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Sperm chromatin: Evaluation, epigenetic signatures and relevance for embryo development and assisted reproductive technology outcomes

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ABSTRACT

Sperm chromatin is distinct from somatic cell chromatin, as a result of extensive remodeling during the final stages of spermatogenesis. In this process, the majority of histones is replaced with protamines. The chromatin is consequently highly condensed and inert, which facilitates protection of the DNA. The sperm epigenomic landscape is shaped by histone retention, histone and protamine modification, DNA methylation, and RNAs. In recent years, sperm chromatin integrity and its epigenetic marks have been increasingly studied, and the constitution of sperm chromatin is steadily being uncovered. This growing body of research prompts assessment of the frequently overlooked involvement of sperm in fertility and embryonic development. Moreover, numerous endogenous and exogenous factors are known to affect sperm chromatin, which may in turn impact the reproductive success. Concerns have been raised about the effects of assisted reproductive technology (ART) on the sperm epigenome, embryonic development and offspring health. This review examines the structure and epigenetic signatures of sperm chromatin in the context of fertility and early embryonic development. Additionally, sperm chromatin evaluation and causes of aberrant integrity are outlined. Building on the knowledge discussed in the current review, future research should aim to elucidate the intricate relationship between all aspects of sperm chromatin and embryo development. This could lead to the uncovering of new targets for treating infertility, as well as the acquisition of much needed insights into the possible reciprocal association between ART and sperm chromatin integrity.

1. Introduction

Spermatogenesis occurs in three phases: mitotic, meiotic and post-meiotic, which are also referred to as spermacytogenesis, spermatidogenesis, and spermiogenesis, respectively. Spermatogonia, derived from spermatogonial stem cells, undergo mitosis to generate primary spermatocytes and daughter stem cells. Two consecutive meiotic cell divisions then create secondary spermatocytes and haploid round spermatids, successively. During spermiogenesis, the post-meiotic stage of spermatogenesis, the nuclei of round spermatids elongate to form elongating spermatids and subsequently condense into mature sperm (Neto et al., 2016). During mid-to-late spermiogenesis, sperm chromatin undergoes drastic remodeling which involves the replacement of most histones by protamines, thus facilitating transcriptional silencing and hypercondensation of the nucleus. This allows for the polarization of the nucleus to one side of the cell and shapes the head in a species-specific manner. The chromatin of ejaculated sperm consists of protamine-bound DNA, histone-bound DNA, and matrix attachment regions (MARs). Altered sperm chromatin structure or its epigenetic marks are postulated to underlie some cases of infertility.

Assisted reproductive technologies (ART) are the collective technologies employed to treat infertility, a health condition from which one in six couples is estimated to suffer globally (World Health Organization, 2023). Semen parameters conventionally measured in ART clinics include sperm motility, concentration, count, and semen volume. While several methods exist to evaluate sperm chromatin integrity, they are

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not included in the standard semen analysis performed in clinically ART. Concerns have been raised regarding the effect of ART on sperm chromatin and its epigenetic modifications, as several studies have reported increased numbers of imprinting disorders among individuals conceived using ART, compared to naturally-conceived counterparts (Hattori et al., 2019). Moreover, critics highlight the risk of transmitting epigenetic mutations by using advanced ART techniques such as intracytoplasmic sperm injection (ICSI). Recently, the body of literature examining sperm chromatin has been expanding, and novel sperm chromatin marks have been reported, as well as crosstalk between the different facets of the sperm epigenome. Herein, the current knowledge on sperm chromatin and infertility, embryonic development and ART is outlined, and the need for a continued effort to gain a more comprehensive understanding of the interconnection between these realms is also highlighted. Importantly, not only could this facilitate improvement of the ART through uncovering new targets for infertility treatment and diagnosis, but it might also allow for the monitoring of the epigenetic health of ART-conceived individuals.

2. Sperm chromatin

2.1. Histone-to-protamine transition

A major event of chromatin remodeling during spermiogenesis is the replacement of histones with protamines. Protamines are basic, positively charged arginine-rich proteins that associate with the negatively charged DNA's phosphate backbone. Moreover, cysteine residues of protamines can form disulfide bonds for further chromatin compaction (Mukherjee et al., 2021). The majority of mammals express one type of protamine: protamine 1 (P1), whereas primates and several rodents express an additional protamine: P2. In mice and humans, the genes coding for P1 (*PRM1*) and P2 (*PRM2*) are located on chromosome 16 (Wykes and Krawetz, 2003). Moreover, and as studies in mice indicate, while translation of *Prm1* results in the mature P1 protein, P2 is synthesized as a precursor, which requires cleaving of the N-terminal domain to obtain the mature P2 protein (mP2) (Yelick et al., 1987). Interestingly, the ratio of P1-to-P2 expression is species-specific, and was determined to be 1:1 in humans (de Mateo et al., 2009), and 1:2 in mice (Corzett et al., 2002). The P1:P2 ratio is also linked to fertility, as demonstrated in previous mouse studies. In addition to *Prm1*$^{-/-}$ mice being infertile and *Prm1*$^{+/-}$ mice being subfertile, P1 was observed to play a vital role in maintaining the species-specific P1:P2 ratio, as the ratio was skewed in *Prm1*$^{+/-}$ and *Prm1*$^{-/-}$ mice, where increased levels of P2 precursors were detected (Merges et al., 2022). In a later study, *Prm2*$^{+/-}$ mice were reported to be fertile, whereas *Prm2*$^{-/-}$ mice were infertile (Schneider et al., 2016). Acrosome formation, motility, and DNA hypercondensation were severely impacted in these *Prm2*-deficient mice. On the other hand, not just the mature carboxyterminal domain of the P2 is required for proper chromatin processing, but, as a recent mouse study demonstrated, the cleaved N-domain (cP2) is also required for complete protamination (Arévalo et al., 2022). Indeed, sperm lacking cP2, but not mP2, showed increased histone and TNP retention, as well as an altered protamine ratio (Arévalo et al., 2022). This domain thus serves an important role in the interaction between histones, transition proteins, and protamines. In humans, associations between an aberrant P1:P2 ratio and sub/infertility were also identified. It is, however, worth noting that the ratio in fertile normozoospermic men ranges from 0.54 to 1.43, suggesting that a strict 1:1 ratio is not required for proper spermatogenesis (Nanassy et al., 2011; Francis et al., 2014).

Somatic cell chromatin consists of nucleosomes associated with ~150 bp of DNA, interconnected through ~50 bp of linker DNA (Luger et al., 1997). At the onset of the histone-to-protamine transition in post-meiotic sperm, canonical histones are partially replaced with testis-specific histones such as H2A.L1/2, testis-specific histone H2B (TH2B), and H3T. A wave of histone hyperacetylation is then observed, which is thought to facilitate the introduction of transient double-stranded breaks by topoisomerase II as a means of opening the chromatin (Laberge and Boissonneault, 2005). Histones are subsequently displaced by transition nuclear proteins TNP1 and TNP2 and protamines P1 and P2, successively. Opposing this view, Barral et al (Barral et al., 2017). proposed a function of TNP1 and TNP2 as mediators of protamine association rather than supplanting histones. Protamines in this model are responsible for the displacement of histones from the DNA. A histone variant, H2A.L.2, is understood to play a crucial role in opening the nucleosomes and allowing for TNP loading (Barral et al., 2017). Following protamine-DNA interactions, DNA is looped into toroids and the hypercompaction of sperm chromatin occurs. An overview of histone-to-protamine transition is displayed in Fig. 1a.

2.2. Organization of sperm chromatin

The chromatin of mature sperm consists of the following components: protamine-bound DNA, histone-bound DNA, and matrix attachment regions (MARs) (Fig. 1b). As mentioned previously, human sperm have two protamines, P1 and P2, which constitute the majority of sperm nucleoproteins (85–95 %) (Hammoud et al., 2009; Gatewood et al., 1987; Tanphaichitr et al., 1978). The protamine-associated regions of sperm chromatin are looped into doughnut-like toroids containing approximately 50 kb DNA each (Ward, 2010; Hud et al., 1995). This protamine-rich conformation facilitates the compaction of sperm chromatin. DNA intervals of roughly 50 bp termed toroid linker regions interconnect the toroids. These nuclease-sensitive linkers are associated with the nuclear matrix at the MARs (Shaman et al., 2006). Around 5–15 % of sperm DNA remains histone-bound, in the form of the secondary chromatin solenoid structure or associated with the MARs. The histones retained in sperm chromatin include canonical histones as well as testis-specific histones and histone variants.

A hairpin-loop configuration has been suggested for the higher order organization of sperm chromatin. This model describes chromosome organization in discrete domains called chromosome territories, with their centromeres congregated in the nuclear center to form a chromocenter and their telomeres paired in the nuclear periphery (Mudrak et al., 2005; Zalenskaya and Zalensky, 2004). A more segmented model was recently hypothesized in which multiple chromocenters are formed by the centromeres of preferentially associated chromosomes (Ioannou et al., 2017). Intriguingly, the topologically associated domains (TADs) observed in human somatic cells (Baranello et al., 2014) and mouse sperm (Jung et al., 2017) are not found in human sperm (Chen et al., 2019). How chromatin is organized in ejaculated sperm is an interesting subject for further research, as it is postulated to affect transcriptional silencing and remodeling of the paternal epigenome, factors that are in turn relevant to development and fertility (Ioannou et al., 2017).

3. Epigenetic marks in sperm

The protamine proportion of the sperm genome has been widely studied and, because protamines render the chromatin tightly packed and transcriptionally inert, it was long thought that the spermatozoon was just a vessel for the delivery of the haploid paternal genome to the oocyte. Recently, more attention has been drawn towards the histone-enriched fragment of sperm chromatin and its epigenetic signatures, which are of great relevance in fertility, imprinting, and early development. Moreover, the protamine component of sperm chromatin was also found to bear post-translational modifications (Brunner et al., 2014). The contributors to the sperm epigenome thus include DNA modifications, histone and protamine modifications, and RNAs (Fig. 2). Each of these elements is separately discussed in the following sections of this review.

3.1. DNA methylation

A widely studied aspect of the sperm epigenome is DNA methylation.

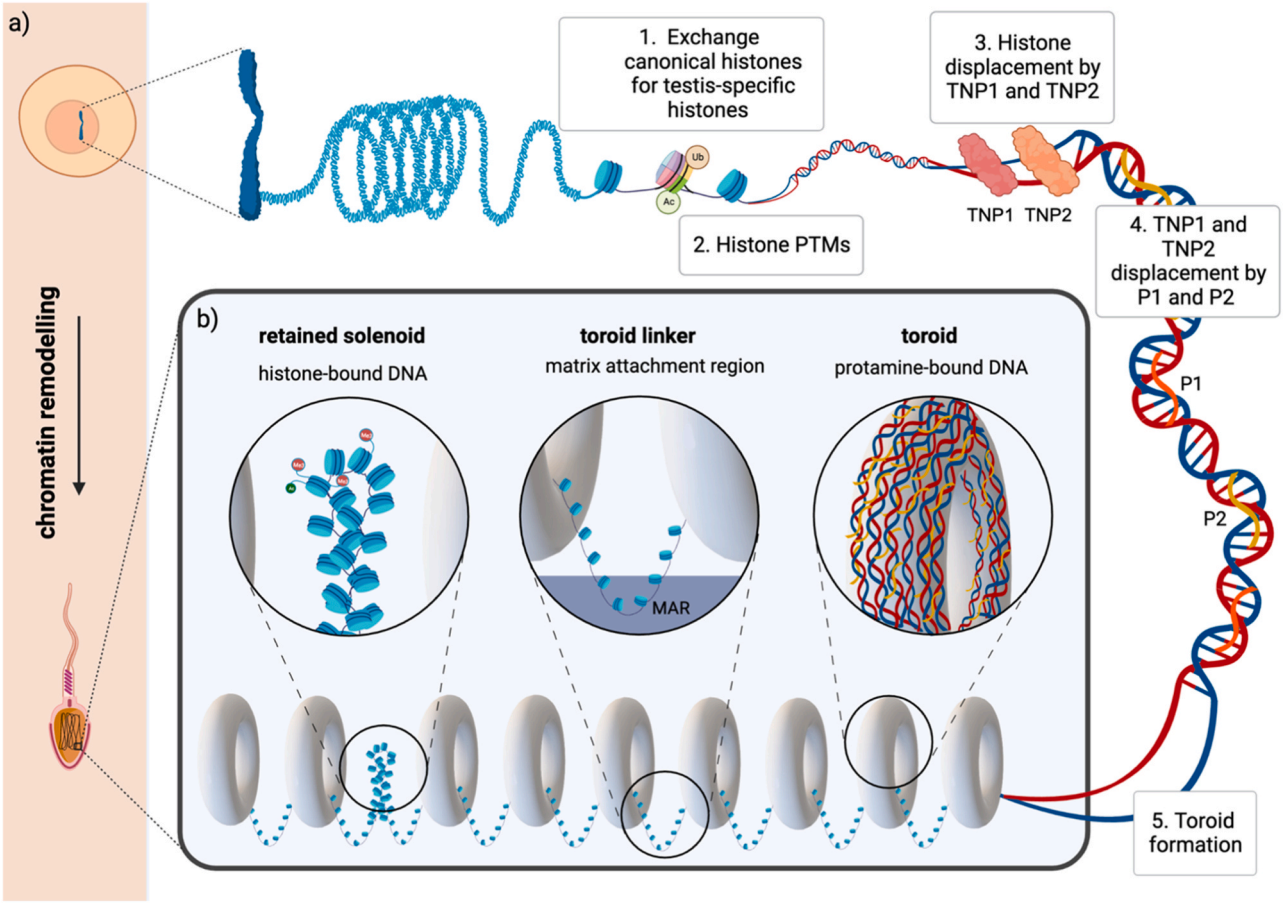

Fig. 1. Changes in the organization of sperm chromatin during spermiogenesis. **(a)** Chromatin remodeling. Some histones are replaced with testis-specific histone variants. Histones then undergo post-translational modifications such as ubiquitylation and acetylation. Transition proteins displace histones, and are in turn replaced by protamines. This allows for hypercompaction of the sperm genome. **(b)** The three components of sperm chromatin from left to right: Histone-bound DNA, MARs, and protamine-bound DNA (most abundant). Histone-bound DNA is present in retained solenoids and toroid linkers. Histones contain post-translational modifications, and DNA is methylated. MARs of toroid linker regions associate to the sperm nuclear matrix. Protamine-bound DNA is coiled into doughnut-like toroids. Abbreviations: MAR, Matrix attachment region; PTM, post-translational modification; P1, protamine 1; P2, protamine 2; TNP, transition nuclear protein. Figure created with BioRender.com.

In mammals, this concerns the methylation of the 5-position of cytosine residues (5mC) in CpG dinucleotides. DNA methylation represses gene expression through inhibition of transcription factor-binding or via association of methyl-CpG-binding proteins which induce chromatin remodeling through transcriptional co-repressor molecules (Klose and Bird, 2006). DNA methylation levels are high during spermatogenesis; however, one study described a transient global reduction in DNA methylation at the onset of meiosis (Gaysinskaya et al., 2018). An association was found between CpG content and methylation status of single-exon genes in mice. Higher and lower CpG content were linked to with hypo- and hypermethylation, respectively (Kato and Nozaki, 2012). Similar patterns that were highly conserved among normozoospermic individuals were observed in human sperm, showing hypomethylated CpGs corresponding to CpG islands or shores, and hypermethylation most prevalent in intergenic open sea regions (Åsenius et al., 2020; Krausz et al., 2012). Two protein families are important in the establishment of DNA methylation patterns: DNA methyltransferases and ten-eleven translocation enzymes.

3.1.1. *DNA methyltransferases (DNMTs)*

DNA methyltransferases (DNMTs) are catalysts of DNA methylation. DNMT1 methylates hemimethylated CG sites at the replication fork, a feature highly important for the maintenance of DNA methylation patterns during cell division (Song et al., 2012). DNMT3a and DNMT3b induce *de novo* methylation (Yagi et al., 2020). DNMT3L lacks the catalytic site for DNA methylation but is involved in the establishment of imprinting patterns through co-localization and interaction with DNMT3a and DNMT3b (Hata et al., 2002). Mouse studies showed that expression of *Dnmt3a* is required for spermatogenesis, and that of *Dnmt3l* is needed for the establishment of the DNA methylation pattern in the testis (Oakes et al., 2007; Kaneda et al., 2004). In humans, expression of *DNMTs* was found during all stages of spermatogenesis (Marques et al., 2011). Interestingly, higher levels of *DNMT3A* and *DNMTB3* transcripts were identified in sperm of oligoasthenoteratozoospermic (OAT) men compared to control subjects, suggesting a link between DNA methylation and (in)fertility (Rahiminia et al., 2021, 2018).

3.1.2. *Ten-eleven translocation enzymes (TETs)*

In 2009, another family of proteins, the one of ten-eleven translocation (TET) dioxygenases, was discovered and found to also contribute to the establishment of DNA methylation patterns. In 2016, successive expression of the three members of this family, TET1, TET2 and TET3, was reported during spermatogenesis at the mRNA as well as the protein level. An association of expression levels with sperm concentration and progressive motility was also observed (Ni et al., 2016). Two transcriptional single-cell atlases of the human testis confirm the expression of *TET1*, *TET2*, and *TET3* in human testicular supporting and immune cells (Guo et al., 2020; Mahyari et al., 2024). Data from theses atlases, however, are not in accordance with the previously mentioned report of successive *TET* expression in spermatogonia, spermatocytes

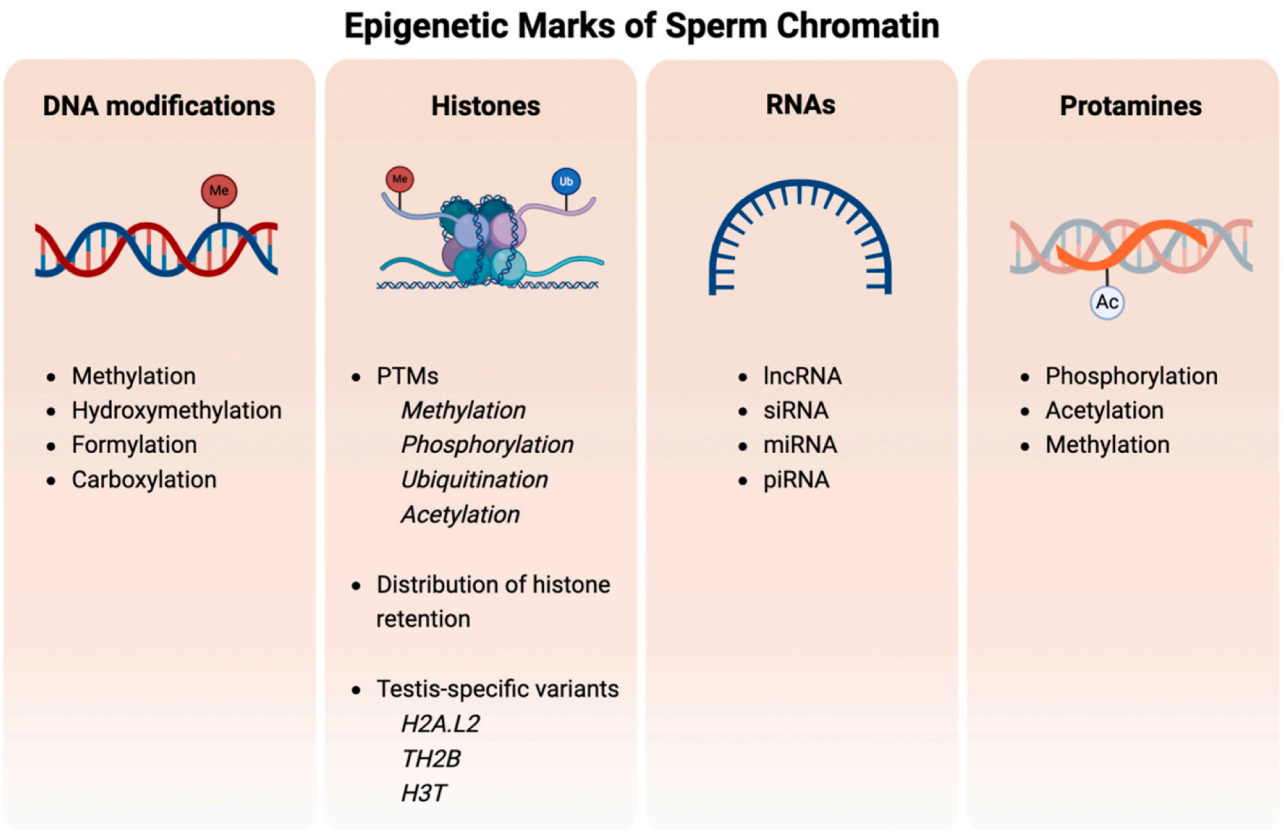

Fig. 2. . Factors shaping epigenomic footprints in sperm. The contributors to the sperm epigenome include modifications to DNA and histones, RNAs and protamines. Abbreviations: lncRNA, long noncoding RNA; miRNA, microRNA; piRNA, piwi-interacting RNA; PTMs, post-translational modifications; siRNA, small interfering RNA. Figure created with BioRender.com.

and spermatids. One of the atlases displays no *TET* expression in spermatogonia and their successors during spermatogenesis (Guo et al., 2020), while detectable expression levels of *TET3*, but not of *TET1* and *TET2* can be observed in the second atlas (Mahyari et al., 2024). The different types and low number of donors used for generating the respective atlases could underlie some of these inconsistencies in the literature. Infant, juvenile, and adult donors were used for the former atlas, whereas the second atlas included data from healthy and infertile donors. Further research into TET expression and its role in spermatogenesis and fertility may shed light into how DNA methylation affects sperm chromatin during different stages of development.

TET1, TET2, and TET3 oxidize 5mC to generate 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC), and 5-carboxylcytosine (5caC). One pathway of active DNA demethylation induced by TET dioxygenases is the excision of 5fC and 5caC by thymine DNA glycosylase, followed by base excision repair (Maiti and Drohat, 2011; Zhang et al., 2012). Additionally, demethylation can be achieved by TETs passively, or in a replication-dependent manner. DNMT1 has a lower catalytic activity in the presence of 5hmC, 5fC and 5caC compared to 5mC which leads to the dilution of 5mC marks generated by DNMT1 in consecutive cell cycles when TET enzymes are active (Seiler et al., 2018).

3.2. Histones

3.2.1. Histone retention

A second contributor to the sperm epigenome is the retention and modification of histones. Although the majority of histones is replaced by protamines in the process of chromatin remodeling during spermiogenesis, approximately 5–15 % of sperm DNA escapes the histone-to-protamine transition and remains histone-bound. The distribution of retained histones following the transition is non-random, and the histones which escape the transition are most prominently enriched at CpG-rich sites in genes related to embryonic development such as imprinted genes, miRNA, HOX-gene clusters, and transcription factors involved in pluripotency networks and early embryonic development, such as SOX2, FOXD3, HLX and MEIS1 (Wykes and Krawetz, 2003; Hammoud et al., 2009; Yamaguchi et al., 2018; Yoshida et al., 2018). Recent studies identified that retained histones H3 and H4 are primarily located at gene-poor or distal intergenic regions (DIGknopcs), as well as associated to transposable elements, including LINEs and SINEs (Yamaguchi et al., 2018; Carone et al., 2014; Samans et al., 2014). Conversely, another study did not identify H3 enrichment at DIGs, but rather at gene promoter regions, thus suggesting a role in transcriptional regulation following fertilization (Yoshida et al., 2018). Different methods employed for histone solubilization might underlie the discrepancy between the outcomes of different studies.

3.2.2. Histone post-translational modifications

Histone post-translational modifications, including methylation, acetylation, and ubiquitination constitute another aspect of the sperm epigenome. Activating di- and tri-methylation of histone H3 lysine 4 (H3K4me2/3) appears to occur in developmental loci and several non-coding RNA (ncRNA) (Hammoud et al., 2009). Reduced levels of H3K4me3 in sperm chromatin was observed to impair fertility and health in mouse offspring (Siklenka et al., 2015; Lismer et al., 2020). Indeed, neonatal survivability was reduced, pregnancy loss increased, and skeletal, skin, and limb abnormalities were observed in offspring of a transgenic mouse model overexpressing the histone H3 lysine 4 (H3K4) demethylase KDM1A. Importantly, transcription start sites of differentially expressed genes had reduced H3K4me3, indicating that transmission of altered gene expression patterns constitutes one mechanism through which histone methylation can transgenerationally

impact offspring health (Siklenka et al., 2015). H3K4me2/me3 was also found to promote histone H2A ubiquitination, a process that facilitates histone-to-protamine transition through intermediate protein binding, revealing an indirect means by which histone methylation can shape the sperm epigenome (Wang et al., 2019). Moreover, repressive tri-methylation of histone H3 lysine 27 (H3K27me3) is enriched in promoters of developmental genes repressed in early embryos. Previous studies observed that H3K4me3, but not H3K27me3, escapes the epigenetic reprogramming that erases paternal histone modifications during early embryonic development. Another widely studied histone modification is H3K9me3, proposed to contribute to paternal chromatin and X chromosome inactivation (van de Werken et al., 2014; Ernst et al., 2019). This mark constitutes another example of the interplay between DNA methylation and histone modifications, as H3K9me3-marked CpG-rich loci with high DNA methylation frequently escape the reprogramming and maintain their DNA methylation during early embryonic development. The knockout of H3K9me3 was reported to exhibit reduced DNA methylation in allele-specific imprinting control regions. Remarkably, the knockout of H3K9me3 imprinted regions affected embryonic development only when passed through the paternal germline. This underlines the fact that imprinting control regions carry out allele-specific regulatory functions, as exemplified by the allele-specific expression of one of the downstream targets of one of the imprinting control regions affected by the KO (Yang et al., 2022).

3.3. Protamine modifications

In addition to post-translational modifications in histones, which have now been studied quite extensively in sperm, several post-translational changes also occur in protamines (Brunner et al., 2014; Chira et al., 1993). These modifications include acetylation, phosphorylation and methylation. As noted by Brunner et al (Brunner et al., 2014)., acetylation and phosphorylation are two marks associated with increased gene expression when observed in histones. This potential function in the case of protamine modifications conflicts with the notion these proteins are responsible for sperm chromatin inertness through tight packaging. Although its implications and mechanisms of action remain unclear, the presence of a 'protamine code' analogous to the histone code of post-translational modifications is intriguing and should not be disregarded when considering the sperm chromatin and its effects on embryonic development and fertility. One proposed function for protamine modifications is the recruitment of maternal histones following fertilization (Brunner et al., 2014). Unlike the enrichment of histone post-translational modifications, which has been associated with specific loci, protamine modification enrichment remains to be interrogated. Much like the other components of the sperm chromatin epigenetic landscape, it is likely that the 'protamine code' does not function independently; its interaction with histone and DNA modifications and RNAs should be investigated to gain a better understanding of this literarily underrepresented aspect of the sperm epigenome.

3.4. RNAs

RNAs constitute the last modulator of sperm chromatin structure. RNAs can act in a regulatory manner by interacting with DNA and include housekeeping RNAs such as transfer RNA, ribosomal RNA and small nuclear RNA, as well as regulatory RNAs such as long noncoding RNA, piwi-interacting RNA (piRNA), small interfering RNA (siRNA), and microRNA (miRNA). RNAs fulfill different regulatory roles in the sperm epigenome. Several nuclear ncRNAs were observed to destabilize H2A-H2B dimers, affecting chromatin structure (Fujita et al., 2020). RNA was also shown to direct the pericentric nuclear localization and stabilization of H2A.L2, which induces preferentially localized histone retention (Hoghoughi et al., 2020). Endogenous siRNAs (endo-siRNA), predominantly derived from double-stranded RNA, were found to be abundantly present in mouse testicular tissue and their main target was DNA genomic regions (Song et al., 2011). Furthermore, mouse sperm with deficient miRNA and/or endo-siRNA were seen to give rise to embryos with reduced developmental potential (Yuan et al., 2015).

P-element induced wimpy testis [PIWI]-interacting RNAs (piRNAs) are a class of small ncRNA in the testis suppressing transposable elements. In humans and mice, piRNAs are the most abundant type of regulatory RNA in sperm (Pantano et al., 2015). piRNAs promote the maintenance of repressive histone modification H3K9me3 on LINEs in mouse germ but not somatic cells (Pezic et al., 2014).

miRNAs associate with the Argonaute subfamily of proteins and direct the translational repression or cleavage of their target mRNA (Kim et al., 2009). Several clusters of miRNAs are of importance in spermatogenesis. In mice, miR-34 and miR-29 clusters exhibit repressive function during meiotic prophase I in the male gonads (Hilz et al., 2017). miRNA expression during late spermatogenesis is predictive of H3K4me3 retention on CpG-islands in mature sperm (Pantano et al., 2015), which delineates the interplay between different forms of epigenetic marks. In humans, a correlation was detected between miR-371–1 expression and sperm count (Radtke et al., 2019), thus suggesting a role for miRNAs in clinical settings.

4. Early embryonic development

4.1. Paternal reprogramming

In the pronuclear stage following fertilization, maternal and paternal nuclei are present separately in the cytoplasm. A period of quiescence is required for the remodeling of chromatin, to allow for the induction of totipotency within the newly combined maternal and paternal genomes (Schulz and Harrison, 2019). The paternal chromatin is widely remodeled in a process referred to as paternal reprogramming, which includes the replacement of protamines with maternal histones stored in the oocyte followed by epigenetic modifications of the DNA and histones. Protamine replacement is thought to be regulated by nucleoplasmin 1–3 (NPM1–3), expressed by the oocyte (Okuwaki et al., 2012). Also, a role was suggested for SR protein-specific kinase 1 (SRPK1) in phosphorylating protamines to weaken protamine interactions and facilitate the protamine-to-histone transition (Gou et al., 2020). Recently, the substitution of a single lysine residue on P1 was found to result in the premature removal of P1 in zygotes (Moritz et al., 2021). Acetylation of this lysine residue, normally acquired in the testis, is disrupted and can therefore be speculated to play a role in early embryonic development. This highlights the importance of epigenetic marks in sperm for not only fertility, but also early embryonic development. The fate of paternally retained histones remains unclear, but the replacement of paternal histones including H3.3 with their maternal counterparts has been reported in mice; this is hypothesized to generate an environment that is permissive of zygotic genome activation (ZGA) due to the elimination of repressive histone marks present on the paternal histones (Kong et al., 2018).

4.2. Global demethylation

Upon the replacement of paternal protamines with maternal histones, active demethylation of the paternal but not of the maternal pronucleus occurs. In mice, this phenomenon takes place prior to the first mitotic division, thus indicating active paternal demethylation (Santos et al., 2002; Wang et al., 2014); human zygotes also possess demethylated paternal pronuclei (Beaujean et al., 2004a; Fulka et al., 2004). Animal studies revealed that a sperm-derived factor and/or components of the male pronuclear chromatin are involved in the demethylation of paternal DNA (Beaujean et al., 2004b). Although general demethylation of the male pronucleus happens, certain regions escape this demethylation, such as those involved in imprinting control and intracisternal-A-particles (Popp et al., 2010; Edwards and Ferguson-Smith, 2007). An overview of early embryonic

reprogramming is given in Fig. 3. In mice, maternal TET3 was found to localize preferentially to the paternal pronucleus, where it induces active DNA demethylation. TET3 or 5mC oxidation have also been shown to prevent transcription, thus suggesting a role for this enzyme in mediating the maternal-to-zygotic transition (MZT) following DNA replication (Guo et al., 2014; Shen et al., 2014; Tsukada et al., 2015). Sperm chromatin fragmentation mediated by topoisomerase II prior to fertilization induces the degradation of the mouse paternal pronuclear genome following the removal of protamines in the zygote, whereas the maternal pronucleus remains unaffected (Yamauchi et al., 2007a). This supports the notion that sperm, rather than just transporting the paternal genome, could modulate fertilization and early embryonic development *via* the properties of their chromatin. It was later found that this degradation of the paternal pronucleus was replication-dependent. The cytoplasmic signal that induces DNA replication in the two pronuclei also mediates the degradation of sperm with fragmented chromatin in mouse oocytes (Yamauchi et al., 2007b).

During the MZT, maternally supplied RNAs and proteins are degraded, and ZGA renders the zygote in control of its own transcriptional regulation. The ZGA, also referred to as embryonic genome activation (EGA), is believed to arise in multiple waves. In human embryos, a minor wave of transcriptional activation occurs at the 2–4-cell stage, and a major wave takes place around the 8-cell stage (Rebuzzini et al., 2021). In mice and zebrafish, increased histone acetylation was detected during ZGA and elevated levels of H3K4me3 preceded the increase of H3K27me3 around ZGA, thus generating a bivalent mark in the promotors of developmental genes which is hypothesized to prepare them for transcription (Vastenhouw et al., 2010). The presence of these signals in humans has yet to be elucidated.

The relevance of the sperm genome and its epigenetic signatures is clear, as not only does it affect fertility but also the phenotypic characteristics of progeny. The transmission of phenotypic traits linked to epigenetic patterns in sperm onto the offspring has been demonstrated in numerous studies. Sperm DNA methylation patterns of intracisternal A particles, transposable elements involved in epigenetic inheritance, were shown to mediate offspring phenotype as early as 1997 (Vasicek et al., 1997); since then, a significant body of research supporting the transgenerational transmission of environmental factors without alterations in the DNA sequence has been conducted (Siklenka et al., 2015; Radford et al., 2014). The importance of epigenetic inheritance from the male germline and its effects on fertility, embryonic development and offspring health was highlighted by these studies, and it is imperative that research continues to uncover the mechanisms by which sperm chromatin confers information into the next generations.

5. Sperm chromatin damage

Sperm chromatin is exceptionally vulnerable to damage, as

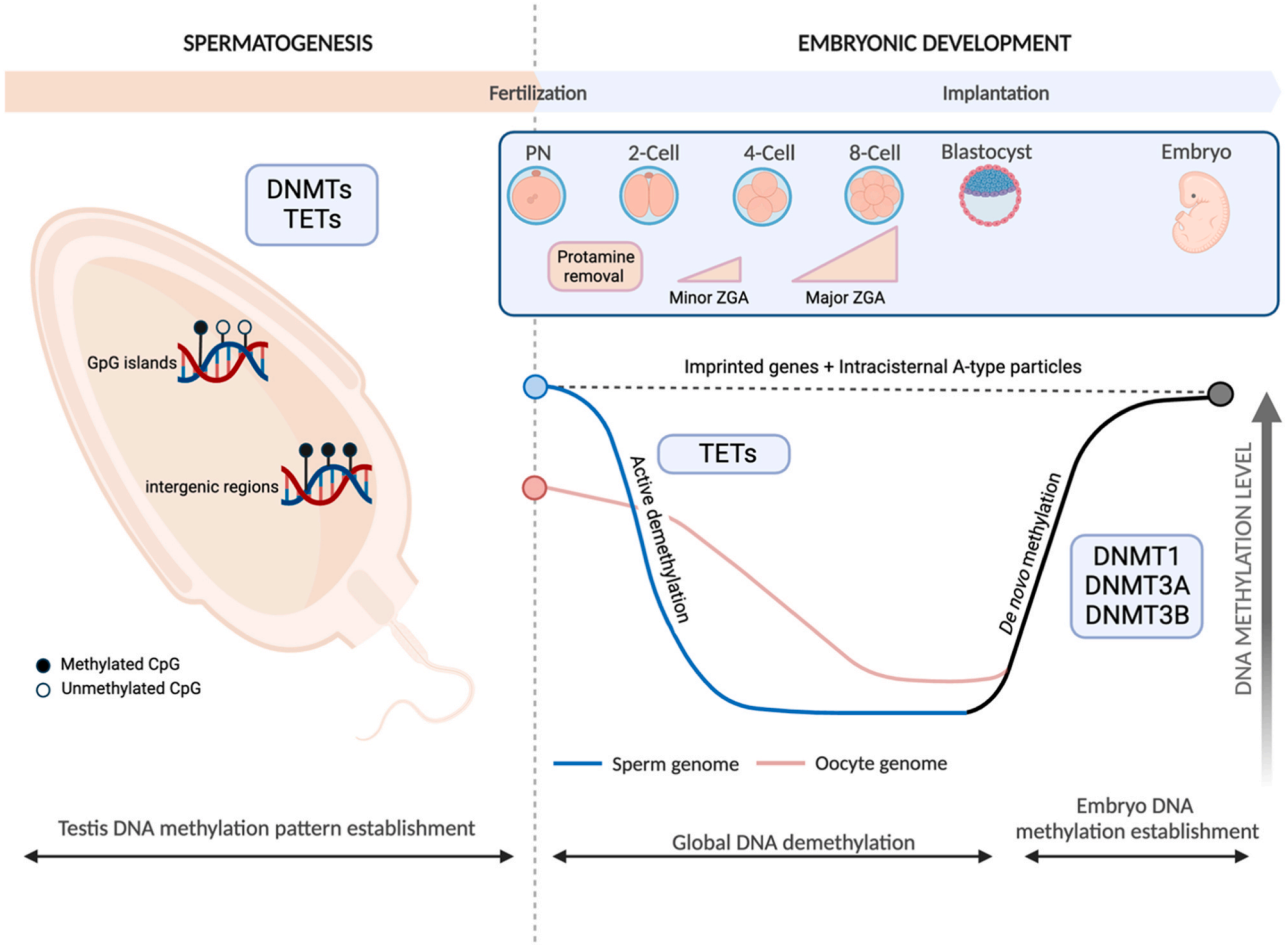

Fig. 3. Early embryonic reprogramming. During spermatogenesis, DNA methylation patterns are established by DNMTs and TETs. CpG islands are hypomethylated, whereas intergenic regions are hypermethylated. Following fertilization, protamine-to-histone exchange occurs and the paternal genome is actively demethylated by TETs prior to the first mitotic divisions. The maternal genome is more slowly demethylated. Several genes, including imprinted genes and intracisternal A-type particles escape demethylation. Minor and major ZGA waves take place at 2–4-cell and 8-cell stages, respectively. After blastocyst formation, *de novo* methylation by DNMTs establishes the DNA methylation pattern in embryos. Abbreviations: CpG, cytosine-guanine dinucleotide; DNMTs, DNA methyltransferases; TETs, ten-eleven translocation enzymes; ZGA, zygotic genome activation. Figure created with BioRender.com.

translational and transcriptional silencing occurs during spermiogenesis and this limits chromatin repair mechanisms available. Sperm are thus reliant on oocyte repair mechanisms following fertilization (González-Marín et al., 2012). There are three types of chromatin damage: DNA fragmentation, abnormal chromatin compaction, and chromosomal aberrations. The different causes of chromatin damage are outlined in the following subsections.

5.1. Meiotic recombination

Meiotic recombination during spermatogenesis is essential for genetic variation and appropriate chromosomal segregation. Meiotic arrest as well as chromosomally imbalanced gametes resulting in aneuploidy play a significant part in male infertility, and lower rates of meiotic recombination are observed in non-obstructive azoospermic compared to normozoospermic men (Gonsalves et al., 2004).

5.2. Defective protamination

The introduction of double-stranded nicks by topoisomerase II during histone-to-protamine transition renders the genome more susceptible to oxidative damage. Moreover, the incorrect repair of these nicks can result in DNA fragmentation associated with reduced fertility. The ratio between *PRM1* and *PRM2* transcript levels correlate negatively with progressive sperm motility, indicating the importance of correct protamination for fertility (Merges et al., 2022; Rahiminia et al., 2021).

5.3. Abortive apoptosis

During the process of spermatogenesis, the apoptotic capacity of male germ cells progressively declines due to translational and transcriptional silencing. This can result in the initiation, but not completion, of apoptosis, thus allowing maturation and the presence of defective sperm in the ejaculate. This process is known as abortive apoptosis (Shen, 2002).

5.4. Exposure to xenobiotics

Xenobiotics are substances found in an organism that are extrinsic to the regular metabolism of this organism. Xenobiotics are known to affect sperm chromatin and can be categorized into pharmacological, occupational, and environmental agents. The pathways of chromatin damage can overlap with the other causes outlined in this review. Examples include antibiotics (Tímermans et al., 2022), environmental pollutants (Hartman et al., 2021; Rubes et al., 2021; Kleshchev et al., 2021), and radiation (Leung et al., 2021; Hassanzadeh-Taheri et al., 2022).

5.5. Oxidative stress

Oxidative stress is defined as the stress caused by an imbalance between the oxidant activity of reactive oxygen species (ROS) and the antioxidant capacity of enzymatic and non-enzymatic antioxidants. ROS are highly reactive molecules due to the presence of one or more unpaired electrons. Although ROS are required for several physiological processes such as sperm capacitation, hyperactivation, the acrosome reaction, and fertilization, elevated ROS levels can infer oxidative damage which has been associated with DNA fragmentation and male infertility. Ample sources are known to induce elevated ROS in the male testis, both endogenous and exogenous, including tobacco (Laqqan and Yassin, 2021; Hammadeh et al., 2010), genitourinary microorganisms (Ho et al., 2022), sperm cryopreservation (Moradi et al., 2022), and varicocele (Mostafa et al., 2009). Interestingly, a correlation was reported between ROS in smokers and increased P1:P2 ratio which, as mentioned previously, is associated with progressive sperm motility. This points to an additional indirect effect of ROS on sperm chromatin (Hammadeh et al., 2010).

5.6. Nutrition and chromatin damage

Several of these mechanisms of chromatin damage have been linked to lifestyle and nutrition, a topic worth considering when discussing sperm chromatin and damage in the context of fertility and embryonic development. A previous systematic review and meta-analysis data revealed a relationship between BMI and conventional sperm parameters such as sperm count, concentration and motility (Guo et al., 2017). Yet, data on sperm chromatin damage were not included in this study. A study in sheep observed a greater DNA Fragmentation Index (DFI) in sperm of sheep that were fed a diet designed for losing body mass, compared to sperm of sheep ingesting a diet designed for the gain of body mass (Guan et al., 2014). Furthermore, a study in rats that employed 8-Hydroxy-2'-deoxyguanosine (8-OHdG) as a biomarker for oxidative DNA damage found increased 8-OHdG levels in sperm and testis of rats that were fed a high-fat diet compared to controls (Billah et al., 2022). In humans, paternal BMI was positively correlated with sperm chromatin damage, immaturity and oxidative stress (Bibi et al., 2022), outlining the importance of metabolic health and nutrition for sperm chromatin.

On the other hand, administration of chemotherapy drugs bleomycin, etoposide and cisplatin (PEBs) to rats leads to increased DNA fragmentation and histone retention rates. Interestingly, previous research reported that rats subject to PEB treatment followed by omega 3 supplementation displayed significantly lower DNA fragmentation and histone retention than those that did not receive omega 3, notwithstanding omega 3 intake did not reduce DNA fragmentation and histone retention rates compared to the control group (Razavi et al., 2021). Similarly, another study found the oxidative damage observed in sperm and testis of rats eating high-fat diets was reduced in both testis and sperm upon supplementing this high-fat-diet with folate, vitamin B6, choline, betaine, and zinc. Micronutrient supplementation compared to control diet also results in reduced 8-OHdG levels in testis but not sperm (Billah et al., 2022). Furthermore, a study by Dattilo et al (Dattilo et al., 2014). in men compared Sperm Chromatin Decondensation Index (SDI) and/or DFI of male partners of couples resistant to ART attempts before and after intake of daily supplements containing zinc, vitamin B, vitamin E and opuntia fig extract. The investigation demonstrated that addition of these supplements to the diet, without any other lifestyle or dietary changes, significantly reduced DFI and SDI; interestingly, the men with a partner suffering from female-factor infertility did not display reduced DFI or SDI (Dattilo et al., 2014). Another study also reported reduced DFI values in men who supplemented their diet with a capsule containing L-carnitine, L-arginine, zinc, vitamin E, glutathione, selenium, coenzyme Q10, and folic acid for three months, compared to men who did not supplement their diet (Lipovac et al., 2021). Notably, the largest effect was noticed in men with an initial DFI >15 %. This is in line with the data from the study by Dattilo et al (Dattilo et al., 2014). in which only female-factor cases showed no decreased DFI and SDI upon diet supplementation. It is also in line with the work performed in rats (Billah et al., 2022), which showed an effect of micronutrient supplementation on oxidative DNA damage in high-fat-diet cohorts, but not in control counterparts. Finally, reduced SDF and oxidation reduction potential (ORP) were observed in idiopathic infertile men following treatment with antioxidant supplement 'FH PRO for Men. The effects was most pronounced in participants positive for ORP and SDF prior to treatment (Arafa et al., 2020).

From all these findings, one can suggest that nutrition and metabolism may play an important role in shaping the sperm chromatin structure and preventing its damage. It is specifically the antioxidant properties of these micronutrients that are hypothesized to affect the sperm chromatin health by reducing oxidative stress. These data point out that the effect of nutrition on sperm chromatin - and subsequently fertility - may be most pronounced in cases initially displaying signs of aberrant chromatin or infertility, making it particularly interesting for future research with the aim of ameliorating male-factor infertility. In

spite of this possibility, it is worth mentioning that a randomized clinical trial in 2020 showed no effect of antioxidant supplementation on DNA fragmentation (Steiner et al., 2020). Another study reported that although prescription of vitamins C and E, β-carotene, zinc and selenium resulted in significantly reduced levels of DFI, it also resulted in an increase of sperm decondensation. The authors hypothesized that this effect could be due to the high redox potential of vitamin C, which could lead to the reduction of cysteine into two cysteines, and consequently the breaking of protamine disulfide bonds (Ménézo et al., 2007). Be that as it may, these observations prove the complex nature of sperm chromatin, and advise that caution should be taken when prescribing antioxidants for the purpose of reducing DNA damage, as other aspects involved in the protection of DNA, like chromatin condensation, may be affected.

6. Evaluation of sperm chromatin

Given the importance of sperm chromatin structure, it is valuable from both a scientific and a clinical viewpoint to have reliable methods for its evaluation. The methods employed for assessing the sperm methylation status include ChIP-seq and bisulfite sequencing. Chromatin integrity can be expressed as the DNA fragmentation index (DFI), a measurement that is frequently acquired by applying the sperm chromatin structure assay (SCSA), terminal transferase dUTP nick-end labeling (TUNEL assay), sperm chromatin dispersion (SCD) test, or the comet assay. Cytochemical assays including chromomycin A3 (CMA3), toluidine blue stain (TB), and aniline blue (AB)-stain sperm chromatin maturation assay (SCMA) can be used for the assessment of chromatin maturation index (Heidari et al., 2020; Dutta et al., 2021). The characteristics of these assays, which are outlined in Table 1, are described below.

6.1. Terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL) assay

The TUNEL assay labels single- and double-stranded breaks using fluorescein-isothiocyanate (FITC) deoxyuridine Triphosphate (dUTP). The terminal deoxynucleotidyl transferase (TdT), a DNA polymerase, attaches the FITC labeled dUTPs to the 3' hydroxyl ends of DNA nicks. The TUNEL assay is considered to be the gold standard for evaluating sperm DNA fragmentation (SDF), and the most widely used technique for analyzing SDF (Baskaran et al., 2019). This method, however, is time-consuming and labor-intensive, especially in the absence of a costly flow-cytometer.

6.2. Sperm chromatin structure assay (SCSA)

In the sperm chromatin structure assay (SCSA), sperm DNA is denatured using an acid detergent, and subsequently stained with purified acridine orange (AO). The metachromatic properties of AO allow for a shift to be observed from green (dsDNA) to red (denatured ssDNA) fluorescence, and for calculation of the DNA fragmentation index (DFI) (Evenson, 2013). This can be measured and calculated using a flow cytometer, permitting the analysis of thousands of sperm. The SCSA is highly standardized and a fixed protocol is utilized for staining. Software that is specifically designed for this test may be employed for the analysis of flow cytometric data. This makes the SCSA a reliable assay that allows for the comparison of its results in different populations and settings.

6.3. Sperm chromatin dispersion (SCD) assay

For the SCD assay, also known as the Halo test, sperm samples are treated with an acid and a lysis buffer in an agarose gel. When DNA is intact, the formation of halos can be observed around the sperm head as a result of DNA generating external loops. When DNA is fragmented, no

Table 1
Methods for evaluating sperm chromatin integrity. Abbreviations: CMA3, chromomycin A3; SCD, sperm chromatin dispersion; SCSA, sperm chromatin structure assay; TUNEL, terminal transferase dUTP nick-end labeling; TB, toluidine blue stain; AB, aniline blue; SSB, single-strand DNA breaks; DSB, double-strand DNA breaks.

Assay	Principle	Advantage	Disadvantage
TUNEL	Labelling of 3' free ends with a TdT transferase. Breaks are directly labelled	Highly standardized protocol Gold standard Detects both SSB and DSB	Need of flow cytometer for the analysis of a high number of sperm cells Sensitivity for the detection of DNA breaks in sperm cells No detection of MAR-region attached DSB Time consuming
SCSA	Acid denaturation followed by staining with Acridine Orange. DNA with breaks is more susceptible to denaturation	Standardized and fast protocol Differentiation of immature sperm cells (%HDS)	Need of a flow cytometer Need of a skilled technician No detection of MAR-region attached DSB
SCD	Acid denaturation, lysis of sperm membranes and extraction of protamines using detergent and salt. In the human version, non-fragmented sperm cells form a halo whereas fragmented sperm cells do not	Highly standardized protocol	Non-standardized analysis Number of analyzed sperm cells No detection of MAR-region attached DSB
Alkaline Comet	Lysis of sperm membranes and extraction of protamines, alkaline denaturation and electrophoresis at alkaline pH. DNA breaks migrate towards the cathode forming a DNA tail	Differentiation of mostly single strand DNA breaks after electrophoresis Allows quantification of DNA breaks with a specific software	Technique and analysis are not standardized between laboratories No detection of DSB in MAR regions Studies comparing different electrophoresis times are needed
Neutral Comet	Lysis of sperm membranes and extraction of protamines and electrophoresis at neutral pH. DNA breaks migrate towards the cathode forming a DNA tail	Differentiation of MAR-region specific DSB	Technique and analysis not standardized between laboratories
Two-tailed comet	Lysis of sperm membranes and extraction of protamines. First, neutral electrophoresis and, after alkaline denaturation and rotation of slide, alkaline electrophoresis. Sperm present two DNA tails	Detection of single and double strand DNA breaks in the same sperm cell	Technique not standardized Difficult interpretation Requires experienced observer
CMA3	Competitive binding of CMA3 to DNA. Indirect visualization of protamine-deficient DNA	Simple Cheap Fast Reliable	Interobserver variability Does not provide information about DNA fragmentation

(continued on next page)

Table 1 (*continued*)

Assay	Principle	Advantage	Disadvantage
AB	Metachromatic dye with affinity for lysine-rich histones	Simple Cheap Fast	Intra- and interobserver variability No standardized values Does not provide information about DNA fragmentation
TB	Metachromatic dye with affinity for DNA phosphate residues of loosely packed DNA	Simple Cheap Fast	Interobserver variability Does not provide information about DNA fragmentation

looping DNA is present and the halo is absent or small. The protocol is fast and cost-effective, especially when a bright field microscope is used for analysis (Fernández et al., 2018).

6.4. Comet assay

The Comet assay is a single-cell gel electrophoresis test. 'Comet'-like structures can be observed in agarose gel following the electrophoretic movement of DNA fragments of different sizes. Non-fragmented and intact DNA is present in the comet head, whereas DNA fragments migrate towards the opposite pole of the field and form the tail-like structure. The length of the tail provides the DNA fragmentation index (DFI) (Olive and Banáth, 2006).

Several versions of the comet assay are currently in use. Firstly, a distinction can be made between the alkaline and the neutral comet assay. The use of a pH-neutral electrophoresis buffer results in the unwinding of dsDNA loops when DNA is damaged, and this test can thus be used for the detection of dsDNA breaks. In the alkaline comet assay, both dsDNA and ssDNA breaks can be detected due to the exposure of alkali-labile sites on ssDNA strands. A third adaptation of the comet assay is the two-tailed comet assay. This technique requires electrophoresis at a neutral pH, followed by denaturation and electrophoresis at alkaline pH. The second part of this assay is performed after rotating the slide by 90°. Due to the turning of the slide, the 'comets' present two tails. This technique allows for the detection of ssDNA and dsDNA breaks in a single spermatozoon. Protocols for the performance and analysis of comet assays are not standardized which leads to the need for specialized personnel and increases the subjectivity of the test (Olive and Banáth, 2006).

6.5. Chromomycin A3 (CMA3) assay

This assay is used to assess the chromatin maturation index of sperm. Unlike the previously mentioned tests, the CMA3 assay is cytochemical in nature. This assay utilizes CMA3, an anthraquinone glycoside produced by *Streptomyces griseus* which binds to DNA in the presence of Mg^{2+}. CMA3 competes with protamines for protamine binding sites on the DNA, such that a CMA3-positive spermatozoon is protamine-deficient (Manicardi et al., 1995). The evaluation of this test, when not combined with flow cytometry, may be subjective, and the number of sperm that can be evaluated is small. While these factors represent major limitations, this assay is cheap, fast, and easy to execute.

6.6. Aniline blue (AB) staining

A second cytochemical assay involves aniline blue (AB) staining. AB is an acidic dye with a high affinity for the lysine-residues that are found in histones, and low affinity for the cystine/arginine-residues of protamines. AB can consequently be used to visualize sperm with inadequate levels of protamination, or low chromatin condensation (Terquem and Dadoune, 1983). Like the CMA3 assay, this method is simple, quick and non-expensive. The accuracy of sperm counting, particularly in oligozoospermic samples, remains a major obstacle when performing this assay (Sellami et al., 2013).

6.7. Toluidine blue (TB) staining

The last metachromatic dye commonly used for the assessment of sperm integrity is toluidine blue (TB). This dye is a basic thiazine molecule with high affinity for the phosphate residue of immature sperm DNA (Erenpreisa et al., 2003). As with the AB and CMA3 assays, TB staining is inexpensive, simple and fast.

7. Sperm chromatin and assisted reproductive technology

Sperm chromatin, by virtue of its nature, is inextricably linked to male fertility. Interestingly, routine semen analysis before conducting ART exclusively assesses sperm concentration, morphology and motility, whereas sperm chromatin often remains unevaluated. Moreover, the utility of sperm chromatin evaluation in predicting ART outcomes remains controversial. The final section of this review outlines the implications of sperm chromatin in the field of ART, with regard to imprinting disorders and the clinical value of chromatin evaluation.

7.1. Imprinting disorders in ART

While ART procedures, such as *in vitro* fertilization (IVF) and more recently intracytoplasmic sperm injection (ICSI), are considered safe and have been performed routinely since the birth of the first IVF-born baby Louise Brown in 1978, some studies have reported an increased risk of imprinting disorders in individuals conceived by ART when compared to naturally conceived individuals. Imprinting disorders are caused by genetic or epigenetic mutations that disturb the allele-specific expression of imprinted genes in zygotes. The effect of ART on the epigenome of the offspring is a controversial topic due to the wide range of ART patients and procedures applied in different regions of the world, thus resulting in inconclusive or contradictory findings when comparing studies globally. Several reports, nevertheless, have indicated an increased prevalence of certain syndromes, such as Beckwith-Wiedemann, Angelman, Silver Russel (SRS) and Prader-Willi in ART-conceived individuals compared to those conceived naturally (Uk et al., 2018; Cortessis et al., 2018; Mussa et al., 2017; Hiura et al., 2012). One study detected an increased odds ratio for Beckwith-Wiedemann Syndrome, but not Angelman Syndrome or SRS in individuals conceived by ART (Henningsen et al., 2020). Moreover, different methylation patterns were detected in ART-conceived SRS patients when compared to patients with SRS who were conceived naturally (Hattori et al., 2019). Culture medium, ovarian stimulation, *in vitro* fertilization, and even cryopreservation, have all been purported to impact the epigenome of gametes and embryos. Notably, some studies have suggested that factors inherent to couples struggling with in- or subfertility, rather than ART procedures themselves, might underlie these associations between epigenetic conditions and ART (Matsubara et al., 2016).

Although some argue that ART-derived imprinting disorders most likely originate from the oocyte (Owen and Segars, 2009), others report increased variation of DNA methylation in the paternal-specific methylation domains of ART-conceived SRS patients, thus suggesting that imprinting changes may occur after fertilization (Hattori et al., 2019). It is indeed likely that the effects of ART on the paternal epigenome would occur during post-fertilization epigenetic reprogramming since ejaculated sperm are genetically inert. Recent work in mice, however, indicated continuous post-translational modification during epididymal maturation, as demonstrated by differing modifications in sperm from the epididymal cauda when compared to those from the caput (Bedi et al., 2022). Interestingly, an increased first-trimester placental volume was found following testicular sperm-ICSI when

compared to ejaculate-ICSI pregnancies (Hoek et al., 2021). This posits that the putative epigenetic differences between testicular and ejaculated sperm, as observed in mice, may escape early epigenetic reprogramming. While there is no direct evidence of the involvement of imprinted genes here, these striking findings immediately bring to mind the 'tug-of-war' hypothesis that stipulates that paternally expressed imprinted genes promote the growth of extraembryonic tissues, whereas maternally expressed imprinted genes restrain this process to promote maternal survival (Moore, 1991). These data indicate that the role of sperm chromatin in the development of imprinting disorders remains elusive and should not be overlooked when assessing the effects of ART on the offspring epigenome.

7.2. Sperm chromatin and ART outcomes

Numerous studies have reported an association between conventional ART parameters, including sperm count, concentration, and motility, and sperm chromatin integrity measured as DNA fragmentation, DNA and histone methylation levels and chromatin condensation (Rahiminia et al., 2018; Antonouli et al., 2019; Sun et al., 2018; Hekim et al., 2021; Hologlu et al., 2022; Pourmasumi et al., 2019; Schon et al., 2019). It is, therefore, clear that sperm chromatin is linked to fertility and ART, at least theoretically. Yet, whether chromatin evaluation confers any clinical value and predictive power in ART remains a more controversial matter. A large body of literature reports an association between DNA fragmentation rates and reduced ART outcomes (Wang et al., 2022; Esbert et al., 2018; Zheng et al., 2018). Interestingly, some studies observed an effect in IVF but not ICSI procedures (Vončina et al., 2021), whereas others reported the opposite trend (Xue et al., 2016). Conversely, several works did not identify a significant effect of sperm chromatin on ART outcomes (Antonouli et al., 2019; Sun et al., 2018; Khalafalla et al., 2021). One study revealed that chromatin maturity, as measured by AB or CMA3 staining, did not affect ICSI success rates while TB staining was associated with reduced fertilization rates (Gill et al., 2018). This highlights the difficulty of interpreting chromatin assessment due to varying methods. Recent research found no effect of chromatin condensation defects on ART outcomes, but did detect an increased speed of embryonic development associated with chromatin condensation defects (Jumeau et al., 2022). The role of the paternal (epi)genome in directing early embryonic development suggested by these results should not be overlooked when employing ART technologies to treat in- or subfertility. Another factor that may affect the relationship between sperm parameters and clinical outcomes is the maternal contribution to the process. A previous study reported that the maternal age may affect the relationship between sperm DNA fragmentation (SDF) and ICSI success rates, thus suggesting a potential role for oocyte repair mechanisms (Setti et al., 2021). Moreover, a recent study comparing a healthy donor - donor (sperm-oocyte) cohort to a donor - patient (sperm-oocyte) cohort found that while DNA damage detrimentally affects ICSI fertilization rates, female factors may mask male infertility (Ribas-Maynou et al., 2022).

7.3. Chromatin evaluation in ART

The use of chromatin evaluation in ART would only be beneficial if it has the potential to increase success rates, or arguably to reduce the number and duration of cycles necessary to achieve a live birth. In the UK, no traffic-light rating from the Human Fertilization and Embryo Authority (HFEA) is currently assigned to the evaluation of chromatin integrity or SDF in ART; this is due to the lack of randomized control trials (RCTs) and a specific system for rating diagnostic tests (HFEA, 2022). The Society for Translational Medicine recommends the use of SDF testing in patients with varicocele or borderline normal semen parameters for the selection of candidates for varicocelectomy (Agarwal et al., 2017). When conventional sperm parameters are unfavorable, ICSI, the injection of immobilized sperm directly into the ooplasm, is frequently applied to improve the chances of fertilization. Despite ICSI having improved the clinical pregnancy rates of ART (Bhattacharya et al., 2013), critics have voiced their concerns regarding the vertical transmission of genetic defects underlying the ability of sperm to fertilize. A novel add-on, known as physiological ICSI (PICSI), is now available which selects sperm with the lowest SDF through hyaluronic acid binding. While several RCTs failed to find increased birth rates using PICSI when compared to ICSI (Miller et al., 2019; Majumdar and Majumdar, 2013), a recent RCT revealed that PICSI resulted in greater clinical pregnancy rates when compared to conventional ICSI for men with increased SDF (Hozyen et al., 2022). This study supports another use for the evaluation of sperm chromatin, namely for the assessment of the most appropriate sperm selection technique prior to ICSI.

8. Conclusions

With a growing body of literature delving into the sperm chromatin epigenome comes the opportunity and responsibility to investigate the relationship between sperm chromatin and embryonic development and fertility. Sperm chromatin and its epigenetic marks, including DNA and histone methylation, other histone and protamine modifications, and RNA activity play an important role in fertilization and embryonic development. Consequently, both sperm chromatin integrity and its epigenetic marks have implications in the field of ART and for natural conception. Several methods exist to evaluate both aspects of sperm chromatin, but these are not regularly employed for ART treatment. Standardization of chromatin evaluation, and consistent application in the clinic may facilitate the acquisition of useful data that is necessary to draw conclusions regarding sperm chromatin and its effects on fertility. Epigenetic marks in sperm chromatin are known to affect embryonic development, and it is conceivable that sperm chromatin might not significantly change the outcome of ART, although it does affect the embryo. Importantly, ART procedures such as ICSI, despite being effective at achieving pregnancy, may exert negative effects on the genetic and epigenetic health of offspring. Globally, an estimate of one million children conceived through ART are born each year, and ICSI is becoming increasingly common, now accounting for over 70 % of all treatments (European Society of Human Reproduction and Embryology, 2023). It is therefore essential that the relationship between sperm chromatin and the long-term effects of ART is explicated. Chromatin evaluation in future longitudinal studies could be valuable to this end. Moreover, the epigenetic marks in sperm found to affect embryogenesis in animal studies could be assessed in humans to further our understanding of early embryonic reprogramming and health in later life. This may reveal potential targets for treatment of infertility cases currently classified as idiopathic. The clinical use of chromatin evaluation in ART remains elusive, but previous studies suggest that SDF might underlie unexplained infertility in some couples, and that certain sperm selection procedures might be more effective in these couples than others. More RCTs are required to gain consensus on how and when to apply chromatin evaluation in the clinic.

Ethics approval and consent to participate

Not applicable, as this is a review article.

Authors contribution

P.B. wrote the Manuscript. M.Y. supervised the work and critically revised the Manuscript. C.J. and K.C. made a critical revision of the Manuscript. All authors approved the final version.

Consent for publication

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The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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