

REVIEW ARTICLE

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Epigenetic markers in the embryonal germ cell development and spermatogenesis

Amadeusz Odronec, Marta Olszewska* and Maciej Kurpisz

Abstract

Spermatogenesis is the process of generation of male reproductive cells from spermatogonial stem cells in the seminiferous epithelium of the testis. During spermatogenesis, key spermatogenic events such as stem cell self-renewal and commitment to meiosis, meiotic recombination, meiotic sex chromosome inactivation, followed by cellular and chromatin remodeling of elongating spermatids occur, leading to sperm cell production. All the mentioned events are at least partially controlled by the epigenetic modifications of DNA and histones. Additionally, during embryonal development in primordial germ cells, global epigenetic reprogramming of DNA occurs. In this review, we summarized the most important epigenetic modifications in the particular stages of germ cell development, in DNA and histone proteins, starting from primordial germ cells, during embryonal development, and ending with histone-to-protamine transition during spermiogenesis.

Keywords Sperm epigenetics, DNA methylation, Histone methylation, Histone acetylation, Spermatogenesis, Fertilization, Spermatozoa

Résumé

La spermatogenèse est le processus de génération de cellules reproductrices mâles à partir de cellules souches spermatogoniales, dans l'épithélium séminifère du testicule. Au cours de la spermatogenèse, des événements spermatogéniques clés tels que l'auto-renouvellement des cellules souches et l'engagement dans la méiose, la recombinaison méiotique, l'inactivation méiotique du chromosome sexuel, suivis d'un remodelage cellulaire et chromatique des spermatides allongées se produisent, conduisant à la production de spermatozoïdes. Tous les événements mentionnés sont au moins partiellement contrôlés par les modifications épigénétiques de l'ADN et des histones. De plus, au cours du développement embryonnaire, une reprogrammation épigénétique globale de l'ADN se produit dans les cellules germinales primordiales. Dans cette revue, nous avons résumé les modifications épigénétiques les plus importantes dans les étapes particulières du développement des cellules germinales, dans l'ADN et les protéines histones, en partant des cellules germinales primordiales, au cours du développement embryonnaire, jusqu'à la transition histone-protamine pendant la spermiogenèse.

Mots-clés Epigénétique des Spermatozoïdes, Méthylation de l'ADN, Méthylation des Histones, Acétylation des Histones, Spermatogenèse, Fécondation, Spermatozoïdes

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Introduction

Epigenetics can be described as heritable alterations that do not change the DNA sequence [1–5]. In comparison to genetic changes (which affect the structure of proteins mediated by DNA mutations), epigenetic changes affect the gene expression, and in consequence protein product amount within the cell. They are reversible and associated with the response to the environment in which cells reside [1–5]. Three groups of epigenetic changes are being manifested in mammalian cells: covalent modifications of DNA bases, histone posttranslational modifications and non-coding RNA (ncRNA) (Fig. 1).

One of the first discovered and the best-known epigenetic marks to date is DNA methylation [5–7]. 5-methyl-cytosine (5mC), together with the products of its degradation: 5-hydroxymethyl-cytosine (5hmC), 5-formyl-cytosine (5fC) and 5-carboxy-cytosine (5caC), are examples of covalent modifications of DNA bases [8]. DNA methylation preferably occurs at CpG sites that are dinucleotides with cytosine followed by guanine. DNA methylation can be present at CpG sites in the intergenic region, where methylation prevents the formation of DNA mutation by silencing of the retroviral elements, or in the promotor region of the gene within the so-called CpG island (region of DNA with higher CpG density), where methylation is responsible for the control of gene expression. Additionally, methylation in the CpG island plays an important role in the paternal and maternal gene imprinting [7, 8].

Another form of DNA methylation demonstrated in the mammalian genome is non-CpG methylation. Non-CpG

methylation is almost absent in adult somatic cells (0,02% of global 5mC), but the level of non-CpG methylation is elevated in embryonal stem cells, induced pluripotent stem cells, glial cells and neurons [9]. Unfortunately, the mechanisms and functions of non-CpG methylation are still not well-understood [9].

Histone posttranslational modifications are another group of epigenetic modifications. Histone modifications are being formed via the addition of a functional group to an amino acid, most commonly lysine, present within the histone tail of core histones [6]. The main role of histone modifications is the control of gene expression by chromatin condensation and decondensation [10]. Histone modifications can also provide a binding site for various proteins [11]. The already described histone modifications demonstrated in mammalian organisms are methylation, acetylation, phosphorylation, ubiquitination, SUMOylation, ADP ribosylation, short-chain lysine acylation [6, 12, 13].

The last group of epigenetic modifications is non-coding RNA (ncRNA). ncRNA is not translated into a protein and fulfills the regulatory role [14]. ncRNA can be divided into housekeeping RNAs and regulatory RNAs [15]. Housekeeping RNAs can be further divided into the following: ribosomal RNA (rRNA), transfer RNA (tRNA), small nuclear RNA (snRNA), and small nucleolar RNA (snoRNA). The housekeeping RNAs partake in the translation of mRNA into proteins and RNA processing [15]. Additionally, regulatory RNAs are involved in the regulation of gene expression toward gene silencing [14]. Regulatory RNAs can be divided into: small interfering RNA

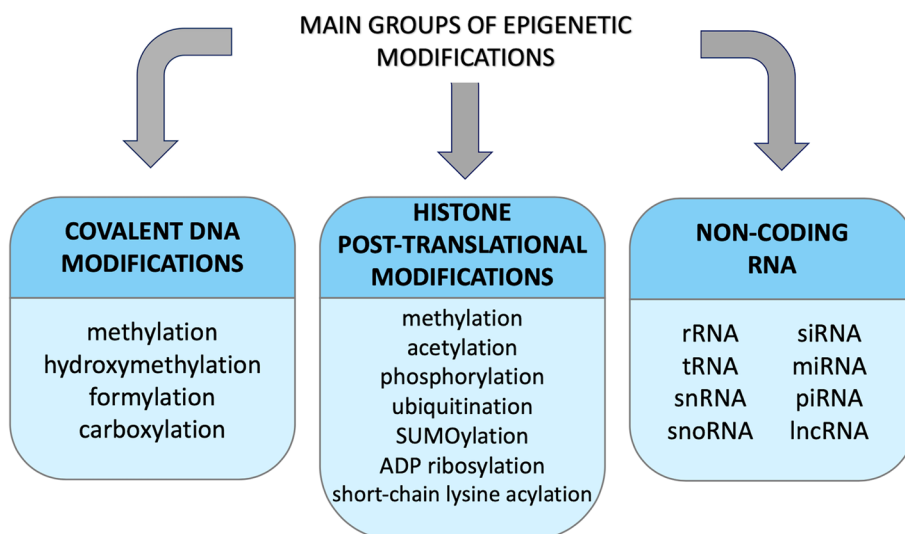


Fig. 1 Three main groups of epigenetic modifications observed in mammalian cells. rRNA – ribosomal RNA, tRNA – transfer RNA, siRNA – silencer RNA, miRNA – micro RNA, snRNA – small nuclear RNA, snoRNA – small nucleolar RNA, piRNA – piwi-interacting RNA, lncRNA – long non-coding RNA, ADP – adenosine diphosphate, SUMO – small ubiquitin-like modifier

(siRNAs), microRNA (miRNAs), Piwi-interacting RNA (piRNAs), and long non-coding RNAs (lncRNAs) [14].

It is already known that roles of parental genomes are distinct after fertilization. This sex-determined diversity is being established during gametogenesis and comes out of gametic imprinting, which is a distinct methylation patterning of parental alleles determining epigenetic mechanisms in the proper development of an organism [4, 16–19]. The maternal genome is responsible for embryonic development, while the paternal one is accountable for early placental progress [4, 16–19]. Additionally, disturbances in proper methylation and demethylation rounds in gametogenic cells, followed by disruption of methylation and/or acetylation of sperm histones may lead to a lack of activation of genes crucial for normal development, resulting in occurrence of certain developmental disturbances [4, 18, 20, 21]. It is also known, that implementation of immature spermatozoa (with misaligned methylation patterns and inadequate chromatin integrity) for fertilization in assisted reproductive technologies (ART) may increase the risk of reproductive failure or offspring health status [2, 4, 22–31]. The unique epigenetic marks in sperm cells may play a key role in poisoning of specific gene activation in the early embryo [2, 4, 21, 25, 32]. Disturbed synchronization of the embryo genome expression may be caused by hypomethylation which may switch the process of cellular differentiation [4, 19, 33]. In this light, a cognition of the mechanisms and sense of disturbances in gametic epigenome seems to be significant, due to the relatively high frequency of ART births today (approximately 1–3% of all live births) [25, 34].

It is also documented that male infertility may be linked with changed methylation pattern in human spermatozoa, both: at the level of sperm DNA (global or in particular genes – imprinted or nonimprinted; reviewed in [35, 36]), as well as in cases with disrupted methylation in particular histones' residues [37, 38]. Alterations in the methylation pattern were also described for males with decreased sperm chromatin integrity (disturbed protamines P1/P2 ratio), regarding to sperm apoptosis, in male ageing, in patients subjected to in vitro fertilization (IVF) procedures, in carriers of chromosomal aberrations, and in males with decreased semen quality [19, 23, 25, 39–44]. For example, in oligozoospermia where genetic causes are responsible only for 2.5–10% of observed infertility [45], the epigenetic evaluation revealed that in some patients from this group aberrant methylation patterns or imprinting errors may be causative for reduced efficiency of fertilization and elevated abortion rates [25, 33, 36, 46, 47].

Spermatogenesis is a complex process of male reproductive cells generation, that occurs within the

seminiferous epithelium of male testis [48]. Spermatogenesis can be divided into several stages: spermatogonial stem cells (SSCs) self-renewal (via mitosis), differentiation of SSCs into spermatocytes, reductional division of spermatocytes into spermatids (via meiosis), and morphological transformation of spermatids into sperm cells (via spermiogenesis supported by spermiation) [48, 49]. The entire spermatogenetic process in mammals ranges from 30 to 78 days [49, 50], approximately 40 days in mice [49, 51] and 74 days in humans [52, 53]. The process of spermatogenesis is followed by the maturation of released sperm cells within the lumen of epididymis [54]. During epididymal maturation, spermatozoa acquire the ability of *zona pellucida* recognition, the acrosome reaction and progressive motility, which are necessary for proper oocyte fertilization [54]. Each step of spermatogenesis is characterized and determined by particular epimarks, both: of germ cell DNA, as well as histone residues. Taking into account the growing amount of data concerning linkage between epigenetic disturbances and reproductive problems, in this review we have been focused on summarizing the role of main epimarks crucial for the process of spermatogenesis, such as DNA methylation, and the most important histone modifications. Additionally, in a Table 1 we have collected data concerning mouse knockout models of epigenetic-related enzymes with negative effect on spermatogenesis. Thus, this review emphasizes also the epigenetic significance for reproduction besides of the strictly genetic causes, and the high number of developmental stages at which epimarking may be disturbed leading to male fertility or fertilization problems.

Major epigenetic modifications of DNA

Methylation (5mC)

DNA methylation is a result of a transfer of a methyl group from S-adenosyl-L-methionine (SAM) to the fifth carbon atom of the cytosine residue [101]. This reaction is performed by a group of specialized enzymes called DNA methyltransferases (DNMTs). In mammals, three of them coordinate DNA methylation: DNMT1 (DNA methyltransferase 1), DNMT3a (DNA methyltransferase 3 alpha), and DNMT3b (DNA methyltransferase 3 beta) [102]. During spermatogenesis, two distinct types of methylation occur: de novo methylation, and so-called maintenance methylation [103]. The first of them is performed by DNMT3a and DNMT3b and allows the methylation of previously unmethylated DNA regions [103]. The latter uses the DNMT1 enzyme to sustain DNA methylation after meiotic division. DNMT1 has an affinity to hemi-methylated DNA in the replication fork [103]. DNMT3L (DNA methyltransferase 3 like) is the next methyltransferase present in mammals, but it lacks

Table 1 Knockout mouse models concerning epigenetic-related enzymes with negative effect on spermatogenesis (including gene IDs from: NCBI – National Center for Biotechnology Information [55] and MGI – Mouse Genome Informatics identifying number [56])

Gene	Full name	NCBI/MGI number	Epigenetic effect	Knockout phenotype	Reference
Embryonic development					
<i>Kdm6a (Utx)</i>	lysine (K)-specific demethylase 6A	22,289/1095419	Persistence of H3K27me3	Disturbed PGC embryonic development due to lack of PGC marker genes expression and aberrant chromatin dynamics	[57]
<i>Mthfr</i>	methylenetetrahydrofolate reductase	17,769/106639	Disturbed SAM synthesis	Reduced number of early germ cell due to failure of mitotic reactivation and enhanced apoptosis resulting in reduced fertility or infertility (depending on mouse model)	[58, 59]
<i>Nsd1</i>	nuclear receptor-binding SET-domain protein 1	18,193/1276545	Reduction of H3K36me2	Absence of spermatogonia in adult testis due to aberrant de novo DNA methylation in prospermatogonia	[60]
<i>Prmt7</i>	protein arginine N-methyltransferase 7	214,572/2384879	Loss of histone arginine monomethylation	Reduced number of PGC	[61]
<i>Prmt5</i>	protein arginine N-methyltransferase 5	27,374/1351645	Reduction of symmetric H4R3me2	Reduced number of PGC due to lack of transposon repression during the hypomethylated DNA state	[62]
<i>Setdb1</i>	SET domain, bifurcated 1	84,505/1934229	Reduction of H3K9me3 and H3K27me3 within endogenous retrotransposon regions	Decreased number of PGCs due to reactivation of endogenous retroviruses	[63]
Self-renewal and commitment to meiosis					
<i>Dnmt3a</i>	DNA methyltransferase 3A	13,435/1261827	Disturbed DNA methylation	Complete spermatogenesis arrest at the spermatogonia level	[64, 65]
<i>Fancd2/Fanci</i>	Fanconi anemia, complementation group D2 MGI:2,448,480 Fanconi anemia, complementation group I	211,651/2384790	Altered H3K9me3 and H3K4me2 in diplotene	Apoptosis of undifferentiated spermatogonia with unknown underlying mechanism, decreased number of mature sperm cells, meiotic progression not affected	[66]
<i>Kdm1a</i>	lysine (K)-specific demethylase 1A	99,982/1196256	Increase of H3K4me2	Complete spermatogenesis arrests upon exit from the stem cell state due to persistent expression of <i>OCT4</i> gene	[67, 68]
<i>Kmt2d (Mll2)</i>	lysine (K)-specific methyltransferase 2D	381,022/2682319	Loss of H3K4me3	Complete spermatogenesis arrests upon exit from the stem cell state due to lack of repression of <i>Magoh2</i> promoter	[69]
<i>Tet1</i>	tet methylcytosine dioxygenase 1	52,463/1098693	Decrease of 5hmC in spermatogonial cells	Premature reproductive aging (decreased SSCs, increased apoptosis, compromised imprinting)	[70]

Table 1 (continued)

Meiosis	Gene	Full name	NCBI/MGI number	Epigenetic effect	Knockout phenotype	Reference
	<i>Cxhc1 (Cfp1)</i>	CXXC finger 1	74,322/1921572	Reduction of H3K4me3	Complete meiotic arrest at the prophase I due to reduced expression of meiotic genes and reduced homologous recombination process	[71]
	<i>Dnmt3l</i>	DNA (cytosine-5)-methyltransferase 3-like	54,427/1859287	Disturbed DNA methylation	Complete meiotic arrest at pachytene checkpoint due to loss of chromatin compaction and unpaired chromosomes	[72]
	<i>Ehmt2 (G9a)</i>	euchromatic histone lysine N-methyltransferase 2	110,147/2148922	Reduction of H3K9me1/2	Meiotic arrest at early pachytene stage due to incomplete synaptonemal complex formation	[73]
	<i>Hdac3</i>	histone deacetylase 3	15,183/1343091	Persistence of H3K9ac and H3K27ac	Spermatogenesis arrest upon exit from meiosis due to upregulation of meiosis related genes and downregulation of spermiogenesis related genes in the epigenetically independent manner (deletion of catalytic domain does not result in infertility)	[74]
	<i>Hrb6 (Ube2b)</i>	ubiquitin-conjugating enzyme E2B	22,210/102944	Disturbed H2Aub	Increased apoptosis of primary spermatocytes due to synaptonemal complex aberration and increased crossing-over frequency	[75]
	<i>Kat8 (Mof)</i>	K(lysine) acetyltransferase 8	67,773/1915023	Loss of H4K16ac	Meiotic arrest due to impaired chromatin-wide expansion of γH2AX phosphorylation in leptotene and zygotene stage of meiosis	[76]
	<i>Kdm2b (Fbxl10)</i>	lysine (K)-specific demethylase 2B	30,841/1354737	Change in H3K4me3 distribution in testicular germ cells in the age-dependent manner	Gradual loss of fertility due to progressive inhibition of cell cycle	[77]
	<i>Paxip1 (Ptip)</i>	PAX interacting (with transcription-activation domain) protein 1	55,982/1890430	Reduction of H3K4me	Meiotic arrest at the zygotene stage	[78]
	<i>Prdm9</i>	PR domain containing 9	213,389/2384854	Reduction of H3K4me3 and H3K36me3 within recombination hotspots	Disturbed homologous recombination due to DSB formation impairment within recombination hotspots	[79]
	<i>Prmt1</i>	protein arginine N-methyltransferase 1	15,469/107846	Loss of asymmetric H4R3me2	Complete meiotic arrest at the zygotene-like stage due to an elevated number of DSB	[80]

Table 1 (continued)

Gene	Full name	NCBI/MGI number	Epigenetic effect	Knockout phenotype	Reference
<i>Rnf20</i>	ring finger protein 20	109,331/1925927	Reduction of H2Bub	Meiotic arrest at the pachytene stage due to impaired programmed double-strand break (DSB) repair	[81]
<i>Setdb1</i>	SET domain, bifurcated 1	84,505/1934229	Reduction of H3K9me3 within pericentromeric heterochromatin and X chromosome	Meiotic arrest at zygotene stage due to aberrant centromere clustering, bouquet formation, failure in pairing and synapsis of homologous chromosomes and MSCI failure	[82, 83]
<i>Suv39h1/2</i>	suppressor of variegation 3–9 1/2	20,937/1099440 64,707/1890396	Reduction of H3K9me	Meiotic arrest at mid- to late pachytene stage due to delayed chromosome synapsis or incomplete pairing	[84]
<i>Ubr2</i>	ubiquitin protein ligase E3 component n-recognin 2	224,826/1861099	Disturbed H2Aub	Increased apoptosis of primary spermatocytes due to synaptonemal complex aberration and increased crossing-over frequency	[85]
<i>Uhrf1</i>	ubiquitin-like, containing PHD and RING finger domains, 1	18,140/1338889	Loss of H3K9me3 at the retrotransposon region and global loss of 5mC	Meiotic arrest at pachytene stage due to reactivation of retrotransposons	[86]
<i>Camk4</i>	calcium/calmodulin-dependent protein kinase IV	12,326/88258	Loss of Protamine 2 phosphorylation	Spermiogenesis arrest in late elongating spermatids stage due to specific loss of protamine-2 and prolonged retention of transition protein 2	[87]
<i>Cdy1</i>	chromodomain protein, Y chromosome-like	12,593/1339956	Reduction in histone lysine crotonylation	Reduction of male fertility with a decreased epididymal sperm count and sperm cell motility due to aberrant reactivation of sex chromosome-linked genes in round spermatids	[88]
<i>Chd5</i>	chromodomain helicase DNA binding protein 5	269,610/3036258	Reduction of H4ac	Spermiogenesis arrest due to aberrant histone removal during histone-to-protamine replacement	[89]
<i>Epc1</i>	enhancer of polycomb homolog 1	13,831/1278322	Reduced H4 hyperacetylation	Spermiogenesis arrest at the transition from round spermatids to elongating spermatid due to aberrant histone to protamine transition	[90]

Spermiogenesis

Table 1 (continued)

Gene	Full name	NCBI/MGI number	Epigenetic effect	Knockout phenotype	Reference
<i>Jmjd1c</i>	jumonji domain containing 1C	108,829/1918614	Reduction of H4K16ac and HSP90 hypermethylation	Reduction of germ cells, abnormal histone-protamine remodeling and incomplete elongation of spermatids	[91]
<i>Kat5 (Tip60)</i>	K(lysine) acetyltransferase 5	81,601/1932051	Reduced H4 hyperacetylation	Spermiogenesis arrest at the transition from round spermatids to elongating spermatid due to aberrant histone to protamine transition	[90]
<i>Kdm3a (Jhmd2a/Jmjd1a)</i>	lysine (K)-specific demethylase 3A MGI:	104,263/98847	Persistence of H3K9me1/2 in promoter region of Tnp1 and Prm1 genes	Spermiogenesis arrest due to incomplete chromatin condensation, extensive apoptosis and blocked spermatid elongation caused by reduced cAMP-response element modulator (CREM) regulated gene expression	[92, 93]
<i>Parp1/2</i>	poly (ADP-ribose) polymerase family, member 1/2	11,545/1340806 11,546/1341112	Abnormal ADP ribosylation of DNA damage response proteins	Reduced fertility caused by abnormal sperm morphology due to abnormal histone retention	[94]
<i>Pygo2</i>	pygopus 2	68,911/1916161	Reduction of H3K9/14ac	Spermiogenesis failure due to reduced expression of Prm1, Prm2, Tnp2 and H1fnt	[95]
<i>Rnf8</i>	ring finger protein 8	58,230/1929069	Reduction of H2Aub and H2Bub	Spermiogenesis arrest at late spermatid stage due to aberrant histone removal during histone-to-protamine replacement	[96]
<i>Scml2</i>	Scm polycomb group protein like 2	107,815/1340042	Persistence of H2AK119ub on sex chromosome	Disturbed reactivation of escape genes located on sex chromosome during spermiogenesis	[97]
<i>Setd2</i>	SET domain containing 2	235,626/1918177	Loss of H3K36me3	Complete spermatogenesis arrests at round-spermatid stage due to acrosomal malformation and histone-to-protamine replacement malfunction	[98]
<i>Sirt1</i>	sirtuin 1	93,759/2135607	Reduction of H4 hyperacetylation	Reduced fertility due to defects in the histone to protamine transition	[99]
<i>Sly Dot1l</i>	Sycp3 like Y-linked DOT1-like, histone H3 methyltransferase	382,301/2687328 208,266/2143886	Reduction of H3K79me2 and H4 hyperacetylation	Impaired histone to protamine transition due to aberrant chromatin structure	[100]
<i>Tssk6</i>	testis-specific serine kinase 6	83,984/2148775	Loss of γH2AX phosphorylation	Spermiogenesis arrest due to retention of histone H3 and H4 during histone-to-protamine transition	[12]

a catalytic domain, and therefore has no catalytic activity [101]. DNMT3L recognizes the unmethylated region of histone H3 and activates DNMT3a and DNMT3b methyltransferases by acceleration of DNA and S-adenosyl-L-methionine (SAM) binding to methyltransferases [104]. Passive or active demethylation is further responsible for removal of DNA methylation. Passive demethylation is coupled with cell division and lack of methylation maintenance, while active demethylation is associated with the oxidation of methyl groups to hydroxymethyl group [105, 106].

The role of DNA methylation depends on its location within the DNA structure. In the intergenic region, bulk DNA methylation prevents the expression of potentially harmful genetic elements like incorporated retroviral genetic material [8]. Methylation within CpG island shores regulates tissue-specific gene expression, while methylation in CpG islands contributes to genomic imprinting by the stable silencing of one copy of the gene [8]. The level of DNA methylation remains roughly constant throughout the life of an individual [107]. The highest level of DNA methylation was detected in the brain and thymus, while the lowest levels were recorded in mature sperm cells and placenta [107], but the difference is negligible [108].

Hydroxymethylation (5hmC)

DNA hydroxymethylation is a well-described intermediate product of active demethylation. Ten-Eleven Translocation (TET) family proteins can oxidize the 5mC to 5-hydroxymethylcytosine (5hmC) [106, 109–112]. The same enzymes can further oxidize 5hmC to 5-formylcytosine and 5-carboxylcytosine, from which the carboxyl group is removed by thymine-DNA glycosylase (TDG) coupled with base excision repair (BER) mechanism to restore unmethylated cytosine [106, 109, 111, 112]. The level of DNA hydroxymethylation is tissue-specific and has been mainly demonstrated within regulatory elements of the gene [113, 114]. 5hmC level has been observed as the highest in brain tissue [108]. Also, in primordial germ cells (PGCs) the 5hmC level is observed at a high level [115]. In embryonal cells, 5hmC level originates out of genome-wide demethylation, which leads to epigenetic reset and the 5mC landscape restructuring during the development of specific tissues [113, 115]. Additionally, hydroxymethylation presence is associated with transcription of tissue-specific genes [113, 114], which can be especially important in constantly changing organs that must adapt to environmental signals (such as the brain), where the 5hmC level is the highest among the tissues. In sperm cells, 5hmC level is four times lower than that in somatic cells [116].

Major epigenetic modifications of histones

Methylation

Histone methylation is a modification resulting from the transfer of the methyl group from SAM to ϵ -amino group of lysine (K) or ω -guanidino group of arginine (R) residues, mostly on the N-terminal tail of histones H3 or H4 [117]. The lysine residue can be mono-, di- or trimethylated, while an arginine residue can be mono- or dimethylated [117]. Methylation does not change the charge potential of modified histone and can be associated either with activation or repression of gene expression [10]. Histone methyltransferases (HMTs) are the catalyzers for the methylation of histones [10]. HMTs that add a methyl group to the lysine residue are called lysine methyltransferases (HKMTs), while the methylation of arginine residue is performed by protein arginine methyltransferases (PRMTs) [118]. Most lysine methyltransferases contain the SET (Su(var)3–9, Enhancer-of-zeste and Trithorax) domain as their catalytic domain [119, 120]. However, the DOT1L (disruptor of telomeric silencing-1-like) is a unique lysine methyltransferase that lacks a SET domain and catalyzes the methylation of only lysine 79 residue of histone H3 (H3K79) in the histone core [120]. HMTs methylate their substrate to a defined level and specific changes in the catalytic site of the amino acid sequence can alter the level of methylation activity [121]. For example, the mutation F281Y in *Neurospora crassa* defective in methylation-5 (*Dim5*) histone H3 lysine 9 methyltransferase gene can change the enzyme activity from trimethylase to monomethylase [121]. A similar effect is observed for the equivalent mutation F1205Y in human *Dim5* homolog euchromatic histone-lysine N-methyltransferase 2 gene (*G9A*), where this mutation changes activity from demethylase to monomethylase [121]. Histone demethylation is performed by histone demethylases (HDMTs) [120]. Demethylation can also be performed using protein-arginine deiminase type-4 (PADI4) enzyme in the process of deamination of monomethylated arginine residue to citrulline without arginine regeneration [122].

For example, the abbreviated form of methylation description is H3R8me2 for dimethylation of arginine 8 residue in histone H3, and H3K36me3 for trimethylation of lysine 36 residue in histone H3.

Acetylation

Histone acetylation is a modification resulting from the transfer of the acetyl group from acetyl-CoA to ϵ -amino group of the lysine side chains in N-terminal tail of core histones (H2A, H2B, H3, H4) [117]. Acetylation neutralizes the positive charge potential from lysine residues, consequently weakening the interaction between DNA and histones. Those changes cause loosening of

chromatin and lead to transcriptional activity [123]. Additionally, histone acetylation regulates protein–protein interaction via bromodomains and in consequence takes part in histone deposition and DNA repair [123]. Acetylation is performed by histone acetyltransferases (HATs) [124]. HATs are classified as type A or type B transferases. Enzymes included in type A are localized in the cell nucleus and contain bromodomains, which allow them to bind and acetylate histones already embedded in chromatin structure [124]. However, type B acetyltransferases are located in the cytoplasm and can modify only newly synthesized histones. All type B enzymes mainly acetylate newly synthesized histones in the cytoplasm and are more conservative [124]. Deacetylation is performed using histone deacetylases (HDACs). They are less site-specific when compared to HATs, and commonly create large complexes with each other and additional proteins [125].

For example, the abbreviated form of acetylation description is H4K5ac for the lysine 5 residue in histone H4.

Phosphorylation

Histone phosphorylation is a modification resulting from the transfer of the phosphate group (PO_4) from ATP to the hydroxyl group of serine, threonine, tyrosine side chain, mainly in the N-terminal tail of histones [117]. Phosphorylation of these amino acids introduces an additional negative charge potential to the histone, which then changes chromatin structure [117]. A phosphate group presence increases the ability for DNA binding by transcriptional factors and enzymes. Next, the attached enzymes can add new post-transcriptional modifications (PTMs) or participate in double-strand breaks (DSBs) repair [126]. The phosphate group can be attached to the histone tails by kinases and detached by phosphatases [117].

For example, the abbreviated description of phosphorylation is H3S10ph for serine 10 residue in histone H3.

Ubiquitination

Histone ubiquitination is a process of an addition of a small 76 amino acid protein called ubiquitin mainly to the ϵ -amino group of lysine residue in a side chain via the covalent isopeptide bond [127]. Ubiquitination is catalyzed by a sequence of 3 enzymes: ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzyme (E2) and ubiquitin ligase (E3) [128]. Poly-ubiquitination is created by the extension of mono-ubiquitination at lysine residues: K6, K11, K27, K29, K33, K48, and K63 or methionine M1 [129]. The function of ubiquitination depends on the location within the histone tail, and whether a histone is mono- or poly-ubiquitinated. Mono-ubiquitination primarily controls gene expression probably via the changing chromatin structure or by providing an interaction surface for other protein complexes [130],

while poly-ubiquitination is involved in a wide range of processes, including protein to protein interaction (i.e., K63-linked ubiquitin chains in DSB repair response), or protein guidance towards degradation in proteasomes (i.e., K48-linked ubiquitin chains) [131]. Ubiquitination is removed by deubiquitinating enzymes (DUBs) [128].

For example, the abbreviated description of ubiquitination is H2BK120ub for lysine 120 residue in histone H2B.

Other histone modifications

Of the described modifications, the less common modifications can also be present on histone tails, i.e., SUMOylation (a modification by small ubiquitin-like modifiers), a group of short chain lysine acylations including: crotonylation, or butyrylation(s), (widely reviewed in [132]), or PARsylation poly(ADP-ribose) metabolism. However, there is only little literature data available concerning their role, so far [133, 134], thus we made only a brief description here. It was already shown that SUMOylation represents a marker of defective sperm quality (motility and morphology), is linked to chromatin remodeling and constitutive heterochromatin [135–139]. In *D. melanogaster* a general lysine crotonylation depends on the acetylation status of the spermatid chromatin, while in mice crotonylation was strictly linked to transcription regulation of sex chromosome-linked genes in round spermatids and genome-wide histone replacement in elongating spermatids [88, 140, 141]. Additionally, dysregulation of crotonylation by Cdy1 (chromodomain Y-like transcription corepressor) resulted in a lower sperm count and motility [88]. A role of other modifications from butyrylation subgroup, have been linked to active gene transcription in meiotic and post-meiotic cells in male germ cells, [142]. PARsylation has been found in elongating spermatids (mouse study) as an important player for proper sperm nucleoprotein exchange and correct sperm head formation [143, 144].

Epi-changes in primordial germ cells

Spermatogenesis is a complex process that starts from spermatogonial stem cells (SSCs) and ends with mature spermatozoa [50]. Primordial Germ Cells (PGCs), which are the more primitive ancestors of SSCs, are at least equally important for sperm production from an epigenetic standpoint [145]. At this stage, major DNA methylation modifications occur, which ensure appropriate epigenetic patterns in developing gonocytes [145, 146]. The pluripotency of PGCs and their open chromatin state give rise to SSCs by undergoing asymmetrical cell divisions [145, 146]. There are also suggestions (but still disputable), that PGCs have been reported to survive in low numbers in multiple adult tissues (including testes, both mice and human) as VSELs (very small embryonic like

stem cells), that are epiblast-derived cells deposited during early gastrulation playing an important role in turnover of tissue specific/committed stem cells, and expressing several markers characteristic for pluripotent stem cells [147–150]. The pluripotent VSELs express variety of PGCs markers, and their number is increased in testicular pathologies (i.e., cancer), revealing reduced 5mC expression and altered *Igf2-H19* (H19 imprinted maternally expressed transcript – insulin like growth factor 2) pattern and thus influencing the epigenetic profile [147–150].

Three stages can be listed during PGC development: specification, migration, and colonization [151]. They are timed, as follows: embryonic day E6.0-E8.0, E8.0-E10.0, and E10.0-E13.0 in mice, while in humans: 2.0–3.0 weeks, 5.5–8.0 weeks, and 8.5–9.0 weeks of embryonal development [146, 151]. PGCs undergo massive DNA demethylation, mainly in a replication-coupled manner. This change is associated with rapid cell cycle progress, the lack of a specific gene expression (i.e., developmental pluripotency-associated protein 3 gene – *STELLA*, ubiquitin like with PHD and ring finger domains 1 gene—*UHRF1*), and repression of DNMT3a, and DNMT3b activity [102]. This results in the absence of both maintenance and de novo methylation. Demethylation is essential for the erasure of genomic imprints, which could affect the particular stages of spermatogenesis [102]. The wave of demethylation in mice happens between E6.5-E10.5 and E10.5-E12.5 and

includes approximately 90% of genome-wide loss of 5mC [146], while in humans starts before 7 weeks of embryonal development, with global methylation decreasing from more than 80% to approximately 20% (Fig. 2) [145, 152]. In the 11-week human embryos, DNA methylation reaches its lowest level at approximately 8%. This status is maintained until at least until week 19th, which indicates that de novo methylation occurs later in embryo development [145]. More importantly, the differentially methylated regions (DMRs) of imprinted genes are completely demethylated from week 10th at least until the week 19th [145]. However, some regions in DNA retain relatively substantial methylation, namely the alpha satellite regions in the centromeric and pericentromeric regions of the chromosomes (36.5% of DNA methylation retained), and evolutionary young and active transposable elements with 24% of methylated DNA [145]. Also, hydroxymethylation of DNA was observed in PGCs in male 10-week embryos at a low level of approximately 2%. The presence of 5hmC indicates an auxiliary role of the active process in global DNA demethylation [145].

After PGCs colonize gonads, they undergo a sex-specific transformation toward gonocytes, and between the weeks 9–26 they gradually enter the mitotic arrest for the remaining phase of fetal development [153, 154]. Mitotic divisions resume 8–12 weeks after birth, and gonocytes transform into self-renewing spermatogonial stem cells

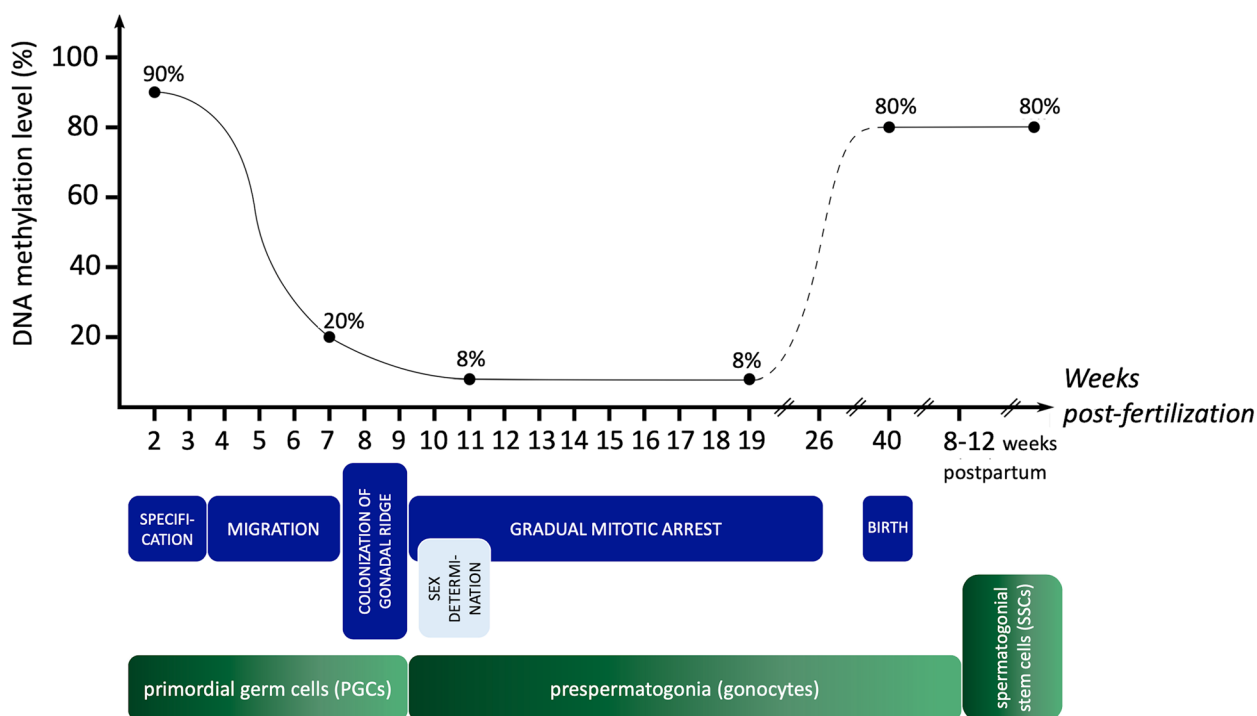


Fig. 2 Global DNA methylation changes during human embryonal development. Dashed line means the estimated (but still unknown) time points of the remethylation phase in arrested gonocytes. The full methylation pattern is established either until birth or before puberty, and is sustained to the end of spermatogenesis. Dots represent the most important steps of DNA methylation. PGCs – primordial germ cells, SSCs – spermatogonial stem cells

(SSCs) [155]. De novo methylation starts to emerge in mitotically arrested gonocytes [156]. The global methylation pattern is established before birth [157], while full methylation of paternal imprints (*H19-IGF2* and *DLK1-DIO3*: delta like non-canonical Notch ligand 1 – iodothyronine deiodinase 3) is fully established only before the onset of meiosis during puberty [156]. Simultaneously, maternally imprinted genes remain fully unmethylated

from the PGC stage onwards [156]. Also, the expression of DNA methyltransferases changes in time. All the three of them follow a similar expression pattern. In mice, high levels of DNMT1, DNMT3a, and DNMT3b are being observed in spermatogonium A, leptotene/zygotene spermatocytes, and round spermatids [101].

PGCs also exhibited dynamic changes in histone modification patterns (Fig. 3). Three main modifications occur

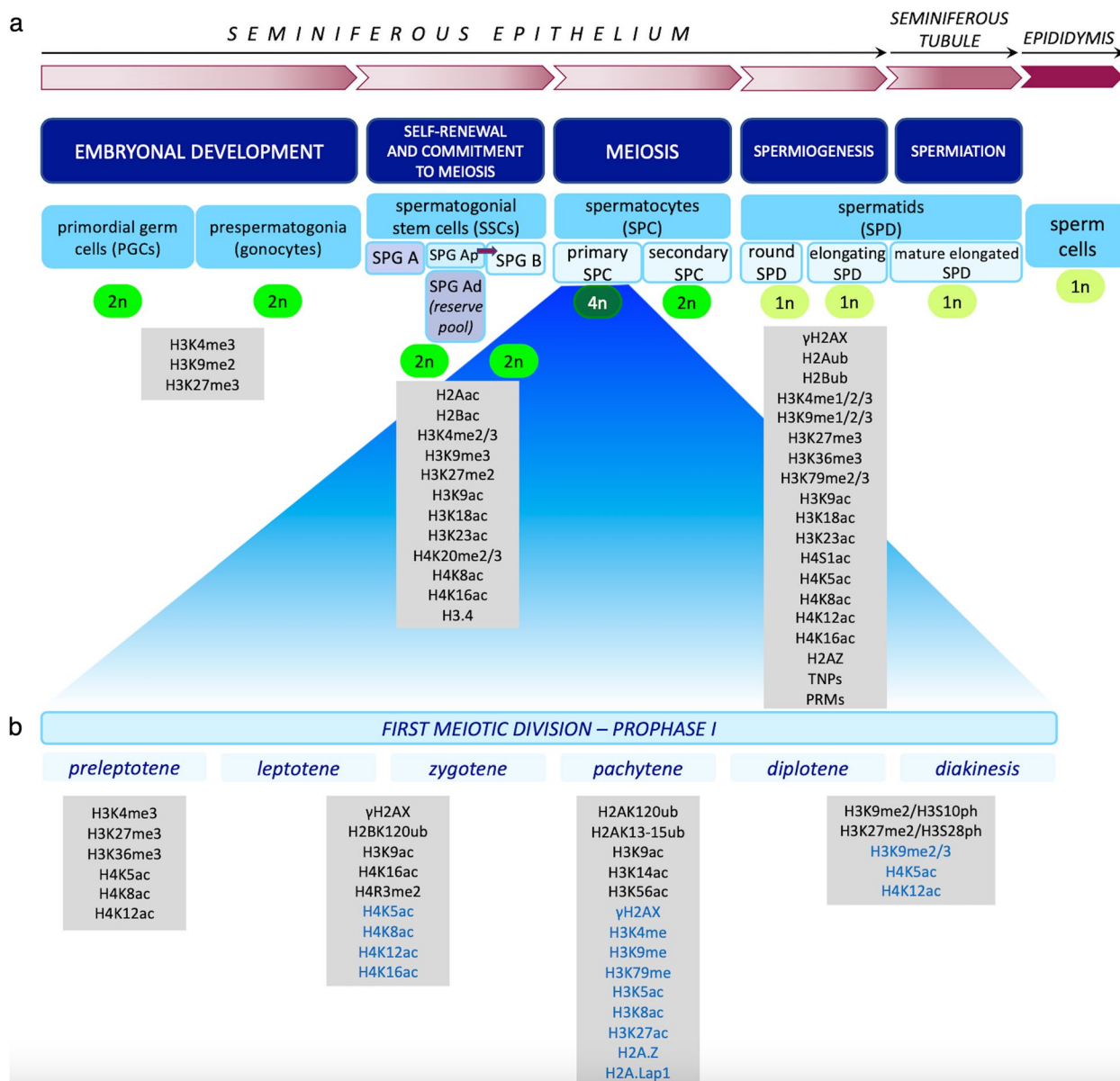


Fig. 3 The most important histone post-translational modifications at particular stages of human spermatogenesis. **a** The main histone post-translational modifications during embryonal development, mitotic proliferation, and spermiogenesis, together with indication of the cell type, their ploidy and morphology. **b** The main histone post-translational modifications during prophase I (the most important stage of first meiotic division in primary spermatocytes). Epigenetic modifications found on autosomal chromosomes are marked in black, while modifications representative for sex chromosomes are marked in blue. PGCs – primordial germ cells, SSCs – spermatogonial stem cells, SPC – spermatocyte, SPD – spermatid, ac – acetylation, me – methylation, ph – phosphorylation, ub – ubiquitination, 1n – haploid genome, 2n – diploid genome, 4n – tetraploid genome

at lysine 4, 9 and 27 residues (K7, K9, K27) of histone H3. Both H3K4me3 (activating) and H3K27me3 (repressive) are present in the promoter region of somatic genes (i.e., *Hox*: Homeobox proteins gene, *Gfap*: Glial fibrillary acidic protein gene) as a bivalent histone modification and they ensure gene repression in an absence of DNA methylation [37, 158]. However, in the promoter region of PGC-specific genes only activating H3K4me3 modification was observed, which corresponds to an active gene expression [158]. Additionally, bivalent marks are distributed differently in female and male PGCs, suggesting their role in establishing male and female-specific methylation patterns [159]. Furthermore, H3K4me3 is expected to be involved in the blocking of the de novo DNA methylation, as Dnmt3L binds only to unmethylated H3K4 [104, 160]. In PGCs the global reduction of H3K9me2 and increase in repressive H3K27me3 are being observed in mice at E8.5, while in humans after week 9 of gestation, and these changes are likely associated with the activation of PGC-specific genes, such as *Ddx4* (DEAD-box helicase 4), *Dazl* (deleted in azoospermia-like), and *Stra8* (stimulated by retinoic acid gene 8) [161–163]. Simultaneously, H3K9 acetylation increases, while H3K9 trimethylation remains at a high level [162].

Self-Renewal of spermatogonia and commitment to meiosis

Spermatogenesis starts with spermatogonial stem cells (SSCs), also known as ‘spermatogonia A type’ (SPG A), ready for replication via mitosis [102]. This process enables the cells to multiply continuously and maintain a constant number of stem cell reservoir within the gonad. Two types of SPG A in human testes can be found: SPG A dark (SPG Ad), and SPG A pale (SPG Ap). The first one is associated with the self-renewal of SSCs and constitutes a reserve pool of gonadal stem cells [164]. Pale spermatogonia A can also be subjected to mitotic self-renewal divisions, but in addition, they can differentiate into spermatogonia B (SPG B) [164]. In the next step, SPG B transform into primary spermatocytes, which enter the first meiotic division. Those transitions are controlled by the activation and repression of specific genes by post-translational modifications of histone tails [117]. Acetylation of histone H4 (H4K8, H4K16) prefers the neighbourhood of transcription start sites (TSSs) in SSCs, but it controls only constitutively active genes [165]. Also, the acetylation of histone H3 (i.e., H3K9, H3K18, H3K23), and variants: H2A, and H2B is present in both SPG A and SPG B cells [166]. Those modifications might be necessary during DNA replication [117]. Histone methylation fulfils the critical role in maintaining the balance between self-renewal of spermatogonia and commitment to meiosis. Commonly occurring histone methylation sites are

H3K27, H4K20 in their dimethylated states, and H3K9, H4K20 in trimethylated states [167]. Those PTMs interact with promyelocytic leukaemia zinc finger transcriptional repressor (PLZF), which is essential in the maintenance of the stem cell pool [167]. But the most important modification responsible for the stem cell persistence in a pluripotent state is the methylation of lysine 4 residue in histone H3 (H3K4me2/me3) [37, 38, 67]. This modification can exist in constitutive heterochromatin (in the dimethylated state) or facultative heterochromatin (in the trimethylated state). It is present at the promoter and enhancer regions of genes associated with pluripotency (i.e., octamer-binding transcription factor 4—*OCT4*, Nanog homeobox – *NANOG*) and activates their transcription [38, 67]. H3K9me works oppositely to H3K4me and is added by histone lysine methyltransferase G9a in a promoter region of the same genes. However, it acts through the blocking gene transcription and – in consequence – promoting cell differentiation [73]. Demethylation of H3K9me restores self-renewal phenotype in SSCs [117]. After the commitment to meiosis, SSCs undergo numerous changes in histone profile, among which the most prominent ones are modifications in H3 and H4 methylation profiles, and incorporation of a new variant of histone H3 called H3.4 in human, and H3t in mice [168–170]. The changes in methylation pattern mainly occur in the following: H3K4, H3K9, H3K27, and H4K20. In early spermatogonia, repressive methylations like H3K9 and H3K27 occur. Their role is to impede genes’ action associated with pluripotency, such as *NANOG* (Nanog homeobox), *SOX2* (SRY-box transcription factor 2), *LEFTY* (left–right determination factor), and *PRMD14* (PR/SET domain 14) [117]. In late spermatogonia, transcriptionally activating modifications as H3K4me strengthen, and are probably linked with an increase in the expression of genes necessary at the early stages of meiotic division [118]. The histone variant H3t in mice (H3.4 in human) replaces canonical H3 in SPGs B and early spermatocytes. H3t is probably introduced during mitotic divisions throughout the entire genome, except for sex chromosomes [168, 169]. The incorporation of H3t into chromatin structure causes its decondensation due to more flexible entry-exit regions of H3t compared to canonical H3 histone [168, 169]. The absence of histone H3t in mice results in a suspension of SPG differentiation into spermatocytes [168, 169]. An interesting – but still scantily described – is variant H3.5 observed in testes of patients with normal spermatogenesis in spermatogonia or preleptotene/leptotene-stage primary spermatocytes, where accumulated around TSS sites may play a role in the chromatin loosening [171, 172]. The general representation of histone variants during particular stages of spermatogenesis has been summarized in Fig. 4 and detailed reviews [169, 170].

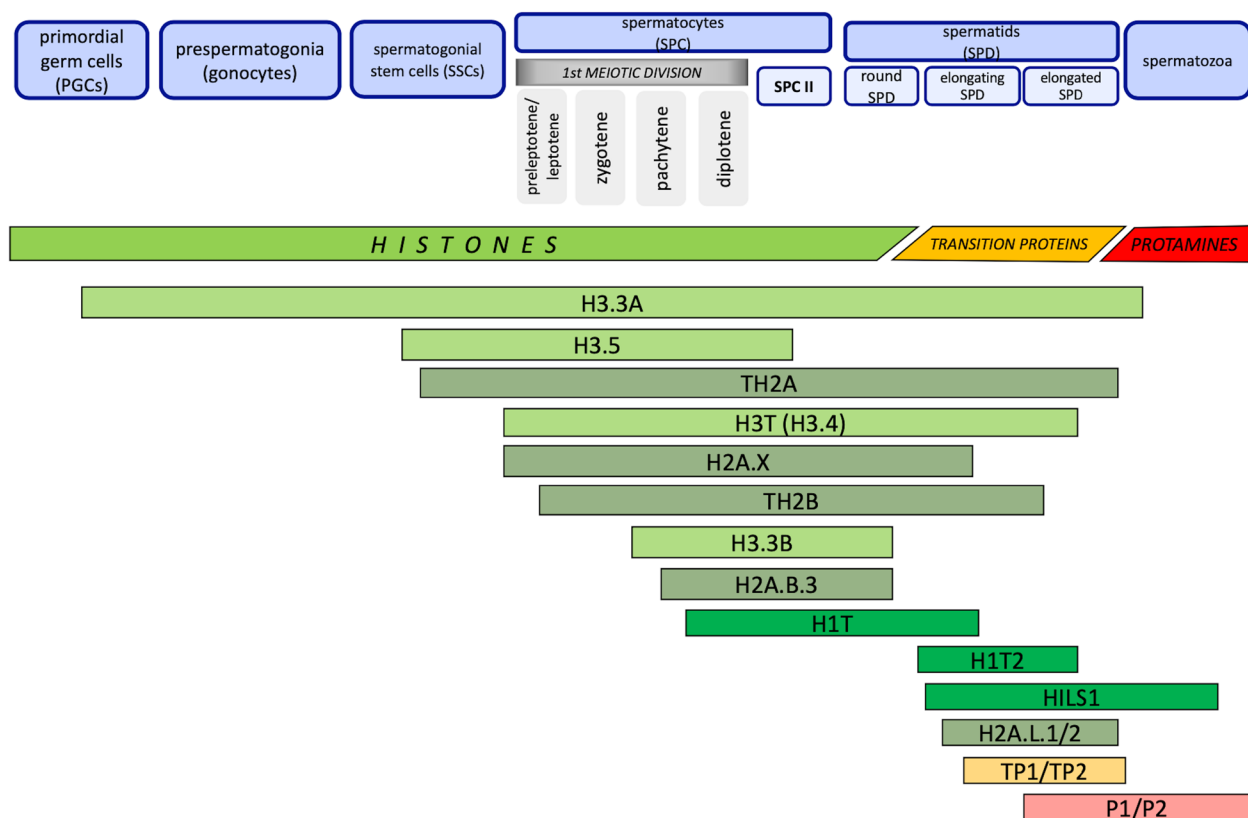


Fig. 4 Main variants of the histones during the spermatogenic process (human and mice)

Meiosis

The next step of spermatogenesis is meiosis – a complex process with the main goal to reduce cell ploidy from 2n (diploid) to 1n (haploid) [173]. One tetraploid (4n) primary spermatocyte enters meiosis, and after two meiotic divisions results in four haploid spermatids [174]. Spermatogenesis consists of two consecutive divisions with the intermediate product in the form of diploid secondary spermatocytes [174]. During spermatogenesis, the most important point is the first phase of meiosis I called prophase I. Dividing cells spend overwhelming majority of meiosis time in prophase I, the phase full of actions crucial for proper cell division [173, 175]. The first one is meiotic recombination, known as the crossing-over [176]. This process is critical for the maintenance of genetic diversity in the offspring. Lack of crossing-over events leads to disturbances in maintenance of proper ploidy in gametes, and then to infertility problems. In some regions of DNA, so-called: ‘hot spots’, recombination occurs more often [176]. The other important process is meiotic sex chromosome inactivation (MSCI). The absence of MSCI induces meiotic arrest at the pachytene stage of prophase I due to numerous unrepaired DNA double-strand breaks (DSB) [177]. The third one crucial

for prophase I is the synapsis of homologous chromosomes, which is necessary for later phases of meiosis I. It also allows mechanisms mentioned above to be carried out correctly [176, 177]. Also during prophase I, great changes in the expression of meiotically associated genes are being observed, such as: *TEX19* (testis expressed 19), *PRDM9* (PR/SET domain 9), *SYCP3* (synaptonemal complex protein 3), and *BRDT* (bromodomain testis associated) [126, 178]. All those events are completely or partially controlled by histone epigenetic modifications. Prophase I can be divided into 5 major stages: leptotene, zygotene, pachytene, diplotene and diakinesis.

Preleptotene

During the preleptotene stage of prophase I, preparation for meiotic recombination of primary spermatocyte begins. In this stage of prophase I, chromosomes are loosely packed and appear singly [179]. To keep the chromatin stable in a loose state, mostly the histone modifications with decondensing type of influence are present, mainly acetylation of histone H4 lysine residues 5, 8, and 12 (H4K5ac, H4K8ac, H4K12ac) [180]. In both: human and mice, the main role of H4 acetylation is to unlock access to recombination sites, creating recombination

hot spots by DNA-binding zinc-finger protein (PRDM9) and trimethylation of histone H3 on lysine 4 residue (H3K4me3) [181, 182]. This modification is the most important mark of recombination hot spots, and it is used for further loosening of the chromatin structure [182]. In mice, Prdm9 can also trimethylate lysine 36 residue (H3K36me3), and then leads to the creation of the bivalent signature exclusively in the region of recombination [182]. Repressive histone marks are mostly absent in the recombination hot spots, in the exception of the coincidence of H3K27me3 and H3K4me3 in bivalent recombination sites [183].

Leptotene and Zygotene

At the leptotene stage, the first step of homologous recombination (HR) occurs. In both: human and mice, topoisomerase Spo11 induces double-strand breaks (DSBs) within hot spots of relaxed DNA chromatin [176]. Two copies of the Spo11 enzyme create an asymmetric DNA break, while each copy cuts one strand of DNA [176, 184]. After the appearance of DSB, the most important modification for this stage – the phosphorylation in serine 139 residue of the histone variant H2AX is added (also known as γ H2AX, expressed especially at preleptotene, but weakly also in spermatogonial cells, primary pachytene spermatocytes and in elongated spermatids) [126, 185]. Three waves of phosphorylation of the γ H2AX have been observed during prophase I, two of which are related to HR [76]. The first wave occurs during leptotene and is performed by ATM serine/threonine kinase, while the second one depends on ATR serine/threonine-protein kinase and is present from the early to mid-zygotene stage [76]. Ubiquitination of lysine 120 residue on histone H2B (H2BK120ub) by ring finger protein 20 (RNF20) is another active modification during the zygotene [186]. Both γ H2AX and H2BK120ub are responsible for loosening of chromatin structure and recruitment of repair proteins like: the mediator of DNA damage checkpoint protein 1 (MDC1), Mre11, Rad50, and Nbs1 (MRN) complex, and breast cancer type 1 susceptibility protein (BRCA1) and this process is similar in human and mouse [117, 186]. For correct phosphorylation and ubiquitination, proper methylation and acetylation of histone H4 must be established. Especially, the dimethylation of arginine 3 residue in histone H4 (H4R3me2) performed by protein arginine methyltransferase 5 (Prmt5) [187], and acetylation of lysine 16 residue in histone H4 (H4K16ac) by MOF (male absent on the first) histone acetyltransferase [76]. The absence of Prmt5 and MOF results in a lack of proper epigenetic marking, leading to inappropriate γ H2AX phosphorylation as a consequence [76, 187].

During the leptotene and zygotene stages of the prophase, the acetylation of lysine 9 residue in histone H3

(H3K9ac), and lysine 5, 8, 12, 16 residues in histone H4 (H4K5ac, H4K8ac, H4K12ac, H4K16ac) occurs in X and Y chromosomes [188]. Those modifications resemble transcriptionally active chromatin and might be associated with the activation of genes located within sex chromosomes [188]. Correspondent modifications, namely H4K5ac, H4K8ac, and H4K12ac, can also be found in autosomal chromosomes where they play a similar role [180].

Pachytene

During the pachytene stage of prophase, DSBs induced in leptotene are repaired using Holliday junctions (HJs) or synthesis-dependent strand annealing (SDSA) mechanisms [176]. However, only an HJ pathway results in crossing-over [176]. At the pachytene stage, chromatin decondensation in hot spots is promoted. A loosening of DNA structure allows the enzymes to bind properly to DSB sites. Acetylation of H3K9, H3K14, and H3K56 is responsible for relaxation and recruitment of repair enzymes [189]. Additionally, ubiquitination H2AK119 intensifies, and H2AK13-15 start to emerge. Those alterations take part in the recruitment of repair proteins, such as RAP80 (receptor-associated protein 80), BRCA1 (breast cancer type 1 susceptibility protein), and 53BP1 (tumor protein p53 binding protein 1) [190]. Finished DSB repair marks the end of homologous recombination (HR) in meiosis [176].

On the other hand, MSCI starts to emerge in the pachytene [177]. MSCI is a meiotic silencing mechanism of asynapsed chromatin (MSUC) that affects only X and Y chromosomes. MSUC action ensures that asynapsed regions of autosomes are not transcriptionally active [177]. MSCI performs the same function, but it occurs in the sex chromosomes, and it is responsible for creating the sex body, also known as XY body [177]. In pachytene, chromatin condensation and gene repression are promoted by the reduction of histone modifications responsible for marking histones with activation epimarks on sex chromosomes [188]. Among those, the most prominent is a reduction of acetylation at: H3K9, H4K12, and H4K16, and reduction of methylation at H3K4me3 [188]. In contrast, repressive modifications like: methylation at H3K9 and H3K4, and hyperacetylation at H3K5, and H3K8 are present [188]. In pachytene, the third wave of γ H2AX phosphorylation occurs on asynapsed fragments of sex chromosomes [76, 177]. DNA damage response precedes γ H2AX phosphorylation [191]. First, BRCA1 accumulates on asynapsed sex chromosome axes and allows the recruitment of TOPBP1 (DNA topoisomerase 2-binding protein 1) and ATR kinase. In the next step, the γ H2AX phosphorylation by ATR occurs, which in consequence attracts MDC1 (mediator of DNA damage checkpoint protein 1) [191]. MDC1 then coordinates

spread of γ H2AX to the subsequent nucleosomes [191]. The absence of γ H2AX modification on sex chromosomes leads to defects in XY synapsis, MSCI failure, and complete arrest in the pachytene stage [177, 191]. Additionally, to ensure that gene silencing will persist, new histone variants are introduced from pachytene onwards. One of them is H2A.Z, which replaces canonical H2A histone [126].

The so-called: 'pachytene checkpoint' occurs in the late pachytene stage [192]. In response to HR or synapsis formation failures, the pachytene checkpoint is involved in arrest or delaying of spermatocytes progress through the pachytene stage. This mechanism prevents the formation of aneuploid spermatids [192]. Dot1 histone methyltransferase is responsible for the methylation of lysine 79 residue in histone H3 (H3K79me), which controls this checkpoint. The absence of Dot enzyme decreases the level of H3K79me, and thus leads to meiotic arrest at the pachytene stage of prophase I [192, 193]. This quality-control mechanism is well characterized in yeast and is evolutionary-conserved from yeast to mammals [192].

The activation of some genes located on sex chromosomes (~13%) is necessary for the later phases of meiosis and spermiogenesis [194, 195]; however, the MSCI process inactivates the expression of all genes located within the sex body [194, 196]. In the dividing cell, special mechanisms allow certain genes to be re-expressed, and this reactivation starts at the pachytene stage. Two major changes in the histone code are responsible for this reactivation. The first of them is the replacement of the H2A histone with H2A.B.3 (known also as H2A.Lap1) variant, which is mouse orthologue of human H2A.B variant [194, 197]. This reinstatement occurs around transcription start sites (TSS), and is associated with the relaxation of chromatin structure (less compacted nucleosome states) and gene transcription [194, 197, 198]. This histone variant is selectively located within X chromosome genes active in round spermatids [194]. The second modification that helps to restore the transcriptional activity later in spermatogenesis, is the polyubiquitination of an unknown substrate by RNF8 (ring finger protein 8) with deubiquitination of H2AK119 by SCML2 (Scm polycomb group protein like 2) [97]. As shown in mouse study, those modifications promote H3K27 acetylation of the enhancer region of escape genes in the late pachytene stage, and H3K4me2 of the promoter region in early diplotene. Histone ubiquitination is removed from sex chromosomes at the late diplotene stage, while both H3K27ac and H3K4me2 persist in spermiogenesis as an epigenetic memory, where they facilitate the activation of escape genes such as *Gm9* (predicted gene 9) or *Prdx4* (peroxiredoxin 4) [97].

Diplotene and diakinesis

From diplotene through metaphase, bivalent methylation/phosphorylation post-translational modifications will be imposed, namely: H3K9me2/H3S10ph and H3K27me2/H3S28ph [199]. Those modifications could facilitate specific condensed chromatin conformation during meiosis, as well as mitosis [199]. Additionally, the presence of H3K9me2/3 on sex chromosomes ensures chromatin condensation of XY chromosomes, which is necessary for MSCI persistence and transition to post-meiotic status of sex chromosomes [200]. Starting from diplotene until the end of meiosis, histones H3 and H4 are underacetylated on sex chromosomes, except for H4K16 throughout the X and Y chromosomes, and H4K5 only in the pericentromeric region [188].

After the end of prophase I, primary spermatocytes complete the following phases of the first meiotic division: metaphase I, anaphase I, telophase I and cytokinesis, and become secondary spermatocytes. Secondary spermatocytes quickly finish the second meiotic division, which results in haploid spermatids.

Spermiogenesis

In a process of spermiogenesis, the last step is the transformation of round spermatids into sperm cells, which are the final differentiation point of male reproductive cells [201]. During this transformation, immature spermatids undergo chromatin condensation, acrosome and tail formation, elongation, and cytoplasm reduction [201]. This transformation is possible due to histone-to-protamine exchange, which is the most important event performed during spermiogenesis. Transition nuclear proteins (TNPs: TNP1 and TNP2) mediate the transition from histones to protamines. In the first step, TNPs substitute histones, and are later replaced by protamines [134, 201, 202]. Protamines are small, arginine-rich proteins, expressed specifically in spermiogenesis starting from elongating spermatids onwards [203]. In mammals, two protamines P1 and P2 (in human encoded by *PRM* and *PRM2*, respectively) are incorporated into the chromatin structure. The arginine-rich DNA-anchoring domain allows protamines to wrap around the major groove of DNA helix. This neutralizes the negative charge of DNA backbone and allows nucleo-protamine chromatin to be coiled and condensed much tighter than nucleohistone chromatin type of packaging [203]. Most of the sperm chromatin is associated with protamines [204]. In mouse sperm cells, only 1% of the whole genome remains attached to histones, while in humans, the histones are present in 10%-15% of the sperm chromatin [35, 134, 204, 205]. Disturbances in ratio between particular protamines or between protamines and histones lead to

decrease of semen parameters and to reproductive problems [134, 205, 206]. The remaining nucleo-histone chromatin is present principally around the centromeric and telomeric regions of the chromosomes [204, 207]. The main histone variants and their involvement in modifications during the histone-to-protamine exchange, followed by relevant mouse models have been summarized in [134].

Histone acetylation is essential for the histone-to-protamine transition. H4K5, H4K8, and H4K12 acetylation are the first markers of the histone H4 hyperacetylation, which facilitates chromatin opening at the early stages of spermatid elongation [90, 208, 209]. In mouse round spermatids, the histone H4 phosphorylation of the serine 1 residue (H4S1) forego hyperacetylation [210]. This modification may help to compact DNA before histone-to-protamine transition [211]. Additionally, histone H3 hyperacetylation, mainly at lysine 9, 18, 23 residues (H3K9ac, H3K18ac, H3K23ac), has been demonstrated in spermatids, but its role in histone-to-protamine exchange is less prominent [166].

Thanks to histone hyperacetylation, DNA topoisomerase II beta (TOP2 β) binds to DNA and induces DSB formation [212]. Similar to HR and MSCI, both ATM and ATR enzymes catalyze the formation of histone γ H2AX, but during spermiogenesis also additional protein kinase TSSK6 (testis-specific serine/threonine kinase) is required [12]. γ H2AX then binds to MDC1 protein, which facilitates ubiquitination of histones H2A and H2B by recruitment of RNF8 E3 ubiquitin ligase [96, 117]. Additionally, MDC1 replaces H2A/B histones for a H2AZ/H2B dimer [117]. Those modifications lead to chromatin opening and promote H4K16ac by MOF histone acetyltransferase [96, 213]. H4K16 acetylation indicates global histone removal [96]. This process is performed by bromodomain testis-specific protein (BRDT), which binds to tetra-acetylated H4 histone and guides the histone removal [208, 214, 215].

In elongating spermatids, histone methylation is also present, and mainly consists of H3K4me2/3, H3K9me2/3, H3K27me3, and H3K79me2/3 [117, 193]. During this stage, both repressive and activating histone modifications are present. Activating H3K4me2/3 helps in the ubiquitination of histone H2A by providing a binding place for PHD finger protein 7 (PHF7) E3 ubiquitin ligase [213]. In round spermatids, H3K4me1/2/3 is located in euchromatin and activates gene expression of genes important for spermiogenesis [117]. Then in elongating spermatids, H3K9me1/2 and H3K36me3 regulate the expression of genes coding transition nuclear proteins and protamines. H3K9me1/2 presence in the promoter

region of those genes downregulates their expression [93], while H3K36me3 acts in the opposite manner [98].

After final changes in chromatin compaction and necessary epigenetic modifications, the non-motile elongated spermatids are released into the lumen of the seminiferous tubules in the process of spermiation. Next, during the transition through epididymis, the contact with unique microenvironmental lumen factors leads to the maturation of the sperm cells – assessment of motility and ability to fertilize [216–220].

Conclusions

The ever-growing number of studies of epigenetic changes in both male and female gametogenesis leads to the detailed understanding of the mechanisms governing the contribution and teamwork of the paternal and maternal genomes during the fertilization process and embryo development, also at the level of genetic and epigenetic cooperation. In this review, we have summarized the most important epigenetic modifications of DNA and histones crucial for the process of spermatogenesis. Following the facts, that each step of spermatogenesis is characterized by determined epimarks, and the complexity of male gametogenesis is intricate, the important role of epigenetic changes in male germ cell development is clearly highlighted. It is also significant in the context of male reproductive failures, with unknown or unclear genetic background, so far, and the rising number of cases with epigenetic background of the infertility as the main reason.

It is known that the majority of data have been provided from mouse studies. It is caused by the fact that still there is a low availability of human material from particular gametogenesis stages (incl. ethical issues) or embryonal ones. There is also no availability (or are problems with culturing) for cell lines related to human spermatogenesis that could be used for genetic or epigenetic research. Of course, even if it is known that mice phenotype of predicted model is often with a less severe phenotype than human (because of alternative ways of compensation for the loss of a proper protein), methylation patterns and reprogramming events are relatively conserved between human and mouse and mouse still is a good model organism for inferring general mechanisms. Translation of mouse model results into human male infertility enhances understanding of fertility pathways, and is able to mimic some aspects of primary human infertility (examples of the mouse-to-human modelling has been widely reviewed in [221, 222]). It seems to be important to expand further studies and to involve mouse models also for epididymis evaluation in

the epigenetic manner. The increasing number of data indicates changes of epigenetic markers of sperm DNA also during the maturation in epididymis (and its particular parts), and thus puts attention also on the epigenetic role of this organ, leading to the need of revision of the statement that the histone PTMs are completed before the release of the sperm from the male gonad [21, 217, 223]. So, in the context of male reproduction there is a strong need to deep evaluation of the epididymal processes in the epigenetic manner, particularly in the light of the known rich epididymal microenvironment and its already described influence both: on sperm maturation (motility, fertilization capability), as well as on the rate of sperm DNA damage [21, 202, 217–220, 223]. Thus, the link between proper epimarks and sperm chromatin integrity perhaps will possess part of its principles also from the epididymal side. Additionally, the adult diseases of the offspring linked to paternal transmission of dysfunctions related to epigenetic patterns (i.e., obesity), should also be evaluated more extensively, with the special attention to the environmental influence and the life style of the future father [68, 224–226].

From the clinical view point, the sperm epimarks should also be checked for azoospermic or cryptozoospermic patients subjected to fertilization with gametes aspirated from testicular biopsy – one step before epididymal maturation. Maybe there would be an answer for cases with successful fertilization rate (= we have an embryo) but unsuccessful embryo development (why it is not developing, when preimplantation genetics is fine), aberrant imprinting patterns, birth defects or poor health outcome of ART-born children [222, 224–227]. Thus, there is a strong need for further evaluation of epigenetic marks and mechanisms in male reproductive context, not only on human samples but also with the mouse models, because of the fact that animal studies give better accessibility of biological samples to develop a great variety of experimental pathways (specifically in testis-derived gametes and in embryo evaluation after ART). Recently expanding range of high-resolution methods and big data analyses seem to give great capabilities of a detailed and complex data acquirement at the single cell level, including genetic and epigenetic data among the whole genome and methylome, and thus leading to getting of priceless evidences for reproduction, and also for developing of novel routes for disease aetiology and its prevention or treatment in the future. The exploration of this area concerning potential linkage between male reproductive epigenome and infertility or other disease phenotypes in the offspring (not necessarily related to fertility) should be more extensive in a practical and theoretical challenges.

Abbreviations

1n	Haploid genome
2n	Diploid genome
4n	Tetraploid genome
53BP1	Tumor protein p53 binding protein 1
5caC	5-Carboxy-cytosine
5fC	5-Formyl-cytosine
5hmC	5-Hydroxymethyl-cytosine
5mC	5-Methyl-cytosine
ac	Acetylation
ART	Artificial reproductive technique
ATP	Adenosine triphosphate
BER	Base excision repair
BRCA1	Breast cancer type 1 susceptibility protein
BRDT	Bromodomain testis associated
BRDT	Bromodomain testis-specific protein
Camk4	Calcium/calmodulin-dependent protein kinase IV
Cdyl	Chromodomain protein, Y chromosome-like
Cfp1	CXXC finger 1
Chd5	Chromodomain helicase DNA binding protein 5
Dazl	Deleted in azoospermia-like
Ddx4	DEAD-box helicase 4
Dim5	Defective in methylation-5
DLK1-DIO3	Delta like non-canonical Notch ligand 1 – iodothyronine deiodinase 3
DMRs	Differentially methylated regions
DNA	Deoxyribonucleic acid
Dnmt3a	DNA methyltransferase 3A
Dnmt3l	DNA (cytosine-5-)-methyltransferase 3-like
DNMTs	DNA methyltransferases
DOT1L	Disruptor of telomeric silencing-1-like
Dot1l	DOT1-like, histone H3 methyltransferase
DSBs	Double-strand breaks
DUBs	Deubiquitinating enzymes
E1	Ubiquitin-activating enzyme
E2	Ubiquitin-conjugating enzyme
E3	Ubiquitin ligase
Epc1	Enhancer of polycomb homolog 1
Fancd2	Fanconi anemia, complementation group D2
Fanci	Fanconi anemia, complementation group I
Fbxl10	Lysine (K)-specific demethylase 2B
G9A	Euchromatic histone-lysine N-methyltransferase 2 gene
Gfap	Glial fibrillary acidic protein gene
Gm9	Predicted gene 9
H19-IGF2	H19 imprinted maternally expressed transcript – insulin like growth factor 2
HATs	Histone acetyltransferases
Hdac3	Histone deacetylase 3
Hdac6	Histone deacetylase 6
HDACs	Histone deacetylases
HJs	Holliday junctions
HKMTs	Lysine methyltransferases
HMTs	Histone methyltransferases
Hox	Homeobox proteins gene
HR	Homologous recombination
Hr6b	Ubiquitin-conjugating enzyme E2B
Jhmd2a/Jmjd1a	Lysine (K)-specific demethylase 3A (Kdm3a)
JmJd1c	Jumonji domain containing 1C
Kdm1a	Lysine (K)-specific demethylase 1A
Kdm4d	Lysine (K)-specific demethylase 4D
LEFTY	Left–right determination factor
lncRNA	Long non-coding RNA
MDC1	Mediator of DNA damage checkpoint protein 1
me	Methylation
Mettl21a	Methyltransferase like 21A
miRNA	MicroRNA
MII2	Lysine (K)-specific methyltransferase 2D
KAT8	Lysine acetyltransferase 8
MOF	Male absent on the first
MRN	Mre11, Rad50, and Nbs1
MSCI	Meiotic sex chromosome inactivation

MSUC	Meiotic silencing mechanism of asynapsed chromatin
Mthfr	Methylenetetrahydrofolate reductase
Mtrr	5-methyltetrahydrofolate-homocysteine methyltransferase reductase
NANOG	Nanog homeobox
ncRNA	Non-coding RNA
Nsd1	Nuclear receptor-binding SET-domain protein 1
OCT4	Octamer-binding transcription factor 4
PADI4	Protein-arginine deiminase type-4
Parp1/2	Poly (ADP-ribose) polymerase family, member 1/2
PGCs	Primordial germ cells
ph	Phosphorylation
PHF7	PHD finger protein 7
piRNA	Piwi-interacting RNA
PLZF	Promyelocytic leukaemia zinc finger transcriptional repressor
Prdm9	PR domain containing 9
PRDM9	PR/SET domain 9
Prdx4	Peroxiredoxin 4
PRM1	Protamine 1
PRM2	Protamine 2
PRMD14	PR/SET domain 14
Prmt1	Protein arginine N-methyltransferase 1
Prmt5	Protein arginine methyltransferase 5
Prmt5	Protein arginine N-methyltransferase 5
Prmt7	Protein arginine N-methyltransferase 7
PRMTs	Protein arginine methyltransferases
Ptip	PAX interacting (with transcription-activation domain) protein 1
PTMs	Post-transcriptional modifications
Pygo2	Pygopus 2
RAP80	Receptor-associated protein 80
RNA	Ribonucleic acid
RNF20	Ring finger protein 20
RNF8	Ring finger protein 8
rRNA	Ribosomal RNA
SAM	S-adenosyl-L-methionine
SCML2	Scm polycomb group protein like 2
SDSA	Synthesis-dependent strand annealing
SET	Su(var)3–9, Enhancer-of-zeste and Trithorax
Setd2	SET domain containing 2
Setdb1	SET domain, bifurcated 1
siRNA	Small interfering RNA
Sirt1	Sirtuin 1
Sly	Sycp3 like Y-linked
snoRNA	Small nucleolar RNA
snRNA	Small nuclear RNA
SOX2	SRY-box transcription factor 2
SPC	Spermatocyte
SPD	Spermatid
SPG A	Spermatogonia A
SPG Ad	Spermatogonia A dark
SPG Ap	Spermatogonia A pale
SPG B	Spermatogonia B
Spo11	SPO11 initiator of meiotic double stranded breaks
SSCs	Spermatogonial stem cells
STELLA	Developmental pluripotency-associated protein 3 gene
Stra8	Stimulated by retinoic acid gene 8
SUMO	Small ubiquitin-like modifiers
Suv39h1/2	Suppressor of variegation 3-9 1/2
SYCP3	Synaptonemal complex protein 3
TDG	Thymine-DNA glycosylase
TET	Ten-Eleven Translocation
Tet1	Tet methylcytosine dioxygenase 1
TEX19	Testis expressed 19
Tip60	Lysine acetyltransferase 5 (Kat5)
TNPs	Transition nuclear proteins
TOP2β	DNA topoisomerase II beta
TOPBP1	DNA topoisomerase 2-binding protein 1
tRNA	Transfer RNA
Tssk6	Testis-specific serine kinase 6

TSSs	Transcription start sites
ub	Ubiquitination
Ubr2	Ubiquitin protein ligase E3 component n-recognin 2
UHRF1	Ubiquitin-like with PHD and ring finger domains 1 gene
Utx	Lysine (K)-specific demethylase 6A

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