The Sperm Epigenome

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Abstract

The development of male germ cells from the primordial germ cell stage to that of the mature spermatozoon is a key time of epigenetic reprogramming. Orchestrated by specialized enzymes, DNA methylation and histone modifications undergo dynamic changes throughout gametogenesis. Alterations to any level of the sperm epigenetic coding may affect fertility and the sperm's contribution to normal embryo development. In support of an important role for normal genomic methylation patterns in human sperm, a number of recent studies have reported abnormal DNA methylation in imprinted and other sequences in infertile men. As well, a number of genomic imprinting disorders in offspring, associated with underlying DNA methylation alterations in imprinted genes, have been linked with infertility and the use of assisted reproductive technologies (ARTs). In this chapter, we discuss different aspects of the sperm epigenome, from the timing and mechanisms underlying the acquisition of epigenetic patterns to the consequences of perturbing such patterns. The focus here is on DNA methylation, since it is not only one of the most well-studied epigenetic modifications taking place during male germ cell development but also one that has been clearly linked to infertility in men.

Keywords

Sperm epigenome • Male germ cells, Epigenetics • DNA methylation • Assisted reproductive technologies

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The development of male germ cells from the primordial germ cell stage to that of the mature spermatozoon is a key time of epigenetic reprogramming. Orchestrated by specialized enzymes, DNA methylation and histone modifications undergo dynamic changes throughout

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gametogenesis. Male gamete epigenetic programming plays multiple roles not only in spermatogenesis, including gene expression programs and meiosis, but also in preparing the sperm for its role post fertilization in embryogenesis. Alterations to any level of the sperm epigenetic coding may affect fertility and the sperm's contribution to normal embryo development. In support of an important role for normal genomic methylation patterns in human sperm, a number of recent studies have reported abnormal DNA methylation in imprinted and other sequences in infertile men [1]. As well, a number of genomic imprinting disorders in offspring, associated with underlying DNA methylation alterations in imprinted genes, have been linked with infertility and the use of assisted reproductive technologies (ARTs) [2, 3]. Most of the evidence demonstrating the importance of proper epigenetic marks to reproduction and the general health of the embryo come from the use of animal models. In this chapter, we discuss different aspects of the sperm epigenome, from the timing and mechanisms underlying the acquisition of epigenetic patterns to the consequences of perturbing such patterns. The focus here is on DNA methylation, since it is not only one of the most well-studied epigenetic modifications taking place during male germ cell development but also one that has been clearly linked to infertility in men.

Epigenetics and the Roles of DNA Methylation

The term epigenetics refers to heritable mechanisms that help to control gene expression without an actual change in the underlying DNA sequence. These mechanisms include histone modifications (discussed in Chap. 3), noncoding RNAs (discussed in Chap. 8), and DNA methylation. The different types of epigenetic modifications interact in numerous ways to influence gene expression. The covalent addition of a methyl group to the cytosine residue in DNA is the best studied of the epigenetic modifications. This mark is found at 60–80% of CpG dinucleotides in the genome and plays important roles in many cellular processes. Methylation of the promoter region of genes is invariably associated with gene repression. Deviations from normal epigenetic patterns can result in diseases such as cancer and developmental disorders, fueling the development of a new area of epigenetic therapeutics [4].

The large majority of methylated cytosines is found within transposons and repeat sequences. DNA methylation prevents expression from transposons and their remnants within the genome. These elements have the potential to disrupt gene expression; demethylation of such sequences results in transposon reactivation in animal models [5, 6]. Along with its role in silencing such repeat sequences, DNA methylation may have functions in chromosome organization and structure. Heterochromatin, a densely packed form of DNA, has been associated with mainly gene-free regions and areas of high DNA methylation [7]. By contrast, euchromatin is generally rich in genic sequences showing active transcription, including sequences with low levels of methylation [8].

DNA methylation also contributes to the process of X-inactivation during embryogenesis. The silencing of the second X chromosome is accomplished by repression of genes located on the chromosome, associated with DNA hypermethylation of the underlying sequences [9, 10]. Similarly, genomic imprinting is a phenomenon in which DNA methylation marks at differentially methylated regions (DMRs) allow for the monoallelic expression of genes in a parent-of-origin specific manner [11]. These marks, which are initiated in the germ line, play an important role during embryonic growth and development [12, 13]. In humans, a number of disorders are associated with altered expression of imprinted genes, including the imprinting syndromes Beckwith–Wiedemann, Silver–Russell, Angelman, and Prader–Willi Syndromes, as well as several cancers [14, 15]. Outside of imprinted genes, abnormal methylation is frequently associated with cancers; both genome-wide DNA hypomethylation and site-specific hypermethylation have been reported, associated with the silencing of tumor suppressor genes and the activation of oncogenes [16].

Many mammalian promoter regions contain a high CpG content with approximately 40% containing regions known as CpG islands [17]. Methylation within promoter regions has been

shown to affect the transcriptional regulation of genes, mainly through repression. Different mechanisms by which DNA methylation mediates its effect on gene regulation include direct interference with the transcriptional machinery or the recruitment of methyl CpG binding proteins containing transcriptional repression domains [18–22]. DNA methylation may also interact with other epigenetic marks, such as histone modifications, in order to regulate gene expression. Histone 3 lysine 4 (H3K4) methylation and histone acetylation, which are marks of active chromatin structure, are normally associated with a lack of DNA methylation. By contrast, methylation at CpG dinucleotides promotes a closed chromatin structure, blocking H3K4 methyltransferases and thus resulting in transcriptional inhibition [23]. Other histone modifications such as H4K20 and H3K8 methylation are associated with the presence of DNA methylation within the DMRs of imprinted genes [24].

Enzymes Involved in DNA Methylation

The DNA (cytosine-5)-methyltransferases (DNMTs) are the enzymes involved in catalyzing the reaction in which methyl groups from *S*-adenosylmethionine (SAM) are transferred to cytosine residues. Members of this group have been characterized and classified into three groups: DNMT1, DNMT2, and DNMT3 [25]. DNMT1, the first DNA methyltransferase discovered, has a high affinity for hemimethylated sequences and plays a role in maintaining methylation patterns at the time of DNA replication (maintenance methylation) [26–28]; it was also found to be able to *de novo* methylate unmodified DNA residues [29]. DNMT1 is the major form of methyltransferase and is found in all somatic tissues, although the highest levels of mRNA expression are in the testis [30]. DNMT2 has no known role in DNA methylation but has been determined to methylate tRNAs [31]. The DNMT3 family consists of three members: DNMT3A, DNMT3B, and DNMT3L. While DNMT3A and 3B have DNA methyltransferase activity, DNMT3L does not have any catalytic activity [32]. Despite this, DNMT3L improves the *de novo* methylation abilities of the other DNMT3 members [33–36]. Interestingly, DNMT3L has been shown to have higher affinity for the unmethylated lysine 4 of the histone 3 tail (H3K4), helping to direct DNA methylation and providing evidence of interactions between these two epigenetic marks [37].

Germ Cell Expression

From mouse studies, *Dnmt1* expression has been shown to be highly regulated in both male and female gametogenesis. In males, primordial germ cells show high levels of *Dnmt1* during the proliferative phase up to 13.5 days post coitum (dpc). From 14.5 dpc on, levels drop and are undetectable at 18.5 dpc [38, 39]. Postnatally, increased expression is seen when spermatogonia resume mitotic divisions [38, 40]. DNMT1 protein is present during the early stages of meiosis and is depleted in pachytene spermatocytes.

Dnmt3a and *Dnmt3b* show developmental stage-specific differences in expression during gametogenesis. Isoforms of *Dnmt3a* are highly expressed in the prenatal testes at 16.0 dpc, with continued high expression in early postnatal life [41]. *Dnmt3b*, on the other hand, shows minimal expression in prenatal life, but high levels in type A spermatogonia at 6 days postpartum (dpp) [38, 41, 42]. Human *DNMT3A* and *DNMT3B* are highly homologous to their murine counterparts and are expressed in a variety of tissues, including the testes [43].

Expression of *Dnmt3L* in mouse male germ cells is highest before birth. Time course analysis indicated that expression is detected between 13.5 and 18.5 dpc, with a peak at 15.5 dpc [38, 41]. Gene reporter experiments have shown that *Dnmt3L* is also expressed in spermatogonia but that expression is low by 6 dpp [44, 45]. Another study detected *Dnmt3L* expression later in male germ cell development also, in differentiating spermatocytes [46]. *Dnmt3L* expression patterns mimic those of *Dnmt3a*, providing evidence that these two enzymes work together in male germ cells as they do in somatic cells.

DNA Methylation Patterns in Germ Cells

Recent mouse and human studies of numerous types of sequences throughout the genome have shown that a unique pattern of DNA methylation is observed in male germ cells in comparison to that in somatic tissues [47, 48]. For instance, in a study by Weber et al. examining promoter methylation, a unique pattern of DNA methylation was observed in human sperm when compared with that in somatic cells, and a role in gene function was postulated [49]. Indeed, methylation patterns observed at promoters in sperm, such as hypomethylation, would allow for germ cell-specific expression of genes involved with spermatogenesis, whereas hypermethylation would allow the repression of pluripotency and somatic tissue-specific genes [50–52]. Interestingly, many of the sites that were found to be differentially methylated between sperm and somatic tissues were outside genic regions and CpG islands, and therefore, likely to have other roles in addition to those in controlling gene expression. Germ cell-specific DNA methylation patterns at centromeric and intergenic sequences may be necessary for the specialized chromatin structure found in male germ cells as they undergo meiosis and spermiogenesis [48, 53, 54]. Not only are patterns unique in sperm compared to somatic tissues, but spermatozoa from the same individual also exhibit distinctive DNA methylation patterns [55].

Erasure and Acquisition of Germ Cell Patterns

Somatic cell patterns of DNA methylation are established early during embryonic life and are maintained throughout development and into adulthood. Germ cells also follow the early establishment along with the embryo; however, erasure of these patterns subsequently takes place in primordial germ cells to allow the establishment of sex-specific patterns, such as those found on imprinted genes.

Erasure of the inherited somatic cell patterns occurs in mouse primordial germ cells between 10.5 and 13.5dpc [56]. This primordial germ cell hypomethylation was observed in studies using different techniques including Southern blotting, restriction enzyme digests, and PCR approaches, as well as cellular 5-methylcytosine antibody staining [57–60]. Detailed analysis by bisulfite sequencing of several imprinted and nonimprinted genes was also performed indicating a similar time frame for germ cell DNA demethylation [61–63]. This rapid erasure of the methylation patterns over a short period of time suggests an active demethylation process. However, not all epigenetic marks are erased during this time of epigenetic reprogramming of the germ cells. Maatouk et al. demonstrated that methylation at several nonimprinted genes retained relatively high levels of methylation [63]. As well, it was shown that a number of imprinted genes retained low levels of methylation and that several repetitive elements underwent only partial demethylation of their DNA sequences [64–66]. Together, the incomplete reprogramming of the parental DNA methylation patterns in the primordial germ cells allows for the possibility of epigenetic inheritance.

Subsequent to the erasure of epigenetic patterns in primordial germ cells, remethylation of DNA is acquired in a sex-specific manner in germ cells. In females, germ cells begin to acquire their methylation patterns postnatally, following the pachytene phase of meiosis, with imprinted genes acquiring their sex-specific mark during the oocyte growth phase [67–69]. Conversely, male germ cell epigenetic patterns begin to be acquired prenatally. The timing of the initial acquisition follows the expression of both *Dnmt3a* and *DnmtL*, consistent with the role of the DNMT3 class of enzymes as *de novo* DNA methyltransferases. Increases in 5-methyl cytosine immunostaining were observed in gonocytes from 17 to 19 dpc embryos, and bisulfite analysis of the imprinted genes *H19*, *Dlk1-Gtl2*, and *Rasgrf* indicated that acquisition of their paternal methylation imprints occurred between 15.5 and 18.5 dpc [58, 59, 61, 65, 70] The male germ cell methylation patterns are completed after birth by the pachytene phase of meiosis. While most DNA methylation is acquired by the type A spermatogonial phase, several loci still undergo acquisition and loss of methylation marks between this time point and

the pachytene spermatocyte phase, at which point similar patterns are observed as those in mature spermatozoa [54].

Compared to studies using animal models, little research has been undertaken on human samples concerning the timing and sequences involved during the erasure, acquisition, and maintenance of DNA methylation marks in male germ cells. However, existing human evidence does support the erasure of methylation patterns in prenatal gonocytes and acquisition and maintenance of such patterns in early and late germ cells. For instance, Kerjean et al. [71] analyzed the DMR of *H19* and found that this sequence was unmethylated in fetal gonocytes and methylated in adult spermatogonia and in later stages of male germ cell development. As discussed in more detail below, imprinted genes that are normally methylated in the female germ line are unmethylated in human sperm as is the case in mouse. Furthermore, DNMT expression shows a similar timing of expression in human fetal gonads as that described in mouse [72].

Histone Modifications and Epigenetic Memory

Several studies have examined the modification of histone marks, in particular histone 3 methylation, during the course of male germ cell development [73–75]. The establishment and the removal of different histone modifications are important for normal spermatogenesis to occur. Transgenic animal models involving the targeting of enzymes involved in histone demethylation have revealed important roles for these enzymes in spermatogenesis and normal fertility [76].

Histone modifications can influence chromatin structure and gene expression in germ cells. In particular for male germ cells, as discussed elsewhere in this volume, extensive chromatin remodeling occurs during spermiogenesis, where histones are replaced by transition proteins, followed by protamines. This replacement allows for the high level of compaction required for packaging the DNA into the sperm head. However, in human sperm, 5–15% of histones remain bound to the genome [77, 78]. Recent studies have suggested that sperm histones and specific methylation modifications of the histones may play important roles post fertilization and "mark" or "poise" genes for expression in the embryo [79, 80]. As well, conservation of these histone modification marks at orthologous genes was seen in mouse spermatozoa [80]. Together, histone modifications in sperm would appear to be important and may contribute to the early stages of embryo development.

Consequences of an Altered Sperm Epigenome for Male Reproductive Function

Animal Models

Gene targeting has been used to examine the function of different DNMT enzymes. Mice with partial ($Dnmt^{n/n}$ and $Dnmt^{s/s}$) and complete ($Dnmt^{c/c}$) loss of function of DNMT1 were developmentally delayed and died at mid-gestation [81], before an effect on germ cells could be examined. DNMT1-deficient embryos also showed abnormal biallelic expression of imprinted genes and expression of normally silent IAP sequences, as well as ectopic X-chromosome inactivation [5, 9, 82]. Embryos obtained from the mating of female mice deficient for the oocyte-specific form of DNMT1, known as DNMT1o, also showed embryonic lethality and abnormal methylation patterns at imprinted loci [83]. Although such studies have not yet been done, with its high and tightly regulated expression in male germ cells, male germ cell-specific targeting of DNMT1 would be likely to help uncover the role of DNMT1 at different times during male germ cell development. Disease-causing mutations in DNMT1 in humans have not been reported yet, with the exception of DNMT1 catalytic domain mutations in certain rare cases of colorectal cancer [84].

DNMT3a-deficient mice do survive to term, although they were underdeveloped and did not survive past the first few weeks of life. While global levels of DNA methylation were normal in these animals, spermatogenesis was impaired [85]. Closer inspection revealed abnormal entry into meiosis as well as decreased methylation at

the imprinted *H19* locus, indicating a crucial role of DNMT3a in male germ cell development [86]. Indeed, conditional inactivation of this enzyme in male germ cells resulted in infertility due to spermatogenic failure [87]. While abnormal DNA methylation was observed at the imprinted loci *H19* and *Dlk1-Gtl2*, as well as some repeat regions in spermatogonia, little effect was found at *Rasgfr* and IAP sequences [66, 87].

Consequences of DNMT3b deficiency in mice were dramatic resulting in mid-gestation lethality and demethylation of minor satellite repeats [85]. By contrast, male germ-line conditional elimination of DNMT3b did not appear to have any phenotypic effect, resulting in normal spermatogenesis; overall DNA methylation levels appeared for the most part to be normal, although slight decreases were observed at the *Rasgrf* locus, as well as in minor and major satellite repeats [66, 87]. In humans, mutations in DNMT3B result in an autosomal recessive genetic disorder characterized by immunodeficiency, centromeric instability, and facial anomalies known as ICR syndrome [88]. Pericentric regions, containing normally methylated satellite DNA, and CpG island on the inactive X-chromosome showed aberrant methylation in ICF patients [89, 90]. No studies on fertility have been reported.

Mice with homozygous deficiency for DNMT3L are viable; however, both males and females were infertile [44, 46]. Males had small testes and were azoospermic following the initial wave of spermatogenesis. Early loss of germ cells was observed at 6 dpp and a lack of differentiated spermatocytes was detected in mice at 4-weeks [45, 46, 91]; this loss of spermatocytes occurred after meiotic failure characterized by extensive chromosomal mispairing [45, 92]. Male germ cells of DNMT3L-deficient mice had a lack of methylation of most repetitive elements, leading to their abnormal transcription in early germ cells, as well as hypomethylation of paternally methylated imprinted loci [45, 66, 92]. Loss of methylation at intergenic loci in type A spermatogonia was also observed [91].

One critical factor for all methylation reactions, including the methylation of DNA, is the availability of the methyl donor, SAM. Factors that may influence cellular methyl pools include enzymes within the folic acid pathway. The impact of altered function of some of these enzymes has been studied and shown to be associated with decreased fertility in men [93]. One such enzyme, methylenetetrahydrofolate reductase (MTHFR), is the link between the one-carbon methyl donors of the folate pathway and the formation of SAM from the methionine pathway. Enzymatic activity of MTHFR is highest in testes as compared to other tissues, suggesting a critical role in reproduction. Indeed, homozygosity for one common polymorphism (677C->T), resulting in a thermolabile form of MTHFR, has been shown to be overrepresented in cases of male idiopathic infertility [94, 95]. As well, mice with MTHFR deficiency were created, in which altered SAM levels were observed along with hypomethylation in several tissues including the testes and ovaries [96]. MTHFR-deficient mice show strain-specific pathologies. MTHFR-deficient males of the BALB/c strain had abnormal seminiferous tubules lacking germ cells and were infertile [97]. With the dietary addition of an alternate methyl donor, betaine, some of the spermatogenic defects in the BALB/c strain MTHFR-deficient mice were alleviated, indicating a critical role of methyl donors in male germ cell development. MTHFR mice of the C57BL/6 strain showed normal early germ cell development; however, adverse reproductive outcomes, including decreased testicular weights and sperm counts, were observed starting at about 3.5 months of age [98]. In addition, while normal imprinted gene methylation was found, global methylation analysis revealed both hyper- and hypomethylation at several loci throughout the sperm epigenome.

Drug Targeting

Since abnormal DNA methylation has been associated with a number of disease states, and cancer in particular, interest in epigenetic therapies has emerged. Two inhibitors of DNA methylation, 5-azacytidine and 5-aza-2'deoxycytidine, were first synthesized as potential cancer chemotherapeutic agents [99]. These drugs are cytidine analogs that

are incorporated into newly synthesized DNA during replication. When bound with DNA methyltransferases, the drugs inhibit the enzyme activity by forming covalent adducts, thereby depleting cellular pools of available DNMTs [100]. Animal exposures to these chemicals have been shown to cause male reproductive abnormalities and DNA hypomethylation. Treatment of male rats with 5-azacytidine interfered with normal germ cell development; mating with untreated females resulted in decreased fertilization and altered embryo development [101]. An increase in apoptotic germ cells as well as a decrease in global DNA methylation was also observed in mature sperm from treated males [102]. Similar effects were seen in male mice treated with 5-aza-2'deoxycytidine. Kelly et al. observed dose-dependent decreases in testicular weights and abnormal histology in the treated males and reduced pregnancy rates and increased preimplantantation loss in females mated with the treated males [103]. A dose-dependent reduction in global sperm DNA methylation was also reported, with the DNA hypomethylation restricted to loci that were shown to acquire methylation marks during spermatogenesis [104]. The results suggested that 5-aza-2'deoxycytidine selectively inhibited *de novo* methylation activity in male germ cells.

Other drugs used for chemotherapy treatment have also been shown to cause epigenetic defects in male germ cells. Cyclophosphamide, an anticancer and immunosuppressive drug, was shown to cause reproductive abnormalities and affect embryo development in a time- and dose-dependent manner [105–107]. Along with increased incidences of chromosomal abnormalities in epididymal rat sperm, epigenetic reprogramming in the early rat embryo was affected [108, 109]. Hyperacetylation of histones and altered DNA methylation were observed in early one- and two-cell rat embryos.

Human Infertility

Idiopathic infertility makes up approximately half of all cases of male infertility. A recent study has looked for genetic causes of infertility examining oligozoospermic, azoospermic, and normospermic men in a genome-wide association study using genotyping microarrays and a gene-centric approach evaluating SNPs associated with male fertility [110]. Results from this and animal models have indicated that although genetics do play a role [111], the causes of male factor infertility are multifactorial and other mechanisms may contribute to the disease. Since epigenetics plays an important role during male germ cell development, and perturbations have been shown to cause abnormal reproductive outcomes, the association of altered epigenetic marks and human infertility has been examined. In particular, the assessment of methylation defects at imprinted gene loci have been the focus of many studies.

One of the first studies analyzed the methylation in sperm at the imprinted locus *H19*, comparing oligozoospermic and normospermic men [112]. Bisulfite sequencing of the *H19* DMR found decreases in methylation at the locus that were associated with decreased sperm numbers; the methylation defects were related to the severity of the oligozoospermia. In a later study, the same researchers analyzed the *H19* locus and a maternally imprinted gene, *PEG1/MEST* [113]. They reported abnormal methylation patterns at both imprinted loci in oligozoospermic men, with a loss and gain of methylation of *H19* and *PEG1/MEST,* respectively, while global methylation (LINE1 transposon) was unaffected. Similarly, a larger study of oligozoospermic men found sperm DNA hypomethylation at *H19* and *GTL2* and hypermethylation of several maternally methylated imprinted loci [114]. In an examination of male idiopathic infertility, Poplinski et al. examined methylation profiles in swim-up purified sperm from 148 idiopathic infertile and 33 normospermic men [115]; again, abnormal methylation at *H19* and *MEST* were associated with low sperm counts. In addition, *MEST* hypermethylation was a marker for decreased motility and abnormal sperm morphology. More widespread changes in DNA methylation were observed in a study of infertile men with abnormal semen parameters, where imprinted loci, gene promoters, and several repetitive elements were shown to

be affected [116]. Finally, one recent study reported that altered methylation at different imprinted loci was associated with two different causes of male infertility [117]. Severely oligozoospermic patients had greater alterations at the *MEST* locus, a gene associated with Silver–Russell Syndrome; patients with abnormal chromatin structure were affected at the imprinting sequences of *KCNQ1OT1* (*LIT1*) and *SNRPN*.

From these studies, questions arise as to whether abnormal methylation of the imprinted and nonimprinted loci in sperm may perturb the normal development of the resulting offspring. Changes in sperm methylation profiles may help explain the low birth weight, preterm birth, and other complications reported in babies conceived using ARTs. In an attempt to answer this, Kobayashi et al. examined the methylation of ART-conceived aborted conceptuses as well as the sperm from their fathers [118]. A total of 17 ART-conceived fetal samples were found to have abnormal methylation at imprinting loci; 7 of the 17 (41%) abnormal patterns in ART-conceived fetuses were also found in the sperm DNA profile of fathers. Interestingly, sequence variations in *DNMT3L* were observed in two of these fathers as well. The results suggest that the abnormalities in DNA methylation of the fetus were transmitted from the father. Further evidence comes from a case study in which an infant conceived through in vitro fertilization was born with Silver–Russell Syndrome [119]. It was suggested that abnormal methylation in the *MEST* locus in the father's sperm may have contributed to the imprinting disorder in the child.

Conclusions and Future Directions

Future studies in both human and animal models may help us to better understand the mechanisms underlying the association between altered sperm DNA methylation and infertility. It is currently unclear whether the DNA methylation defects found in the sperm of infertile men are primary or secondary to the cause of the underlying infertility. Understanding the basis of the sperm DNA methylation defects will be important for the development of effective therapies for the associated infertility. Dietary supplementation of the methyl donor folate has been used in the treatment of infertile men [93] and may act by ameliorating abnormal DNA methylation patterns in male germ cells. The high levels of replication that occur during the course of spermatogenesis require an abundant supply of nucleotides that can be produced from the folate pathway. In addition, folic acid supplementation may provide methyl donors for the production of adequate supplies of SAM for germ-cell methylation reactions, including DNA methylation. However, there may be adverse consequences associated with dietary folate supplementation. Data have started to emerge looking at the impact of folate fortification of foods that became mandatory in North America in the late 1990s. While the main reason for fortification was to reduce the incidence of neural tube defects in pregnant women, studies have shown a concurrent increase in the incidence of colorectal cancer with the time just after implementation has begun [120]. Caution may also be warranted before treating infertile men with high doses of folate without appropriate studies showing that such treatments do not lead to abnormal methylation in sperm that might be transmitted to the offspring.

For the future, more studies are required to better understand the role of epigenetic modifications in normal and abnormal male germ cell development. For instance, as next-generation sequencing and bioinformatic resources become more readily available, it will be possible to determine the DNA methylation status at all of the 20–30 million sites in the genome in patients and in germ cells at different stages of development. Such studies may help identify important sites of epigenetic perturbations in the sperm of infertile patients that may be passed on to the offspring. Additionally, genome-wide sequencing studies may help determine which types of methylated sequences are most sensitive to endogenous factors such as age and exogenous factors such as environmental and drug exposures. There is also concern that some epigenetic defects may be passed across generations despite the genome-wide erasure that takes place within the germ line [121].

The mechanisms and potential for transgenerational passage of epigenetic defects will need further study due to the possible adverse consequences for future generations. A better understanding is also needed of the interactions between the different epigenetic modifications and the enzymes involved, in normal male germ cell development, as well as which modifications are important for embryo development.

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References

1. Filipponi D, Feil R. Perturbation of genomic imprinting in oligozoospermia. Epigenetics. 2009;4(1): 27–30.
2. Gosden R, Trasler J, Lucifero D, Faddy M. Rare congenital disorders, imprinted genes, and assisted reproductive technology. Lancet. 2003;361(9373): 1975–7.
3. Lucifero D, Chaillet JR, Trasler JM. Potential significance of genomic imprinting defects for reproduction and assisted reproductive technology. Hum Reprod Update. 2004;10(1):3–18.
4. Egger G, Liang G, Aparicio A, Jones PA. Epigenetics in human disease and prospects for epigenetic therapy. Nature. 2004;429(6990):457–63.
5. Walsh CP, Chaillet JR, Bestor TH. Transcription of IAP endogenous retroviruses is constrained by cytosine methylation. Nat Genet. 1998;20(2):116–7.
6. Walsh CP, Bestor TH. Cytosine methylation and mammalian development. Genes Dev. 1999;13(1): 26–34.
7. Sanford J, Forrester L, Chapman V, Chandley A, Hastie N. Methylation patterns of repetitive DNA sequences in germ cells of Mus musculus. Nucleic Acids Res. 1984;12(6):2823–36.
8. Bestor TH, Coxon A. Cytosine methylation: the pros and cons of DNA methylation. Curr Biol. 1993;3(6):384–6.
9. Panning B, Jaenisch R. DNA hypomethylation can activate Xist expression and silence X-linked genes. Genes Dev. 1996;10(16):1991–2002.
10. Sado T, Okano M, Li E, Sasaki H. De novo DNA methylation is dispensable for the initiation and propagation of X chromosome inactivation. Development. 2004;131(5):975–82.
11. Reik W, Dean W, Walter J. Epigenetic reprogramming in mammalian development. Science. 2001;293(5532): 1089–93.
12. Smith FM, Garfield AS, Ward A. Regulation of growth and metabolism by imprinted genes. Cytogenet Genome Res. 2006;113(1–4):279–91.
13. Fowden AL, Sibley C, Reik W, Constancia M. Imprinted genes, placental development and fetal growth. Horm Res. 2006;65 Suppl 3:50–8.
14. Paulsen M, Ferguson-Smith AC. DNA methylation in genomic imprinting, development, and disease. J Pathol. 2001;195(1):97–110.
15. Tycko B, Morison IM. Physiological functions of imprinted genes. J Cell Physiol. 2002;192(3): 245–58.
16. Feinberg AP, Tycko B. Timeline – The history of cancer epigenetics. Nat Rev Cancer. 2004;4(2):143–53.
17. Fatemi M, Pao MM, Jeong S, et al. Footprinting of mammalian promoters: use of a CpG DNA methyltransferase revealing nucleosome positions at a single molecule level. Nucleic Acids Res. 2005;33(20): e176.
18. Comb M, Goodman HM. CpG methylation inhibits proenkephalin gene expression and binding of the transcription factor AP-2. Nucleic Acids Res. 1990;18(13):3975–82.
19. Nan X, Campoy FJ, Bird A. MeCP2 is a transcriptional repressor with abundant binding sites in genomic chromatin. Cell. 1997;88(4):471–81.
20. Kaffer CR, Srivastava M, Park KY, et al. A transcriptional insulator at the imprinted H19/Igf2 locus. Genes Dev. 2000;14(15):1908–19.
21. Prokhortchouk A, Hendrich B, Jorgensen H, et al. The p120 catenin partner Kaiso is a DNA methylation-dependent transcriptional repressor. Genes Dev. 2001;15(13):1613–8.
22. Filion GJ, Zhenilo S, Salozhin S, Yamada D, Prokhortchouk E, Defossez PA. A family of human zinc finger proteins that bind methylated DNA and repress transcription. Mol Cell Biol. 2006;26(1): 169–81.
23. Okitsu CY, Hsieh CL. DNA methylation dictates histone H3K4 methylation. Mol Cell Biol. 2007;27(7): 2746–57.
24. Delaval K, Govin J, Cerqueira F, Rousseaux S, Khochbin S, Feil R. Differential histone modifications mark mouse imprinting control regions during spermatogenesis. EMBO J. 2007;26(3):720–9.
25. Goll MG, Bestor TH. Eukaryotic cytosine methyltransferases. Annu Rev Biochem. 2005;74: 481–514.
26. Bestor T, Laudano A, Mattaliano R, Ingram V. Cloning and sequencing of a cDNA encoding DNA methyltransferase of mouse cells. The carboxyl-terminal domain of the mammalian enzymes is related to bacterial restriction methyltransferases. J Mol Biol. 1988;203(4):971–83.
27. Bestor TH. Activation of mammalian DNA methyltransferase by cleavage of a Zn binding regulatory domain. EMBO J. 1992;11(7):2611–7.

28. Yoder JA, Soman NS, Verdine GL, Bestor TH. DNA (cytosine-5)-methyltransferases in mouse cells and tissues. Studies with a mechanism-based probe. J Mol Biol. 1997;270(3):385–95.
29. Okano M, Xie S, Li E. Cloning and characterization of a family of novel mammalian DNA (cytosine-5) methyltransferases. Nat Genet. 1998;19(3):219–20.
30. Trasler JM, Alcivar AA, Hake LE, Bestor T, Hecht NB. DNA methyltransferase is developmentally expressed in replicating and non-replicating male germ cells. Nucleic Acids Res. 1992;20(10):2541–5.
31. Goll MG, Kirpekar F, Maggert KA, et al. Methylation of tRNAAsp by the DNA methyltransferase homolog Dnmt2. Science. 2006;311(5759):395–8.
32. Aapola U, Lyle R, Krohn K, Antonarakis SE, Peterson P. Isolation and initial characterization of the mouse Dnmt3l gene. Cytogenet Cell Genet. 2001;92(1–2): 122–6.
33. Chedin F, Lieber MR, Hsieh CL. The DNA methyltransferase-like protein DNMT3L stimulates de novo methylation by Dnmt3a. Proc Natl Acad Sci USA. 2002;99(26):16916–21.
34. Margot JB, Ehrenhofer-Murray AE, Leonhardt H. Interactions within the mammalian DNA methyltransferase family. BMC Mol Biol. 2003;4:7.
35. Suetake I, Shinozaki F, Miyagawa J, Takeshima H, Tajima S. DNMT3L stimulates the DNA methylation activity of Dnmt3a and Dnmt3b through a direct interaction. J Biol Chem. 2004;279(26):27816–23.
36. Chen ZX, Mann JR, Hsieh CL, Riggs AD, Chedin F. Physical and functional interactions between the human DNMT3L protein and members of the de novo methyltransferase family. J Cell Biochem. 2005;95(5): 902–17.
37. Ooi SK, Qiu C, Bernstein E, et al. DNMT3L connects unmethylated lysine 4 of histone H3 to de novo methylation of DNA. Nature. 2007;448(7154):714–7.
38. La Salle S, Mertineit C, Taketo T, Moens PB, Bestor TH, Trasler JM. Windows for sex-specific methylation marked by DNA methyltransferase expression profiles in mouse germ cells. Dev Biol. 2004;268(2):403–15.
39. Sakai Y, Suetake I, Itoh K, Mizugaki M, Tajima S, Yamashina S. Expression of DNA methyltransferase (Dnmt1) in testicular germ cells during development of mouse embryo. Cell Struct Funct. 2001;26(6):685–91.
40. Jue K, Bestor TH, Trasler JM. Regulated synthesis and localization of DNA methyltransferase during spermatogenesis. Biol Reprod. 1995;53(3):561–9.
41. La Salle S, Trasler JM. Dynamic expression of DNMT3a and DNMT3b isoforms during male germ cell development in the mouse. Dev Biol. 2006;296(1):71–82.
42. Watanabe D, Suetake I, Tajima S, Hanaoka K. Expression of Dnmt3b in mouse hematopoietic progenitor cells and spermatogonia at specific stages. Gene Expr Patterns. 2004;5(1):43–9.
43. Xie S, Wang Z, Okano M, et al. Cloning, expression and chromosome locations of the human DNMT3 gene family. Gene. 1999;236(1):87–95.
44. Bourc'his D, Xu GL, Lin CS, Bollman B, Bestor TH. Dnmt3L and the establishment of maternal genomic imprints. Science. 2001;294(5551):2536–9.
45. Webster KE, O'Bryan MK, Fletcher S, et al. Meiotic and epigenetic defects in Dnmt3L-knockout mouse spermatogenesis. Proc Natl Acad Sci USA. 2005; 102(11):4068–73.
46. Hata K, Okano M, Lei H, Li E. Dnmt3L cooperates with the Dnmt3 family of de novo DNA methyltransferases to establish maternal imprints in mice. Development. 2002;129(8):1983–93.
47. Eckhardt F, Lewin J, Cortese R, et al. DNA methylation profiling of human chromosomes 6, 20 and 22. Nat Genet. 2006;38(12):1378–85.
48. Oakes CC, La Salle S, Smiraglia DJ, Robaire B, Trasler JM. A unique configuration of genome-wide DNA methylation patterns in the testis. Proc Natl Acad Sci USA. 2007;104(1):228–33.
49. Weber M, Hellmann I, Stadler MB, et al. Distribution, silencing potential and evolutionary impact of promoter DNA methylation in the human genome. Nat Genet. 2007;39(4):457–66.
50. Maclean 2nd JA, Wilkinson MF. Gene regulation in spermatogenesis. Curr Top Dev Biol. 2005;71: 131–97.
51. Kitamura E, Igarashi J, Morohashi A, et al. Analysis of tissue-specific differentially methylated regions (TDMs) in humans. Genomics. 2007;89(3):326–37.
52. Farthing CR, Ficz G, Ng RK, et al. Global mapping of DNA methylation in mouse promoters reveals epigenetic reprogramming of pluripotency genes. PLoS Genet Jun. 2008;4(6):e1000116.
53. Yamagata K, Yamazaki T, Miki H, et al. Centromeric DNA hypomethylation as an epigenetic signature discriminates between germ and somatic cell lineages. Dev Biol. 2007;312(1):419–26.
54. Oakes CC, La Salle S, Smiraglia DJ, Robaire B, Trasler JM. Developmental acquisition of genome-wide DNA methylation occurs prior to meiosis in male germ cells. Dev Biol. 2007;307(2):368–79.
55. Flanagan JM, Popendikyte V, Pozdniakovaite N, et al. Intra- and interindividual epigenetic variation in human germ cells. Am J Hum Genet. 2006;79(1): 67–84.
56. Hajkova P, Erhardt S, Lane N, et al. Epigenetic reprogramming in mouse primordial germ cells. Mech Dev. 2002;117(1–2):15–23.
57. Monk M, Boubelik M, Lehnert S. Temporal and regional changes in DNA methylation in the embryonic, extraembryonic and germ cell lineages during mouse embryo development. Development. 1987; 99(3):371–82.
58. Kafri T, Ariel M, Brandeis M, et al. Developmental pattern of gene-specific DNA methylation in the mouse embryo and germ line. Genes Dev. 1992;6(5): 705–14.
59. Coffigny H, Bourgeois C, Ricoul M, et al. Alterations of DNA methylation patterns in germ cells and Sertoli cells from developing mouse testis. Cytogenet Cell Genet. 1999;87(3–4):175–81.

60. Seki Y, Hayashi K, Itoh K, Mizugaki M, Saitou M, Matsui Y. Extensive and orderly reprogramming of genome-wide chromatin modifications associated with specification and early development of germ cells in mice. Dev Biol. 2005;278(2):440–58.
61. Davis TL, Yang GJ, McCarrey JR, Bartolomei MS. The H19 methylation imprint is erased and re-established differentially on the parental alleles during male germ cell development. Hum Mol Genet. 2000;9(19):2885–94.
62. Li JY, Lees-Murdock DJ, Xu GL, Walsh CP. Timing of establishment of paternal methylation imprints in the mouse. Genomics. 2004;84(6):952–60.
63. Maatouk DM, Kellam LD, Mann MR, et al. DNA methylation is a primary mechanism for silencing postmigratory primordial germ cell genes in both germ cell and somatic cell lineages. Development. 2006;133(17):3411–8.
64. Lane N, Dean W, Erhardt S, et al. Resistance of IAPs to methylation reprogramming may provide a mechanism for epigenetic inheritance in the mouse. Genesis. 2003;35(2):88–93.
65. Lees-Murdock DJ, De Felici M, Walsh CP. Methylation dynamics of repetitive DNA elements in the mouse germ cell lineage. Genomics. 2003;82(2):230–7.
66. Kato Y, Kaneda M, Hata K, et al. Role of the Dnmt3 family in de novo methylation of imprinted and repetitive sequences during male germ cell development in the mouse. Hum Mol Genet. 2007;16(19):2272–80.
67. Kono T, Obata Y, Yoshimzu T, Nakahara T, Carroll J. Epigenetic modifications during oocyte growth correlates with extended parthenogenetic development in the mouse. Nat Genet. 1996;13(1):91–4.
68. Lucifero D, Mertineit C, Clarke HJ, Bestor TH, Trasler JM. Methylation dynamics of imprinted genes in mouse germ cells. Genomics. 2002;79(4):530–8.
69. Obata Y, Kono T. Maternal primary imprinting is established at a specific time for each gene throughout oocyte growth. J Biol Chem. 2002;277(7):5285–9.
70. Davis TL, Trasler JM, Moss SB, Yang GJ, Bartolomei MS. Acquisition of the H19 methylation imprint occurs differentially on the parental alleles during spermatogenesis. Genomics. 1999;58(1):18–28.
71. Kerjean A, Dupont JM, Vasseur C, et al. Establishment of the paternal methylation imprint of the human H19 and MEST/PEG1 genes during spermatogenesis. Hum Mol Genet. 2000;9:649–59.
72. Galetska D, Weis E, Tralau T, et al. Sex-specific windows for high mRNA expresion of DNA methyltransferases 1 and 3A and methyl-CpG-binding domain proteins 2 and 4 in fetal gonads. Mol Reprod Dev. 2007;74:233–41.
73. Khalil AM, Boyar FZ, Driscoll DJ. Dynamic histone modifications mark sex chromosome inactivation and reactivation during mammalian spermatogenesis. Proc Natl Acad Sci USA. 2004;101(47):16583–7.
74. Payne C, Braun RE. Histone lysine trimethylation exhibits a distinct perinuclear distribution in Plzf-expressing spermatogonia. Dev Biol. 2006;293(2):461–72.
75. Godmann M, Auger V, Ferraroni-Aguiar V, et al. Dynamic regulation of histone H3 methylation at lysine 4 in mammalian spermatogenesis. Biol Reprod. 2007;77(5):754–64.
76. Carrell DT, Hammoud SS. The human sperm epigenome and its potential role in embryonic development. Mol Hum Reprod. 2010;16(1):37–47.
77. Tanphaichitr N, Sobhon P, Taluppeth N, Chalermisarachai P. Basic nuclear proteins in testicular cells and ejaculated spermatozoa in man. Exp Cell Res. 1978;117(2):347–56.
78. Wykes SM, Krawetz SA. The structural organization of sperm chromatin. J Biol Chem. 2003;278(32):29471–7.
79. Hammoud SS, Nix DA, Zhang H, Purwar J, Carrell DT, Cairns BR. Distinctive chromatin in human sperm packages genes for embryo development. Nature. 2009;460(7254):473–8.
80. Brykczynska U, Hisano M, Erkek S, et al. Repressive and active histone methylation mark distinct promoters in human and mouse spermatozoa. Nature Struct Mol Biol. 2010;17(16):679–87.
81. Li E, Bestor TH, Jaenisch R. Targeted mutation of the DNA methyltransferase gene results in embryonic lethality. Cell. 1992;69(6):915–26.
82. Li E, Beard C, Jaenisch R. Role for DNA methylation in genomic imprinting. Nature. 1993;366(6453):362–5.
83. Howell CY, Bestor TH, Ding F, et al. Genomic imprinting disrupted by a maternal effect mutaion in the Dnmt1 gene. Cell. 2001;104:829–38.
84. Kanai Y, Ushijima S, Nakanishi Y, Sakamoto M, Hirohashi S. Mutation of the DNA methyltransferase (DNMT) 1 gene in human colorectal cancers. Cancer Lett. 2003;192(1):75–82.
85. Okano M, Bell DW, Haber DA, Li E. DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. Cell. 1999;99(3):247–57.
86. Yaman R, Grandjean V. Timing of entry of meiosis depends on a mark generated by DNA methyltransferase 3a in testis. Mol Reprod Dev. 2006;73(3):390–7.
87. Kaneda M, Okano M, Hata K, et al. Essential role for de novo DNA methyltransferase Dnmt3a in paternal and maternal imprinting. Nature. 2004;429(6994):900–3.
88. Xu GL, Bestor TH, Bourc'his D, et al. Chromosome instability and immunodeficiency syndrome caused by mutations in a DNA methyltransferase gene. Nature. 1999;402(6758):187–91.
89. Jeanpierre M, Turleau C, Aurias A, et al. An embryonic-like methylation pattern of classical satellite DNA is observed in ICF syndrome. Hum Mol Genet. 1993;2(6):731–5.
90. Kondo T, Bobek MP, Kuick R, et al. Whole-genome methylation scan in ICF syndrome: hypomethylation of non-satellite DNA repeats D4Z4 and NBL2. Hum Mol Genet. 2000;9(4):597–604.
91. La Salle S, Oakes CC, Neaga OR, Bourc'his D, Bestor TH, Trasler JM. Loss of spermatogonia and wide-spread DNA methylation defects in newborn male mice deficient in DNMT3L. BMC Dev Biol. 2007;7:104.

92. Bourc'his D, Bestor TH. Meiotic catastrophe and retrotransposon reactivation in male germ cells lacking Dnmt3L. Nature. 2004;431(7004):96–9.
93. Forges T, Monnier-Barbarino P, Alberto JM, Gueant-Rodriguez RM, Daval JL, Gueant JL. Impact of folate and homocysteine metabolism on human reproductive health. Hum Reprod Update. 2007;13(3):225–38.
94. Bezold G, Lange M, Peter RU. Homozygous methylenetetrahydrofolate reductase C677T mutation and male infertility. N Engl J Med. 2001;344(15): 1172–3.
95. Lee HC, Jeong YM, Lee SH, et al. Association study of four polymorphisms in three folate-related enzyme genes with non-obstructive male infertility. Hum Reprod. 2006;21(12):3162–70.
96. Chen Z, Karaplis AC, Ackerman SL, et al. Mice deficient in methylenetetrahydrofolate reductase exhibit hyperhomocysteinemia and decreased methylation capacity, with neuropathology and aortic lipid deposition. Hum Mol Genet. 2001;10(5):433–43.
97. Kelly TL, Neaga OR, Schwahn BC, Rozen R, Trasler JM. Infertility in 5,10-methylenetetrahydrofolate reductase (MTHFR)-deficient male mice is partially alleviated by lifetime dietary betaine supplementation. Biol Reprod. 2005;72(3):667–77.
98. Chan D, Cushnie DW, Neaga OR, Lawrance AK, Rozen R, Trasler JM. Strain-specific defects in testicular development and sperm epigenetic patterns in 5,10-methylenetetrahydrofolate reductase-deficient mice. Endocrinology. 2010;151:3363–73.
99. Cihak A. Biological effects of 5-azacytidine in eukaryotes. Oncology. 1974;30(5):405–22.
100. Gabbara S, Bhagwat AS. The mechanism of inhibition of DNA (cytosine-5-)-methyltransferases by 5-azacytosine is likely to involve methyl transfer to the inhibitor. Biochem J. 1995;307(Pt 1):87–92.
101. Doerksen T, Trasler JM. Developmental exposure of male germ cells to 5-azacytidine results in abnormal preimplantation development in rats. Biol Reprod. 1996;55(5):1155–62.
102. Doerksen T, Benoit G, Trasler JM. Deoxyribonucleic acid hypomethylation of male germ cells by mitotic and meiotic exposure to 5-azacytidine is associated with altered testicular histology. Endocrinology. 2000;141(9):3235–44.
103. Kelly TL, Li E, Trasler JM. 5-aza-2'-deoxycytidine induces alterations in murine spermatogenesis and pregnancy outcome. J Androl. 2003;24(6):822–30.
104. Oakes CC, Kelly TL, Robaire B, Trasler JM. Adverse effects of 5-aza-2'-deoxycytidine on spermatogenesis include reduced sperm function and selective inhibition of de novo DNA methylation. J Pharmacol Exp Ther. 2007;322(3):1171–80.
105. Trasler JM, Hales BF, Robaire B. Paternal cyclophosphamide treatment of rats causes fetal loss and malformations without affecting male fertility. Nature. 1985;316(6024):144–6.
106. Trasler JM, Hales BF, Robaire B. A time-course study of chronic paternal cyclophosphamide treatment in rats: effects on pregnancy outcome and the male reproductive and hematologic systems. Biol Reprod. 1987;37(2):317–26.
107. Trasler JM, Robaire B. Effects of cyclophosphamide on selected cytosolic and mitochondrial enzymes in the epididymis of the rat. J Androl. 1988;9(2):142–52.
108. Barton TS, Wyrobek AJ, Hill FS, Robaire B, Hales BF. Numerical chromosomal abnormalities in rat epididymal spermatozoa following chronic cyclophosphamide exposure. Biol Reprod. 2003;69(4): 1150–7.
109. Barton TS, Robaire B, Hales BF. Epigenetic programming in the preimplantation rat embryo is disrupted by chronic paternal cyclophosphamide exposure. Proc Natl Acad Sci USA. 2005;102(22):7865–70.
110. Aston KI, Carrell DT. Genome-wide study of single-nucleotide polymorphisms associated with azoospermia and severe oligozoospermia. J Androl. 2009;30(6):711–25.
111. O'Flynn O'Brien KL, Varghese AC, Agarwal A. The genetic causes of male factor infertility: a review. Fertil Steril. 2010;93(1):1–12.
112. Marques CJ, Carvalho F, Sousa M, Barros A. Genomic imprinting in disruptive spermatogenesis. Lancet. 2004;363(9422):1700–2.
113. Marques CJ, Costa P, Vaz B, et al. Abnormal methylation of imprinted genes in human sperm is associated with oligozoospermia. Mol Hum Reprod. 2008;14(2):67–74.
114. Kobayashi H, Sato A, Otsu E, et al. Aberrant DNA methylation of imprinted loci in sperm from oligospermic patients. Hum Mol Genet. 2007;16(21): 2542–51.
115. Poplinski A, Tuttelmann F, Kanber D, Horsthemke B, Gromoll J. Idiopathic male infertility is strongly associated with aberrant methylation of MEST and IGF2/H19 ICR1. Int J Androl. 2010;33:642–9.
116. Houshdaran S, Cortessis VK, Siegmund K, Yang A, Laird PW, Sokol RZ. Widespread epigenetic abnormalities suggest a broad DNA methylation erasure defect in abnormal human sperm. PLoS One. 2007;2(12):e1289.
117. Hammoud SS, Purwar J, Pflueger C, Cairns BR, Carrell DT. Alterations in sperm DNA methylation patterns at imprinted loci in two classes of infertility. Fertil Steril. 2010;94:1728–33.
118. Kobayashi H, Hiura H, John RM, et al. DNA methylation errors at imprinted loci after assisted conception originate in the parental sperm. Eur J Hum Genet. 2009;17(12):1582–91.
119. Kagami M, Nagai T, Fukami M, Yamazawa K, Ogata T. Silver-Russell syndrome in a girl born after in vitro fertilization: partial hypermethylation at the differentially methylated region of PEG1/MEST. J Assist Reprod Genet. 2007;24(4):131–6.
120. Mason JB, Dickstein A, Jacques PF, et al. A temporal association between folic acid fortification and an increase in colorectal cancer rates may be illuminating important biological principles: a hypothesis. Cancer Epidemiol Biomarkers Prev. 2007;16(7):1325–9.
121. Richards EJ. Inherited epigenetic variation-revisiting soft inheritance. Nat Rev Genet. 2006;7:395–401.