Epigenetics and Male Infertility

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Key Points

- Epigenetics, the study of changes in DNA expression without alterations in the DNA sequence, is now thought to underlie much of human disease, including infertility.
- All four known epigenetic mechanisms, DNA methylation, histone modification and imprinting, non-coding RNAs, and chromatin remodeling, are thought to be active in spermatogenesis.
- Sperm epigenetics has the potential to provide a root cause for much of what is now termed "unexplained" infertility as well as oligospermia and sperm reproductive competence.
- Epigenetic changes that occur in sperm with paternal age appear to be non-random and may involve increased risk of neurodevelopmental diseases in offspring.
- As sperm epigenetic patterns are heritable, their significance to future generations extends to far more than simply infertility and includes familial and *de novo* disease transmission

10.1 Introduction

10.1.1 Definition of Epigenetics

Epigenetics is the study of heritable changes in gene expression that do not alter the underlying DNA sequence. By altering the way in which genes are read, changes in phenotype can occur without changes in genotype. Although every cell in an individual is genotypically identical, epigenetically, each cell's epigenetic signature is distinct, thus facilitating organ-specific differentiation. That is why a nose is not an eye and vice versa, despite having identical copies of DNA. Epigenetic changes are both natural and common occurrences and are influenced by age, environment, lifestyle, and illness. Epigenetic modifications underlie both normal development and also pathologic diseases such as cancer and autoimmunity (Table 10.1) [17].

10.1.2 History of Epigenetics

The term "epigenetics" was first coined by Waddington in 1942 from his work with *Drosophila* fruit flies [18]. He used the word epigenetics to describe the molecular process whereby environmental stress resulted in genetic "assimilation" of phenotypic characteristics. Although conceptually distilled by Waddington, the idea that the environment can influence genetics, that nurture can alter nature, is actually much older with origins ascribed to the eighteenth-century French naturalist Jean Baptiste Lamarck. His concept of "soft inheritance" preceded Darwin's evolutionary theory by 50 years. Whereas Darwin pictured evolution as occurring in rather large, generational "steps," Lamarck had earlier proposed that offspring inherit smaller, environmentally induced changes acquired by parents over their lives. In essence, Lamarck outlined a pathway for evolution that involved passing along traits that were gained from simply living and surviving or the "inheritance of acquired characteristics," an apt description of what we now call epigenetics. Ironically, while Lamarck has historically been considered the one who "got it wrong" in describing the mechanics of evolution, we now believe that certain inheritance patterns are best described by Lamarck's theory.

Our knowledge of epigenetics has exploded over the last quarter century. As outlined in Table 10.1, we now know that epigenetics underlies much of normal cell and tissue function as well as cancer biology, autoimmunity, psychiatric disorders, and intellectual disorders [1, 19]. However, its role as

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Table 10.1 Diseases and disorders in which epigenetic mechanisms have been proposed

Description	Reference
Cancer	
Colorectal	Feinberg and Vogelstein [1]
Breast	Pasculli et al. [2]
Pancreas	Sato and Goggins [3]
Prostate	Ngollo et al. [4]
Intellectual disability	
ATR-X,	Schenkel et al. [5]
Fragile X	Kraan et al. [6]
Rett syndrome	Kubota et al. [7]
Beckwith–Weidman syndrome	Soejima and Higashimoto [8]
Prader–Willi syndrome	Butler [9]
Angelman syndrome	Lalande and Calciano, 2007 [10]
Neurodegenerative	
Schizophrenia	Akbarian [11]
Bipolar disease	Ludwig and Dwivedi [12]
Autism	Loke et al. [13]
Alzheimers	Sanchez-Mut and Gräff [14]
Immunity	
Systemic lupus erythematosus	Xiao and Zuo [15]
Rheumatoid arthritis	Ai et al. [16]

a cause or consequence of infertility is only beginning to be understood. There is a strong sense in the field, though, that epigenetics is critically important to normal human fertility. Factors that complicate the study of epigenetic infertility are the largely continuous nature of the variables involved, the fact that epigenetics can change with age, a serious lack of a defined "normal" cell signatures and the wide variety of epigenetics marks and measures that exist.

10.2 Epigenetics Mechanisms

Four general types of epigenetic modification have been described. All of these are thought to be active in sperm.

10.2.1 DNA Methylation

DNA methylation is one of the oldest and best-characterized epigenetic mechanisms, first described in 1969 [20]. DNA methylation refers to the addition of a methyl (CH_3) group to the DNA strand, typically to a carbon atom of a cytosine ring. It fixes genes in the "off" position and is important for cellular processes like embryonic development, X-chromosome inactivation, genomic imprinting, gene suppression, carcinogenesis, and chromosome stability. Abnormal DNA methylation has been linked to several human diseases including lupus, cancer, muscular dystrophy, and congenital defects [19]. As an example, cancer cell genomes tend to show overall hypomethylation (i.e., are activated) relative to healthy cells which partly explains their malignant behavior.

10.2.2 Chromatin Remodeling

Chromatin is term used to describe the DNA and its associated proteins that are packed within the nucleus of cells. DNA forms chromatin when it is tightly condensed and wrapped around nuclear proteins called histones. The DNA–histone complex is called a nucleosome. When packed tightly in a nucleosome, DNA is relatively inaccessible to transcription factors and therefore unavailable for transcription. In this state, the DNA is called "heterochromatin." When more loosely packed, and accessible for transcription, it is called "euchromatin."

10.2.3 Histone Modification

Epigenetic modifications to histone proteins, also termed histone modification, commonly occur through methylation, phosphorylation, acetylation, ubiquitylation, and sumoylation. These modifications can alter gene expression by grossly or slightly modifying histone structure and are known to underlie biological processes such as transcriptional activation, chromosome packaging, and repair of DNA damage. This process provides another modifiable layer of gene regulation with the potential for heritability.

10.2.4 Non-coding RNA

A non-coding RNA is a functional RNA that is transcribed from DNA but not translated into protein. Non-coding RNAs thought to have epigenetic functions include microRNA (miRNA,) short-interfering RNA (siRNA), piwi-interacting RNA (piRNA), and long-non-coding RNA (lncRNA). In general, non-coding RNAs regulate gene expression at both the transcriptional and post-transcriptional levels and are known to play a role in heterochromatin formation, chromatin and histone modification, DNA methylation targeting, and gene silencing.

10.2.5 Genomic Imprinting

Genomic imprinting is an epigenetic process that involves DNA methylation and histone methylation within the germline (sperm or egg cells) of an organism. After fertilization, these marks are maintained in the early embryo despite the extensive epigenetic reprogramming that takes place early in development. This generates regions in the genome that have DNA methylation present on one parental allele but absent on the other allele. Imprinted areas are then maintained through mitotic cell divisions in the somatic cells of the individual during its lifetime in a parent-of-origin-specific manner. The precise number of genes known to be imprinted is

debated with some studies claiming to have identified over 1000 imprinted genes [21]. The lack of congruence in the data is largely a result of different tissues and species being screened. What is known is that inappropriate imprinting of certain genes has been implicated in several diseases to date, including defective spermatogenesis.

10.3 Sperm Epigenetics

The sperm epigenetic program is uniquely customized to meet the needs of this highly specialized cell. Sperm chromatin structure is one of the most complex structures in the eukaryotic genome, for good reason. Sperm must transport its genome through the male and female reproductive tracts, which necessitates a chromatin structure that is between six and twenty times more dense and robust than somatic cell nucleosome-bound DNA [22, 23]. The extreme compaction of the sperm head is also thought to enhance sperm motility and to protect the DNA from damage in a cellular environment that lacks robust DNA repair abilities [24]. To achieve this uniquely compact chromatin structure, canonical histones are first replaced with transition proteins. Subsequently, two forms of protamines (P1 and P2) take the place of transition proteins in DNA compaction in humans. This process of protamination essentially "blocks" the DNA from any epigenetic change or gene transcription, which make sense given the need to preserve the sperm genome during transport through both the male and female reproductive tract. In fact, the ratio of P1:P2 is tightly regulated at 1:1 in mature sperm and aberrations in this ratio have been correlated with infertility and poor egg fertilization [25–28].

Even more interesting is the fact that the replacement of sperm histones with protamines is typically incomplete, with between 5% and 15% of chromatin remaining histone-nucleosome-bound. Furthermore, the incomplete replacement appears not to reflect random inefficiency but rather a purposeful and programmatic process occurring in deliberate locations [29, 30]. As such, it is thought that histone retention allows for epigenetic modification of genes important for the embryo, including developmental gene promoters, microRNAs, and imprinted loci [29]. These recent findings now suggest that the sperm epigenome, previously considered silent and inaccessible, is actually critical for regulation of early embryo development [31].

10.3.1 Current Technology Used to Evaluate Sperm Epigenetics

The evaluation of sperm DNA methylation profiles is typically based on bisulfite conversion of extracted sperm DNA. This is most commonly assessed in three ways: using arrays, whole genome bisulfite sequencing, and targeted bisulfite sequencing. Among the most popular techniques to screen DNA methylation signatures in humans is the 850K (EPIC) methylation array (Illumina, San Diego, CA, USA). This array assesses the amount of methylation, or lack thereof, at over 850,000 CpGs and reports these methylation signatures as intensity values. Since intensity values reflect a sperm population average, the value effectively represents a "fraction methylation" at each CpG site. Informatic analysis of these data typically includes regional assessments (such as "sliding window" analyses) and point data analyses (assessment of a single genomic site of DNA methylation). This relatively simple format allows for rapid and reliable screening of most known, well-annotated gene promoters, CpG islands, multiple enhancers, and gene body methylation sites with impressive single base pair resolution. The most comprehensive assessment of DNA methylation comes in the form of whole genome bisulfite sequencing. This technology is quite reliable and can cover the entire genome, but has drawbacks including a high cost per sample and the potential loss of sensitivity to identify small methylation changes. One innovative variation on this technology is termed reduced representation bisulfite sequencing (RRBS) that provides similarly high-quality data but with more targeted coverage and at a lower cost. In addition, RRBS can be tailored to cover specific genomic regions of interest depending on the research goals. In addition to the assessment of DNA methylation, newer technologies show great promise in the assessment of sperm RNAs. Because sperm are transcriptionally quiescent, evaluating RNA can be difficult due to very low transcript numbers. However, RNA sequencing methods developed and modified from somatic cell protocols can effectively be used to assess sperm RNAs. These technologies including DropSeq (McCarroll Lab, Harvard Medical School) and the 10× Genomics (San Francisco, CA) platform have shown excellent performance in the evaluation of somatic cell RNAs. Hopefully, they will soon allow for the assessment of single sperm RNA as well.

Sperm chromatin, protamines, and histone modifications have been investigated using several techniques, including simple staining. More advanced techniques including ATAC-Seq or ChIP-Seq allow for the determination of not only the amount of an individual histone present but also the precise genomic location.

10.3.2 Value of Sperm Epigenetics to the Male Infertility Evaluation

The well-recognized inability of the standard semen analysis to predict male reproductive potential [32] needs no emphasis. The fact is that a semen analysis can inform us regarding a potential fertility problem but does not constitute a formal diagnosis. Its ability to predict pregnancy outcomes or to guide clinical decisions is limited. The wide variability in

quality between ejaculates further complicates the potential of the semen analysis to predict "fertility." On the contrary, an understanding of the sperm epigenome has the ability to not only improve the prediction of fertility but also to provide clues to the root cause of the underlying spermatogenetic disorder. Add to this the fact that DNA methylation signatures in mature sperm remain remarkably stable throughout spermatogenesis provides a foundation for a more reliable and relevant diagnostic test for sperm. Limitations in the evaluation of sperm epigenetics include data contamination with somatic cells, which is technically possible to overcome, and the fact the infertility is inherently a couple phenomenon, which makes isolation of male and female factors difficult in most cases.

10.3.3 Sperm Epigenetic–Fertility Phenotypes

The increasing ability of technology to reliably and relatively inexpensively screen the epigenome with high resolution has helped our understanding of the relationship between the sperm epigenome and fertility phenotypes. For example, RNA sequencing has allowed for the assessment of non-coding RNAs, miRNAs, and mRNAs in sperm [33, 34]. While we know that overall RNA content is very low in sperm and that much of the RNA appears to be "remnant" leftovers from spermatogenesis, there appear to be forms of RNA present that may play a role not only in sperm development but also in embryogenesis [34–37]. To date, published research has correlated sperm RNA content to the following fertility phenotypes: decreased IVF success rates [34] and decreased IUI success rates [38]. Similarly, there appear to be signatures in mature sperm methylation patterns that predict the likelihood that an individual will need IVF to conceive or if less invasive therapeutic interventions may be effective [39].

10.3.3.1 Abnormal Semen Analysis

The earliest research on the relationship between epigenetics and semen parameters focused on imprinted loci and measured methylation of sequences in one or only a few genes [40] Marques et al., (2004) examined the *H19* imprinted locus of men with various sperm concentrations and observed abnormal methylation in 0.13% of normozoospermic men, 17% of those with moderate oligospermia, and in 30% of men with severe oligozoospermia. In a study of men with teratozoospermia or abnormal sperm morphology, 11 of 19 patients displayed a loss of methylation at either *IGF2* or both *IGF2* and *H19* genomic sites [40]. Moreover, several studies have confirmed that the abnormal methylation patterns occurring in men with low sperm counts occur at both paternal (hypomethylated) and maternal (hypermethylated) genomic sites [40–43]. Kobayashi et al. (2007) examined the methylation status of seven imprinted genes in the sperm DNA of infertile men and found that when *both* maternal and paternal DNA was abnormally methylated, the finding of severe oligospermia was more common. Thus, abnormal methylation patterns associated with several imprinted genes of both maternal and paternal origin appear to correlate with low sperm concentration and abnormal sperm morphology. At this time, it is unclear whether abnormal DNA methylation among imprinted genes arises from de novo methylation or improper erasure of pre-existing methylation, although the latter seems to be a simpler mechanism [44].

Subsequently, as measures of DNA methylation improved with the advent of methylation arrays, the possibility of examining hundreds or thousands of different methylation markers across the genome was now realizable. In the first study to use a more extensive array of methylation measures, elevated methylation was found at numerous sequences in the DNA of poor-quality sperm from infertile men [44]. The high-throughput analysis addressed hundreds of DNA methylation targets and revealed significant correlations between methylation levels in 35 gene sequences and sperm concentration, motility or morphology. In four gene sequences, *NTF3, MT1A, PAX8, and PLAGL1*, there were striking correlations between methylation levels and abnormalities involving all three semen parameters. Notably, this study was the first to demonstrate that methylation abnormalities in non-imprinted genes are also associated with abnormal semen parameters.

10.3.3.2 Unexplained Infertility

Analyses of DNA methylation patterns in sperm have also identified candidate genetic loci associated with decreased fecundity. In a paired analysis of semen samples from men who had conceived within 2 months of attempting and men unable to achieve a pregnancy within 12 months, two genomic regions were identified as having significantly different methylation patterns between the cohorts [45]. Interestingly, there were no differences in semen volume, sperm concentration or morphology on routine semen analysis testing between the groups. The two sites in which methylation was associated with reduced fecundity are closely related genes that are known to be expressed in sperm: *HSPA1L* and *HSPA1B*. These observations suggest that abnormal epigenetic patterns in sperm might be linked to sperm function, egg fertilization, or embryo development in addition to their previously described association with semen parameters.

A more recent study expanded on the notion that sperm epigenetic patterns correlate with natural fertility and IVF success. Aston et al. [39] studied whether genome-wide sperm DNA methylation patterns can be used to predict male fertility and IVF success. As illustrated in Fig. 10.1, semen samples from a control group of $n = 54$ men with

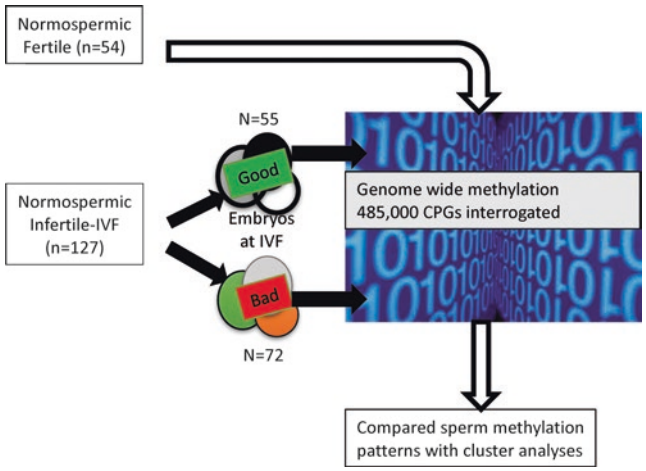

Fig. 10.1 Schematic of study design of genome-wide sperm DNA methylation patterns to predict male fertility and IVF success. Semen samples from a control group ($n = 54$ men) with normal semen quality and proven fertility were compared to infertile men ($n = 127$) with normal semen parameters with none-to-mild female factor infertility undergoing IVF. Genome-wide sperm DNA methylation analysis was performed to measure methylation at >485,000 sites across the genome

normal semen quality and proven fertility were compared to $n = 127$ infertile men with normal semen parameters whose partners were judged to have none to mild female factor infertility and were undergoing IVF. The infertile men were further divided into two groups: men whose partners produced high quality embryos at IVF along with many confirmed pregnancies ($n = 55$ men) and those whose partners produced generally poor-quality embryos ($n = 72$ men) with far fewer pregnancies. Genome-wide sperm DNA methylation analysis was performed to measure methylation at >485,000 sites across the genome. Notably, the sperm DNA methylation patterns were observed to be very stable across semen samples from each individual and maintained consistent difference in methylation patterns across individuals. They observed specific sperm methylation patterns that were highly predictive of fertility status, and somewhat predictive of IVF embryo quality. Predictive models generated based on cluster analysis were capable of correctly classifying male fertility status (fertile or infertile) with 82% sensitivity and 99% positive predictive value. In addition, modeling of the cluster analysis of sperm methylation patterns from infertile couples generating poor quality embryos achieved a positive predictive value of 94%. Finally, a comparison of sperm methylomes of fertile men vs. infertile men revealed >8500 CpGs that had differed significantly. When studying the specific genes with discrepant methylation, several gene classes were involved, including cellular adhesion, cellular morphogenesis and differentiation, and imprinted genes. This study was the first to use large array-based examination of sperm DNA methylation patterns and the first to build predictive models of fertility status using sperm methylation data. It also served as the basis for a commercially available mail in, sperm-based test of male fertility potential (Episona Seed® Assay) that was marketed in the United States from 2016 until 2018 and discontinued due to high testing costs.

10.3.3.3 Embryo Development and Miscarriage

If the sperm epigenome truly influences IVF success, might it act by altering embryo development and effecting miscarriage rates? A study by Denomme et al. [46] has provided early evidence to support the concept that the integrity of the sperm methylome correlates to embryo competence. The study involved comparing the blastocyst methylomes and transcriptomes of 128 couples undergoing IVF for male factor issues characterized by oligoasthenozoospermia to that of 72 surplus banked blastocysts derived from non-male factor patients. Sperm methylomes were not examined. Importantly, all blastocyst were biopsy-euploid to eliminate the influence of maternal or paternal chromosomal disorders on embryo development and pregnancy rates. Although the clinical pregnancy rates were similar after euploid embryo transfer in both male factor and non-male factor embryo transfers, the subsequent miscarriage rate was seven times higher in male factor cases (14.7% vs 2.2%, $p < 0.05$). In addition, there were significant differences in the embryonic methylomes (at 1111 Cpgs) and transcriptome (in 469 transcripts) analyses of embryos between the two cohorts. While the data do not show clear proof of inherited epigenetic dysregulation in blastocysts derived from severe male factor sperm, it does suggest an epigenetic consequence of male factor infertility on embryogenesis and miscarriage rates. The basis for a relationship between sperm DNA methylation patterns and IVF outcomes including miscarriages has now been realized and merits further study.

10.3.4 Sperm Epigenetics and Paternal Age

The relationship between advanced maternal age and pregnancy outcomes is undisputed [47]. Since the advent of epigenetics research, there is now increasing concern that paternal age is associated with non-random alterations in the sperm epigenome that may have implications not only for paternal fertility potential but also on offspring health. Several epigenetic alterations in sperm, particularly DNA methylation defects, have recently been correlated with advanced paternal age [48]. Sperm appear to accumulate hundreds of DNA methylation defects with paternal age that are localized to specific genomic sites, such as CpG regions [48–50]. Strikingly, many of these are found in regulatory or promoter regions and govern neurological, psychiatric, and behavioral disorders, including schizophrenia, bipolar disease, autism, and mood disorders [48–50].

A recent study analyzed age-associated sperm DNA methylation patterns in sperm [48, 51]. In addition to characterizing the type and magnitude of DNA methylation changes, the analysis examined if any specific genomic regions were consistently affected with age. As illustrated in Fig. 10.2, semen samples from men with known fertility were examined at two points in their lives: When they were "younger" (mean age 37.7 years) and "older" (mean age 50.3 years). Global methylation patterns were determined by pyrosequencing, and high-level CpG level array analysis and targeted bisulfite sequencing were performed. Overall, there was a significant global hypermethylation in sperm with paternal age along with localized regions of hypomethylation, which contrasts sharply with patterns of DNA methylation found in somatic tissues with age (i.e., global hypomethylation and localized hypermethylation) [52]. The authors calculated that the average fractional methylation change in sperm was 0.3% per year in hypermethylated regions and 0.28% in hypomethylated regions, both of which appear much higher than the 0.15% annual change in DNA methylation estimated to occur in somatic cells with age [52].

Equally or more intriguing were the study findings that consistently linked altered regions of sperm DNA methylation to genes associated with specific diseases (Fig. 10.1). In a 2014 study, Jenkins et al. found that the genomic loci exhibiting age-associated hyper- or hypomethylation appeared to be enriched at genes associated with bipolar disorder and schizophrenia. This finding suggests that sperm DNA methylation changes observed with paternal age are not randomly distributed within the genome, but could occur more frequently in neurodevelopmental gene sets. This observation is particularly striking when taken in the context of the increased incidence of neuropsychiatric disorders seen in the offspring of older fathers.

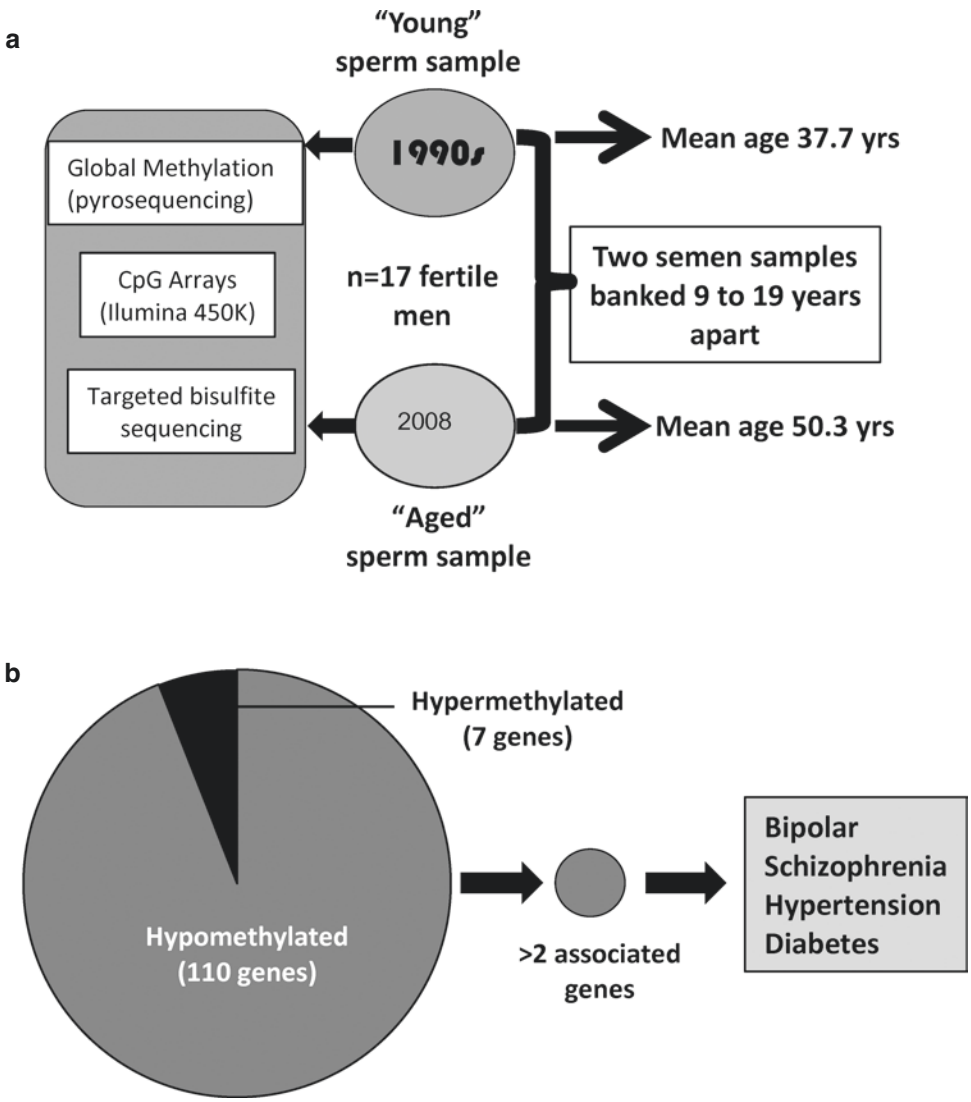

Fig. 10.2 Study of human sperm DNA methylation with age. (**a**) Schematic of study design and epigenetic investigations on sperm. The average difference in subject age between sperm samples was 12.6 years. (**b**) Schematic of study findings. Among the diseased associations with paternal age-related DNA methylation changes, only bipolar disorder reached statistical significance. (Reprinted from Yatsenko and Turek [59]. with permission from Springer Nature)

10.3.5 Lifestyle and Environmental Influences on Sperm Epigenetics

Not only paternal age but nutritional status (obesity) and physical activity levels have also been linked with dynamic epigenetic changes in human sperm [53, 54]. Although potential scientific confounders abound in the examination of environmental influences on sperm epigenetics, including the timing and type of environmental stimulus, the methylation methodology and choice of genomic sites, the type of bioinformatic analysis, somatic cell contamination and the source, purification and fractionation of sperm [55], studies to date are highly suggestive that lifestyle factors significantly modulate the epigenetic health of sperm.

10.3.6 The Hereditability of Sperm Epigenetics

The inheritance of epigenetic alterations in sperm is a plausible way to explain how phenotypic plasticity is transmitted across generations without involving formal genetic mutations [55]. It also lends a molecular mechanism to the mode of inheritance of acquired characteristics postulated by Lamarck more than 200 years ago. Animal models of paternal inheritance have shown that parental dietary factors can affect the metabolism of offspring through epigenetic inheritance [56, 57]. There is also burgeoning evidence from human epidemiological studies that the lifestyle of one generation can modify the risk of chronic disease in offspring through what is now termed "parental effects"[58]. Such modifications have to be transmitted through either sperm or eggs. Currently, the best evidence is that much of human epigenetic inheritance is paternal in nature [57], but this claim may be premature because the investigation of the oocyte epigenome (at least in humans) is ethically and technically far more challenging than studying the sperm epigenome.

10.4 Conclusion

The modern study of epigenetics is based on an old idea that recently found a molecular basis. Sperm epigenetics is a rapidly evolving field that is extremely pertinent to normal and aberrant human reproduction. Abnormal sperm epigenetic profiles appear to correlate not only with semen analysis parameters but also with reproductive competence as defined by embryo quality and miscarriage rates. Sperm epigenetic profiles also change with paternal age and are influenced by paternal lifestyle choices. As sperm epigenetic patterns are uniquely heritable, its significance takes center stage in the study of transgenerational transmission of disease to offspring.

10.5 Review Criteria

In order of importance, randomized controlled trials, scientific studies, meta-analyses, case-controlled cohort studies, and published reviews from 1942 to 2018 were used in this work. Articles published in languages other than English were considered. Data from conference or meeting proceedings, websites, or books were not included.

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