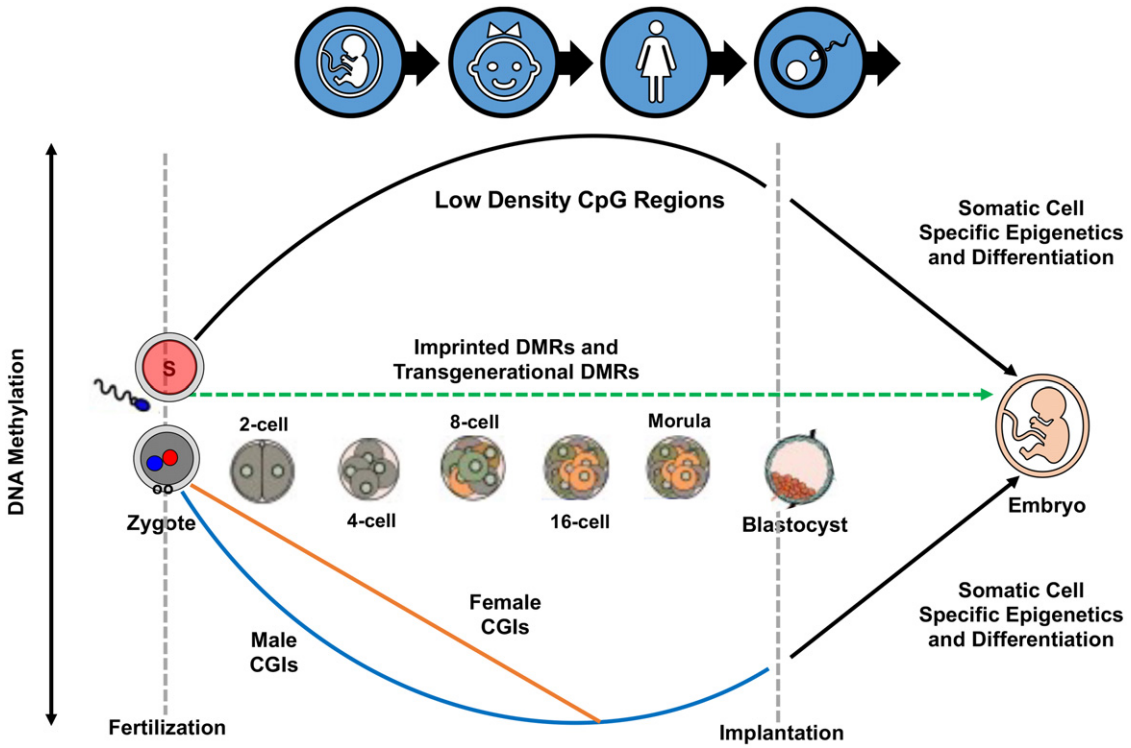

Fig. 2 DNA methylation levels throughout life. Modified from Ben Maamar *et al.* (2023).

gene processes including DNA methylation (Ben Maamar *et al.*, 2023; Jenkins *et al.*, 2017). Methylation events continue to occur throughout development in a cell type and tissue specific manner.

Chromatin Remodeling

The process of fertilization and movement of sperm along the female reproductive tract requires the coordination of many physiological events. Modifications of the sperm structure is essential as it progresses through potentially harmful environment in the female reproductive tract. Regulatory mechanisms following sperm maturation replaces 90–95% of histones with protamines (**Fig. 3**) (Kim, 2014). The replacement of histones with small, arginine rich molecules known as protamine allows for compaction of the nucleus. The condensation of the sperm nucleus, with its decreased surface area, increases sperm motility and protects the sperm genome from oxidation and other chemicals in the female reproductive tract. The compact packaging of the genome prevents the binding of transaction factors prematurely. Immediately following fertilization, protamines are translocated with histones (Gunes and Kulac, 2013).

Histone Modification

Histones – basic proteins rich in lysine and arginine – induce packaging of the DNA into structural units called nucleosomes. H2A, H2B, H3 and H4 histones are found in the nuclei of the nucleosomes (**Fig. 3**). These units are formed by combination of two H3-H4 dimers and two H2A-H2B dimers bound by H1 histone proteins (Campos and Reinberg, 2009). Histones can change the DNA-binding capacities and interaction of other regulatory factors with DNA. Histone modifications include acetylation, methylation, phosphorylation and ubiquitination. During sperm formation, in the haploid phase of spermatogenesis where round spermatids mature into spermatozoa, histone variants are incorporated to restructure chromatin. In human, mouse and fly, testes-specific variants of H1, H2A, H2B and H3 displace canonical histones prior to incorporation of transition proteins and protamines (Godde and Ura, 2009; Govin *et al.*, 2004; Montellier *et al.*, 2013). Some *in vitro* studies have demonstrated that some of these variants cause nucleosome destabilization (Gunes and Kulac, 2013). Other histone variants mark specific chromatin domains. For example, in mouse spermatids, H2AL1/2 are incorporated into heterochromatic pericentric chromatin (Samson *et al.*, 2014).

Acetylation of histone H3 and H4 usually leads to open chromatin configuration and active transcription by facilitating the binding of transcription factors. Deacetylation and methylation are generally correlated, they are associated with an inactivation of the transcription. A methylation of lysines localized on the histone tails is another regulatory mechanism. H3K9 and H3K27

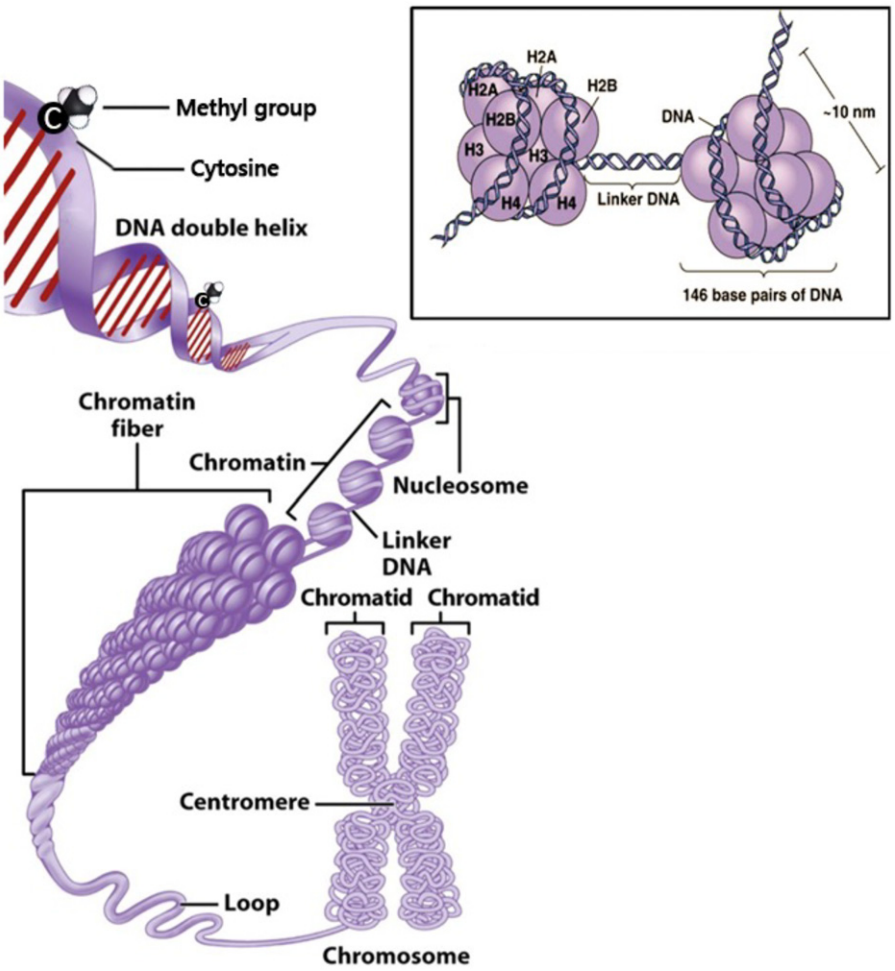

Fig. 3 Structure of DNA, chromatin and histone. Chromatin is an instructive DNA scaffold that can respond to external cues to regulate the many uses of DNA. A principle component of chromatin that plays a key role in this regulation is the modification of histones. Histones are proteins found in eukaryotic cell nuclei. They package and order DNA into structural units called nucleosomes. Modified from Kim (2014) and the cellular level of organization in *Principles of Anatomy and Physiology*, 11th edition by Gerard J. Tortora and Bryan H. Derrickson, John Wiley & Sons.

histones are usually methylated and thus linked with inactivation. However, methylation of histones takes place both in active and inactive chromatin sites. When H3 and H4 histone protein tails undergo modifications, it sometimes regulates transitions between active and inactive chromatin states through a reverse mechanism called histone code.

Histone modification and changes in its composition play important roles in chromatin modifications. These modifications are required for normal meiotic process and later maturation of gametes. Following termination of meiotic divisions, the sperm cells undergo developmental changes. In fact, some histones on the X chromosome label and retain some methylated histones H3K9me2. A global remodeling in haploid spermatids occur at a lower rate. In mature sperm, H2B was found to be the most frequently expressed histone. H3K4me3 is usually found on genes regions involved in spermatogenesis while H3K4me2 in regions rich in developmental genes. Furthermore, the hypermethylation of histone H4 is responsible for the replacement of histones by protamines in haploid spermatids (Gunes and Kulac, 2013).

Thus, the histone packaging process is an evolutional and developmental procedure for spermatogenesis. DNA methylation and histone modification both play an important role in the regulation and control of gene expression.

Sperm Non-Coding RNA

The original observations suggest that DNA methylation alterations and histone modifications in the sperm were crucial; non-coding RNAs have, subsequently, also been shown to be involved (Ben Maamar *et al.*, 2021; Skinner, 2014). Sperm is known to contain a diverse population of RNAs including non-coding RNAs which can be long or small (Ben Maamar *et al.*, 2021; Skinner, 2016). Abundant small non-coding RNAs retained in spermatozoa include miRNAs and piRNAs. Some of them are essential to the early embryo development like miR-34c which is required for the first cellular division. Other non-coding RNAs include

transposable elements, annotated lnc-RNAs, intronic retained elements, exonic elements, chromatin-associated RNAs, small-nuclear ILF3/NF30 associated RNAs, quiescent RNAs, mse-tRNAs and YRNAs (Jodar *et al.*, 2013). Some non-coding RNAs are known to act as epigenetic modifiers, such as histone modifications and DNA methylation, thus playing a role in transgenerational epigenetic inheritance.

This new area of study holds great promise for the development of this field. For example, comparing the differential ncRNAs profiles of infertile versus fertile individuals should help understand the regulatory pathways contributing to male infertility.

Clinical Issues

Aberrant epigenetic changes in male germ cells can lead to clinical issues. Abnormal methylation patterns and aberrant protamine insertions have been known to influence male fertility. CpG islands at gene promoter regions which are usually hypomethylated are especially susceptible to aberrant methylation for specific genes such as DAZL and MTHFR or imprinted loci. Epigenetic abnormalities in these regions are strongly associated with various forms of infertility and sperm defects in men (Carrell, 2012). Proper DNA methylation is especially important in imprinted genes as these genes are inherited directly from the parental germline and may have a transgenerational effect. Men with low sperm counts; oligospermic patients, (less than 10 million spermatozoa per 1 milliliter of semen) have been shown to contain a greater number of DNA methylation errors compared to men containing normal sperm counts. Furthermore, these imprinted loci are suspected to be inherited by the offspring during *in vitro* fertilization (IVF) treatments. Disorders due to defective imprinted gene expression can cause severe developmental defects including Angelman and Prader-Willi syndromes. Furthermore, children born from IVF treatments are found to have slightly greater incidents of imprinting disorders that children who were naturally conceived. This raises questions regarding the status of sperm methylome used in IVF treatments.

The use of assisted reproductive technologies have increased in recent years. Understanding the epigenetic processes underlying male factor infertility will aid proper diagnosis as well as, help develop sperm selection techniques used for IVF (Caroppo and Skinner, 2024; Murphy *et al.*, 2014).

Epigenetic dysfunction can also occur in spermatogenesis and may be a contributing factor in male infertility. The proper replacement of histones with protamines is a crucial step in male gametogenesis as reduced protamine levels are associated with oligospermic men. The differential DNA methylation patterns observed with male infertility is accompanied by altered protamine ratio and indicate the interaction between epigenetic phenotypes. Like the protamine, histones also carry programmatic information and thus can possibly contribute to male infertility. Histone retention has been found to occur sporadically throughout the genome, and loss of histone retention at imprinted loci is often associated with male infertility.

Conclusions

Epigenetic modifications are well studied in both DNA methylation and histone retention. Yet, the topic of chromatin remodeling and of non-coding RNAs have been understudied. More comprehensive understanding of epigenetic influences on male factor infertility can help further sperm selection techniques for artificial reproductive technologies (Caroppo and Skinner, 2024).

References

Anway, M.D., Cupp, A.S., Uzumcu, M., Skinner, M.K., 2005. Epigenetic transgenerational actions of endocrine disruptors and male fertility. Science 308, 1466–1469.
Ben Maamar, M., Nilsson, E.E., Skinner, M.K., 2021. Epigenetic transgenerational inheritance, gametogenesis and germline development. Biol. Reprod. 105, 570–592.
Ben Maamar, M., Wang, Y., Nilsson, E.E., *et al.*, 2023. Transgenerational sperm DMRs escape DNA methylation erasure during embryonic development and epigenetic inheritance. Environ. Epigenetics 9, 1–15. dvad003.
Campos, E., Reinberg, D., 2009. Histones: Annotating chromatin. Annu. Rev. Genet.. 559–599.
Caroppo, E., Skinner, M.K., 2024. Could the sperm epigenome become a diagnostic tool for evaluation of the infertile man? Hum. Reprod. 39, 478–485.
Carrell, D.T., 2012. Epigenetics of the male gamete. Fertil. Steril. 97, 267–274.
Godde, J.S., Ura, K., 2009. Dynamic alterations of linker histone variants during development. Int. J. Dev. Biol. 53, 215–224.
Govin, J., Caron, C., Lestrat, C., Rousseaux, S., Khochbin, S., 2004. The role of histones in chromatin remodelling during mammalian spermiogenesis. Eur. J. Biochem. 271, 3459–3469.
Guerrero-Bosagna, C., Skinner, M.K., 2014. Environmentally induced epigenetic transgenerational inheritance of male infertility. Curr. Opin. Genet. Dev. 26, 79–88.
Gunes, S., Kulac, T., 2013. The role of epigenetics in spermatogenesis. Turk. J. Urol. 39, 181–187.
Jenkins, T.G., Aston, K.I., James, E.R., Carrell, D.T., 2017. Sperm epigenetics in the study of male fertility, offspring health, and potential clinical applications. Syst. Biol. Reprod. Med. 63, 69–76.
Jodar, M., Selvaraju, S., Sendler, E., Diamond, M.P., Krawetz, S.A., 2013. The presence, role and clinical use of spermatozoal RNAs. Hum. Reprod. Update 19, 604–624.
Jodar, M., Soler-Ventura, A., Oliva, R., Molecular Biology of Reproduction and Development Research Group, 2017. Semen proteomics and male infertility. J. Proteom. 162, 125–134.
Kim, Y.Z., 2014. Altered histone modifications in gliomas. Brain Tumor Res. Treat. 2, 7–21.
Kitamura, A., Miyauchi, N., Hamada, H., *et al.*, 2015. Epigenetic alterations in sperm associated with male infertility. Congenit Anom (Kyoto) 55, 133–144.
Montellier, E., Boussouar, F., Rousseaux, S., *et al.*, 2013. Chromatin-to-nucleoprotamine transition is controlled by the histone H2B variant TH2B. Genes Dev. 27, 1680–1692.

Murphy, K.E., Murphy, P.J., Carrell, D.T., 2014. Epigenetic changes in the paternal germline. In: Tollefsbol, T.O. (Ed.), Transgenerational Epigenetics. London: Elsevier Academic Press.
Oliva, R., Castillo, J., 2011. Proteomics and the genetics of sperm chromatin condensation. Asian J. Androl. 13, 24–30.
Portela, A., Esteller, M., 2010. Epigenetic modifications and human disease. Nat. Biotechnol. 28, 1057–1068.
Samson, M., Jow, M.M., Wong, C.C., *et al.*, 2014. The specification and global reprogramming of histone epigenetic marks during gamete formation and early embryo development in C. elegans. PLoS Genet. 10, e1004588.
Sharlip, I.D., Jarow, J.P., Belker, A.M., *et al.*, 2002. Best practice policies for male infertility. Fertil. Steril. 77, 873–882.
Skinner, M.K., 2011. Environmental epigenetic transgenerational inheritance and somatic epigenetic mitotic stability. Epigenetics 6, 838–842.
Skinner, M.K., 2014. Endocrine disruptor induction of epigenetic transgenerational inheritance of disease. Mol. Cell. Endocrinol. 398, 4–12.
Skinner, M.K., 2016. Endocrine disruptors in 2015: Epigenetic transgenerational inheritance. Nat. Rev. Endocrinol. 12, 68–70.
Surani, M.A., 2015. Human germline: A new research frontier. Stem Cell Rep. 4, 955–960.
Tvrda, E., Gosalvez, J., Agarwal, A., 2015. Epigenetics and its role in male infertility. In: Watson, R. (Ed.), Handbook of Fertility. Academic Press.

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