Hindawi
Andrologia
Volume 2024, Article ID 5525839, 6 pages
https://doi.org/10.1155/2024/5525839

Review Article

The Interplay of Varicoceles, Sperm Epigenetics, and Male Infertility: A Focused, Contemporary Review

Grace E. Sollender,[1] Andre Belarmino,[1] Thiago P. Furtado,[1] Vadim Osadchiy,[1] Jesse N. Mills,[1] Matteo Pellegrini,[2] and Sriram V. Eleswarapu[1]

[1]*Division of Andrology, Department of Urology, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA*
[2]*Department of Molecular, Cell and Developmental Biology, University of California, Los Angeles, CA, USA*

Correspondence should be addressed to Sriram V. Eleswarapu; seleswarapu@mednet.ucla.edu

Received 6 November 2023; Revised 26 December 2023; Accepted 23 January 2024; Published 8 February 2024

Academic Editor: Marta Olszewska

Despite the wide recognition of varicocele as a contributor to infertility, its exact pathophysiologic mechanism is not fully understood. As the field of male infertility continues to see progress in new diagnostic and therapeutic technologies, we sought to obtain a robust understanding of the interplay of varicoceles, oxidative stress, DNA methylation, and male infertility. We performed a comprehensive review of the contemporary literature in PubMed using search terms and keywords associated with varicoceles, DNA methylation, and male infertility. The most important findings from the literature review were included in this report. Articles were selected based on quality and relevance to the topic. Eight studies were included in this review. These studies discuss DNA methylation and varicocele-associated male infertility through evaluation of sperm parameters, DNA methylation, DNA fragmentation, and other relevant factors. Our review of the literature on varicocele-associated sperm DNA methylation indicates that global hypomethylation occurs in association with varicoceles. Some data suggest a dose-dependent effect of varicocele grade on sperm DNA methylation, with the greatest magnitude changes seen in grades II and III varicoceles. While changes in DNA methylation patterns would be expected following varicocelectomy, such changes were not found to be statistically significant in one study reviewed here. However, current human studies are limited by small sample size and short follow-up time. Future clinical and preclinical studies with robust sample sizes and hypothesis-driven investigations are necessary to further understand the relationship between DNA methylation patterns and varicocele-associated male infertility.

1. Introduction

A persistent conundrum in the field of male infertility is the mechanism by which varicoceles exert deleterious effects on sperm production and sperm function. A varicocele is an abnormal dilation of the scrotal pampiniform plexus and can cause testicular atrophy, discomfort, and infertility. Approximately 15%–20% of adult and adolescent males have varicoceles, which are directly responsible for 35% of males with primary infertility and up to 80% with secondary infertility [1–4]. Not all men with varicoceles are infertile, and about 80% of men with varicoceles are asymptomatic and have normal fecundity [5]. Despite the wide recognition of varicocele as a contributor to infertility, its exact pathophysiologic mechanism is not fully understood. Recently, there has been burgeoning interest in the role of epigenetics to identify potential biomarkers that can aid with infertility diagnosis, and there is acknowledgment that epigenetic changes may be a potential mechanism by which varicoceles act to impair fertility.

Epigenetics involves the study of gene regulation by processes that alter DNA structure without affecting its sequence. These processes may activate or deactivate transcription of certain genes, thereby altering the expressed phenotype for a given DNA sequence. Epigenetic processes include DNA methylation, posttranslational histone modification, and microRNA regulation [6]. A number of recent investigations have demonstrated that certain patterns of DNA methylation can negatively impact reproductive success [7–11]. Previous work has also revealed a marked difference in DNA methylation patterns between infertile and fertile men [6, 12–20]. Sperm production comprises a complex series of steps which

can be susceptible to epigenetic alterations; for this reason, fertility researchers have begun to focus on risk factors for DNA methylation changes, potentially including varicoceles [21].

Potential causes for varicocele-induced infertility include hypoperfusion leading to hypoxia, heat stress, oxidative stress, hormonal imbalances, and exogenous toxicants. In particular, current evidence supports oxidative stress as a crucial arbiter of varicocele-induced infertility [4, 22, 23]. DNA base modifications, deletions, strand breakage, and chromosomal rearrangements are consequences of oxidative stress. Such alterations may impact the efficiency of DNA methyltransferases, a family of enzymes that mediate the transfer of a methyl group to DNA and serve as key epigenetic regulators [24–27]. Therefore, DNA lesions, such as those caused by reactive oxygen species (ROS), may lead to the inability of DNA to function as a substrate for DNA methyltransferases, resulting in DNA hypomethylation [28]. Previous reports have suggested a negative correlation between ROS and epigenetic alterations in infertile men [28, 29], and sperm from infertile men are more likely to express aberrant DNA methylation patterns [30–34]. These differences in DNA methylation patterns may have implications for oocyte fertilization and embryo development, as the global methylation level of sperm DNA has been demonstrated to influence pregnancy rate after in vitro fertilization (IVF) [17, 19, 35–37].

As the field of male infertility continues to see progress in new diagnostic and therapeutic technologies, we sought to obtain a robust understanding of the interplay of varicoceles, oxidative stress, DNA methylation, and male infertility. To this end, herein, we provide a narrative review of the contemporary literature in this area of interest.

2. Methods

A comprehensive literature search was performed in the PubMed database, without a limit on dates of publication. The following search terms were employed: "varicoceles, DNA methylation, epigenetics, sperm, male infertility." Studies were selected for review that were written in English, focused on varicocele-associated infertility, and discussed epigenetic associations. Emphasis was placed on studies that reported semen analyses, sperm diagnostics, and molecular assessment. Articles were selected based on quality and relevance. A wide range of animal and human studies were reviewed.

3. Results and Discussion

Eight published articles met focused criteria for selection and are summarized in Table 1. Herein, we discuss the findings from these studies.

3.1. Sperm DNA Methylation in Men with Varicoceles. Tavalaee et al. [29] examined the effects of varicocelectomy surgery on sperm DNA methylation, and associated functional characteristics. Conventional semen analysis, sperm DNA fragmentation (SDF), protamine deficiency, oxidative stress, and global DNA methylation were evaluated in 52 men with primary infertility and clinical grade II or III left-sided varicocele, both before and 3 months after varicocelectomy surgery. Sperm concentration, SDF, protamine deficiency, and oxidative stress showed statistically significant improvements after surgery by 42.5%, 47.2%, and 18.5%, respectively. Additionally, the percentage of sperm motility, global DNA methylation, and intensity of DNA methylation also trended toward improvement following surgery, although these differences were not statistically significant compared to preoperative parameters. However, within a subgroup of oligozoospermic men, the increase in global DNA methylation after varicocelectomy surgery was higher than in nonoligozoospermic men. The authors of this study acknowledge several potential contributors to absence of significant differences in DNA methylation in the total population: the limited sample size, inadequate time to analysis due to the number of spermatogenesis cycles needed to observe epigenetic changes, and variations in varicocele effects on each individual, among others.

Santana et al. [35] assessed sperm global DNA methylation, telomere length, and SDF in men with and without clinically palpable varicoceles. In this case–control study, semen samples from 20 men with grade II or III varicocele and 20 healthy controls were collected. Their findings suggest that sperm DNA methylation may be lower in men with varicoceles compared to controls, though the study was ultimately underpowered to detect a statistically significant difference. Rates of SDF and telomere length were not statistically different between the two groups. A considerable limitation of these findings is that the DNA methylation analysis was conducted in only a subset of each group (10 men with varicocele and five control), likely contributing to the lack of statistical significance.

The same group subsequently performed a study with a slightly larger sample size and different methodology. In 2020, Santana et al. [39] assessed the global DNA methylation pattern in sperm and overall semen quality in men with varicoceles. In this prospective case–control study, semen quality and sperm DNA methylation patterns were assessed in 26 men with varicoceles of any grade, regardless of fertility status, and 26 fertile healthy controls. They found that the genomes of men with varicocele were globally hypomethylated when compared to the control group and this difference was statistically significant ($P = 0.0373$). Regional analysis was performed to discern specific regions of the sperm genome that were differentially methylated in males with varicocele. The authors identified 1,695 statistically significant differentially methylated regions, 920 hypermethylated and 775 hypomethylated, in the varicocele group compared with the controls. When regional analyses were subdivided by varicocele grade, 24 methylated regions were common to all grades of varicocele, and all regions were hypermethylated in varicocele patients. No statistically significant difference was found in methylation pattern across varicocele grades, which suggests that severity of clinical phenotype may not predict the degree of impact on fertility.

A case series by Bahreinian et al. [38] evaluated global DNA methylation infertile men with varicoceles and fertile men. Semen samples from 44 infertile men with left-sided grade II or III varicocele and 15 fertile men were collected. Conventional semen analysis, DNA methylation, SDF, oxidative stress, and protamine deficiency were assessed. As

Table 1: Studies included.

	Study	Author	Year	Design	*N*	Notable findings
Sperm DNA methylation findings in men with varicoceles	Effect of varicocelectomy on sperm functional characteristics and DNA methylation	Tavalaee et al. [29]	2015	Cohort	52	Sperm concentration, DNA fragmentation, protamine deficiency, and oxidative stress improved after surgery by 42.5%, 47.2%, 18.5%, respectively. No significant differences in percentage of sperm motility, global DNA methylation, and intensity of DNA methylation following surgery.
	DNA hypomethylation predisposes sperm to DNA damage in individuals with varicocele	Bahreinian et al. [38]	2015	Case–control study	44 cases, 15 controls	Men with varicoceles demonstrated lower sperm concentration (35.8 vs. 78.9 M/mL) and motility (43.1% vs. 57.3%). Having a varicocele was associated with a higher abundance of ROS (assessed with 2′, 7′-dichlorodihydrofluorescein staining: 46.8% vs. 29.3%), more protamine deficiency (assessed with chromomycin A3 staining: 43.5% vs. 32.8%), and DNA fragmentation (assessed with TUNEL assay: 13.2% vs. 8.9%). There was a negative correlation between DNA methylation and DNA fragmentation ($r = -0.3$).
	The relationship among sperm global DNA methylation, telomere length, and DNA fragmentation in varicocele: A cross-sectional study of 20 cases	Santana et al. [35]	2019	Case–control pilot study	20 cases, 20 controls	DNA fragmentation and telomere length were not statistically different between the two groups. Global sperm DNA methylation was lower in men with varicoceles compared to controls; however, this did not achieve statistical significance.
	Differential DNA methylation pattern and sperm quality in men with varicocele	Santana et al. [39]	2020	Prospective, observational case–control study	26 cases, 26 controls	The genomes of men with varicoceles were globally hypomethylated, when compared to the control group ($P = 0.037$). 1,695 statistically significant differentially methylated regions were identified in the varicocele group compared with the controls. Men with grade II or III varicoceles demonstrated lower levels of global DNA methylation compared to those with grade I varicoceles, though this did not achieve statistical significance.
Molecular basis for varicoceles	Is methylenetetrahydrofolate reductase (MTHFR) gene A1298C polymorphism related with varicocele risk?	Ucar et al. [40]	2015	Case–control study	107 cases, 109 controls	There was no significant difference in the frequency of C677T genotypes between patients with varicocele and healthy controls; the MTHFR-1298/A allele frequency in the varicocele group was statistically significantly higher in comparison to the control group. Men with a homozygous genotype (1298AA) for the polymorphic allele A1298C have a 2.3-fold higher risk of having varicocele compared with men who do not have this genotype (OR = 2.3, $P = 0.005$).
	Increased de novo DNA methylation enzymes in sperm of Individuals with varicocele	Rashidi et al. [41]	2021	Case–control study	35 cases, 26 controls	Similar percentage of DNMT1 positive sperm between the two groups; the percentage of DNMT3A and DNMT3B positive sperm were significantly higher in the varicocele group compared to the fertile control group (3A: 65.4% vs. 48.3%; 3B: 76.6% vs. 64.3%). Similar results at the RNA level were found.
Animal studies	Effects of varicocele on DNA methylation pattern of H19 and Snrpn gene in spermatozoa and behavioural characteristics of adult rat offspring	Zhang et al. [42]	2017	Animal study	20 cases, 20 controls	There were no significant differences in DNA methylation levels of H19 and Snrpn genes in spermatozoa among the control group, sham operation group, or varicocele-induced group.
	Aberrant expression of TET2 accounts for DNA hypomethylation in varicocele	Dinani et al. [43]	2023	Animal study	Eight controls, eight sham, eight cases	Rats in the varicocele group demonstrated decreased sperm concentration, motility, and morphology; chromatin maturity and DNA integrity (assessed by degree of DNA damage) compared to the other groups.

expected, sperm concentration and motility were impaired in men with varicoceles, but also the percentages of ROS positive, DNA damaged, and protamine deficient sperm were substantially higher in these individuals. Additionally, the percentage and intensity of DNA methylation were lower in individuals with varicocele compared to fertile men. Decreased DNA methylation was associated with higher rates of DNA fragmentation, but not with the degree of protamine deficiency and ROS susceptibility. The authors conclude that individuals with varicoceles show increased susceptibility to DNA damage with sperm DNA hypomethylation. Ultimately, DNA hypermethylation may serve a protective role in sperm.

3.2. Molecular Basis for Varicocele. DNA methyltransferases are a family of enzymes that mediate the transfer of a methyl group to DNA. In doing so, DNA methyltransferases play a critical role in embryonic development, genomic imprinting, and chromosome stability. This family of enzymes serves as one of the main epigenetic regulators that modifies gene expression [24–27]. The main active DNA methyltransferases are DNMT1, DNMT3A, and DNMT3B. Rashidi et al. [41] performed a case–control study to investigate the relative contributions of DNMT1, DNMT3A, and DNMT3B in sperm of infertile men with grade II or III varicocele ($n = 35$) with fertile individuals ($n = 26$). The DNA methyltransferases were assessed at a protein level with immunostaining and at the mRNA expression level with quantitative RT-PCR. Their findings revealed a similar percentage of DNMT1 positive sperm between the two groups; however, the percentage of DNMT3A and DNMT3B positive sperm were significantly higher in the varicocele group compared to the fertile control group. Similar associations were identified at the mRNA expression level. Of note, DNMT1 is responsible for preservation of methylation pattern through DNA methyltransferase activity during cell division, while DNMT3A and DNMT3B are de novo methyltransferases that play a central role in development. These findings suggest that altered DNA methylation in men with varicoceles may be driven by enzymes involved in de novo DNA methylation and that higher expression of sperm DNMT3A and DNMT3B—both at protein and mRNA expression levels—in men with varicocele may reflect an aberrant physiologic status of the testis in these individuals.

Ucar et al. [40] performed a case–control study of 107 men with varicoceles and 109 fertile and healthy men to explore the impact of gene polymorphisms associated with the methylenetetrahydrofolate reductase (MTHFR) gene. MTHFR is an enzyme involved in folate metabolism and plays a key role in regulation of methyl group balance between DNA synthesis and DNA methylation. C677T and A1298C polymorphisms are the most well-known MTHFR gene variations that lead to reduced enzyme activity, and previous studies suggest that MTHFR polymorphisms may be genetic risk factors for male infertility [40]. Ucar et al. [40] found that MTHFR-1298/A allele frequency in the varicocele group was significantly higher in comparison to the fertile control group. Men with a homozygous genotype (1298AA) for the polymorphic allele A1298C have a 2.3-fold higher risk of having varicocele compared with men who do not have this genotype. These findings suggest a strong genetic component for the development of varicocele in at least a subset of men.

3.3. Relevant Animal Studies. Data on varicoceles and DNA methylation in animal models are quite limited. A relevant study published by Zhang et al. [42] assessed the effect of varicoceles on DNA methylation patterns of two genes in the sperm of adult rat offspring with or without varicoceles: H19 and Snrpn. The product of the H19 gene is an untranslated RNA that is expressed exclusively from the maternal chromosome during mammalian development, and aberrant H19 methylation is associated with growth retardation. Small nuclear ribonucleoprotein polypeptide N (Snrpn) is a maternally methylated imprinted gene. The differentially methylated regions of these two imprinted genes were assessed in sperm to understand the effect of varicocele-induced paternal germline epigenetic alterations on offspring. There were no significant differences in DNA methylation levels of H19 and Snrpn genes in spermatozoa among the control group, sham operation group, and varicocele-induced group. These results indicate that the presence of varicocele did not alter the methylation profiles of H19 and Snrpn genes in this animal model. Further work in animal models of varicocele may have some utility as the field of sperm epigenetics continues to grow.

In a recent study by Dinani et al. [43], 24 rats were assigned to three groups: control, sham, and varicocele. Assessment of sperm quality and DNA methylation patterns of testicular spermatogenic cells showed that rats in the varicocele group demonstrated decreased sperm parameters (worse sperm concentration, motility, and morphology), chromatin maturity, and DNA integrity (assessed by degree of DNA damage) compared to the other groups. Furthermore, these rats also showed increased sperm lipid peroxidation compared to the sham and control groups. DNA methylation can either result in the production of 5-methylcytosine (5-mC) through the action of DNA methyltransferase enzymes or 5-hydroxymethylcytosine (5-hmC) through ten-eleven translocation (TET) enzymes. This study also found that in the varicocele group, the 5-mC signal normally abundant in spermatogonia cells was decreased through a TET-dependent process. This is attributed to aberrant TET2 activity in varicocele but not the control or sham groups given the associated rice in 5-hmC and TET2 mRNA and protein upregulation. The authors suggest that these findings may be leveraged as potential biomarkers of spermatogenesis, especially within the context of a varicocele [43].

3.4. Summary. Varicocele represents an important risk factor or male factor infertility given its strong association with impaired semen parameters, molecular characteristics of spermatozoa, and the testicular microenvironment. Less clear, however, is how varicoceles exert their deleterious effects. Researchers have posited that epigenetic factors may play a role in varicocele-associated infertility, as prior work has demonstrated that certain sperm DNA methylation patterns negatively impact reproductive success [6–20] and have an association with male infertility [30–34]. These differences in DNA methylation patterns may have implications for

future oocyte fertilization and embryo development as the global methylation level of sperm DNA has been demonstrated to influence the pregnancy rate in IVF [17, 19, 35–37]. To our knowledge, this is the first comprehensive review of the literature to summarize the role of DNA methylation in varicocele-associated infertility. Our report highlights the relative ubiquity of global hypomethylation in men with varicoceles. The major limitations of this study are related to the limited published research available given the still-nascent nature of this field.

4. Conclusion

Our review of the literature on varicocele-associated sperm DNA methylation indicates that global hypomethylation occurs in association with varicoceles. Some data suggest a dose-dependent effect of varicocele grade on sperm DNA methylation, with the greatest magnitude changes seen in grades II and III varicoceles [39]. While changes in DNA methylation patterns would be expected following varicocelectomy, such changes were not found to be statistically significant in one study reviewed here [41]. However, current human studies are limited by small sample size and short follow-up time. Future clinical and preclinical studies with robust sample sizes and hypothesis-driven investigations are necessary to further understand the relationship between DNA methylation patterns and varicocele-associated male infertility.

Data Availability

No underlying data were collected or produced in this study.

Conflicts of Interest

J.N.M. has served as a consultant for Boston Scientific, Endo Pharmaceuticals, and Halozyme Therapeutics.

Acknowledgments

This work was supported in part by the H and H Lee Surgical Research Grant via the L.B. Research and Education Foundation and a Scholars in Sexuality Research Grant from the Sexual Medicine Society of North America to G.E.S.

References

[1] B. G. Clarke, "Incidence of varicocele in normal men and among men of different ages," *JAMA*, vol. 198, no. 10, pp. 1121-1122, 1966.

[2] J. I. Gorelick and M. Goldstein, "Loss of fertility in men with varicocele," *Fertility and Sterility*, vol. 59, no. 3, pp. 613–616, 1993.

[3] S. H. Greenberg, L. I. Lipshultz, and A. J. Wein, "Experience with 425 subfertile male patients," *Journal of Urology*, vol. 119, no. 4, pp. 507–510, 1978.

[4] A. Agarwal, A. Hamada, and S. C. Esteves, "Insight into oxidative stress in varicocele-associated male infertility: part 1," *Nature Reviews Urology*, vol. 9, no. 12, pp. 678–690, 2012.

[5] K. F. Green, T. T. Turner, and S. S. Howards, "Varicocele: reversal of the testicular blood flow and temperature effects by varicocele repair," *Journal of Urology*, vol. 131, no. 6, pp. 1208–1211, 1984.

[6] F. Giacone, R. Cannarella, L. M. Mongioì et al., "Epigenetics of male fertility: effects on assisted reproductive techniques," *The World Journal of Men's Health*, vol. 37, no. 2, pp. 148–156, 2019.

[7] K. I. Aston, V. Punj, L. Liu, and D. T. Carrell, "Genome-wide sperm deoxyribonucleic acid methylation is altered in some men with abnormal chromatin packaging or poor in vitro fertilization embryogenesis," *Fertility and Sterility*, vol. 97, no. 2, pp. 285–292.e4, 2012.

[8] S. Laurentino, J. Beygo, V. Nordhoff et al., "Epigenetic germline mosaicism in infertile men," *Human Molecular Genetics*, vol. 24, no. 5, pp. 1295–1304, 2015.

[9] M. Laqqan, S. Tierling, Y. Alkhaled, C. Lo Porto, E. F. Solomayer, and M. Hammadeh, "Spermatozoa from males with reduced fecundity exhibit differential DNA methylation patterns," *Andrology*, vol. 5, no. 5, pp. 971–978, 2017.

[10] H. M. McSwiggin and A. M. O'Doherty, "Epigenetic reprogramming during spermatogenesis and male factor infertility," *Reproduction*, vol. 156, no. 2, pp. R9–R21, 2018.

[11] R. G. Urdinguio, G. F. Bayon, M. Dmitrijeva et al., "Aberrant DNA methylation patterns of spermatozoa in men with unexplained infertility," *Human Reproduction*, vol. 30, no. 5, pp. 1014–1028, 2015.

[12] O. A. Oluwayiose, H. Wu, H. Saddiki et al., "Sperm DNA methylation mediates the association of male age on reproductive outcomes among couples undergoing infertility treatment," *Scientific Reports*, vol. 11, no. 1, Article ID 3216, 2021.

[13] C. Marcho, O. A. Oluwayiose, and J. R. Pilsner, "The preconception environment and sperm epigenetics," *Andrology*, vol. 8, no. 4, pp. 924–942, 2020.

[14] F. Asenius, A. F. Danson, and S. J. Marzi, "DNA methylation in human sperm: a systematic review," *Human Reproduction Update*, vol. 26, no. 6, pp. 841–873, 2020.

[15] T. G. Jenkins, K. I. Aston, T. D. Meyer et al., "Decreased fecundity and sperm DNA methylation patterns," *Fertility and Sterility*, vol. 105, no. 1, pp. 51–57.e3, 2016.

[16] K. I. Aston, P. J. Uren, T. G. Jenkins et al., "Aberrant sperm DNA methylation predicts male fertility status and embryo quality," *Fertility and Sterility*, vol. 104, no. 6, pp. 1388–1397.e5, 2015.

[17] M. Benchaib, V. Braun, D. Ressnikof et al., "Influence of global sperm DNA methylation on IVF results," *Human Reproduction*, vol. 20, no. 3, pp. 768–773, 2005.

[18] R. Cannarella, A. Crafa, R. A. Condorelli, L. M. Mongioi, S. La Vignera, and A. E. Calogero, "Relevance of sperm imprinted gene methylation on assisted reproductive technique outcomes and pregnancy loss: a systematic review," *Systems Biology in Reproductive Medicine*, vol. 67, no. 4, pp. 251–259, 2021.

[19] T. G. Jenkins and D. T. Carrell, "The sperm epigenome and potential implications for the developing embryo," *Reproduction*, vol. 143, no. 6, pp. 727–734, 2012.

[20] N. El Hajj, U. Zechner, E. Schneider et al., "Methylation status of imprinted genes and repetitive elements in sperm DNA from infertile males," *Sexual Development*, vol. 5, no. 2, pp. 60–69, 2011.

[21] J. C. Rotondo, C. Lanzillotti, C. Mazziotta, M. Tognon, and F. Martini, "Epigenetics of male infertility: the role of DNA methylation," *Frontiers in Cell and Developmental Biology*, vol. 9, Article ID 689624, 2021.

[22] C. K. Naughton, A. K. Nangia, and A. Agarwal, "Pathophysiology of varicoceles in male infertility," *Human Reproduction Update*, vol. 7, no. 5, pp. 473–481, 2001.

[23] M. M. Sheehan, R. Ramasamy, and D. J. Lamb, "Molecular mechanisms involved in varicocele-associated infertility," *Journal of Assisted Reproduction and Genetics*, vol. 31, no. 5, pp. 521–526, 2014.

[24] W. Reik, W. Dean, and J. Walter, "Epigenetic reprogramming in mammalian development," *Science*, vol. 293, no. 5532, pp. 1089–1093, 2001.

[25] J. C. Rice and C. D. Allis, "Histone methylation versus histone acetylation: new insights into epigenetic regulation," *Current Opinion in Cell Biology*, vol. 13, no. 3, pp. 263–273, 2001.

[26] B. Jin, Y. Li, and K. D. Robertson, "DNA methylation: superior or subordinate in the epigenetic hierarchy?" *Genes Cancer*, vol. 2, no. 6, pp. 607–617, 2011.

[27] J. Lan, S. Hua, X. He, and Y. Zhang, "DNA methyltransferases and methyl-binding proteins of mammals," *Acta Biochimica et Biophysica Sinica*, vol. 42, no. 4, pp. 243–252, 2010.

[28] O. Tunc and K. Tremellen, "Oxidative DNA damage impairs global sperm DNA methylation in infertile men," *Journal of Assisted Reproduction and Genetics*, vol. 26, no. 9-10, pp. 537–544, 2009.

[29] M. Tavalaee, M. Bahreinian, F. Barekat, H. Abbasi, and M. H. Nasr-Esfahani, "Effect of varicocelectomy on sperm functional characteristics and DNA methylation," *Andrologia*, vol. 47, no. 8, pp. 904–909, 2015.

[30] M. Benchaib, V. Braun, J. Lornage et al., "Sperm DNA fragmentation decreases the pregnancy rate in an assisted reproductive technique," *Human Reproduction*, vol. 18, no. 5, pp. 1023–1028, 2003.

[31] C. J. Marques, F. Carvalho, M. Sousa, and A. Barros, "Genomic imprinting in disruptive spermatogenesis," *The Lancet*, vol. 363, no. 9422, pp. 1700–1702, 2004.

[32] H. Kobayashi, A. Sato, E. Otsu et al., "Aberrant DNA methylation of imprinted loci in sperm from oligospermic patients," *Human Molecular Genetics*, vol. 16, no. 21, pp. 2542–2551, 2007.

[33] S. Houshdaran, V. K. Cortessis, K. Siegmund, A. Yang, P. W. Laird, and R. Z. Sokol, "Widespread epigenetic abnormalities suggest a broad DNA methylation erasure defect in abnormal human sperm," *PLoS ONE*, vol. 2, no. 12, Article ID e1289, 2007.

[34] M. Benchaib, M. Ajina, J. Lornage, A. Niveleau, P. Durand, and J. F. Guerin, "Quantitation by image analysis of global DNA methylation in human spermatozoa and its prognostic value in in vitro fertilization: a preliminary study," *Fertility and Sterility*, vol. 80, no. 4, pp. 947–953, 2003.

[35] V. P. Santana, C. L. Miranda-Furtado, D. C. C. Pedroso et al., "The relationship among sperm global DNA methylation, telomere length, and DNA fragmentation in varicocele: a cross-sectional study of 20 cases," *Systems Biology in Reproductive Medicine*, vol. 65, no. 2, pp. 95–104, 2019.

[36] R. Dada, M. Kumar, R. Jesudasan, J. L. Fernandez, J. Gosalvez, and A. Agarwal, "Epigenetics and its role in male infertility," *Journal of Assisted Reproduction and Genetics*, vol. 29, no. 3, pp. 213–223, 2012.

[37] J. Thilagavathi, M. Kumar, S. S. Mishra, S. Venkatesh, R. Kumar, and R. Dada, "Analysis of sperm telomere length in men with idiopathic infertility," *Archives of Gynecology and Obstetrics*, vol. 287, no. 4, pp. 803–807, 2013.

[38] M. Bahreinian, M. Tavalaee, H. Abbasi, A. Kiani-Esfahani, A. H. Shiravi, and M. H. Nasr-Esfahani, "DNA hypomethylation predisposes sperm to DNA damage in individuals with varicocele," *Systems Biology in Reproductive Medicine*, vol. 61, no. 4, pp. 179–186, 2015.

[39] V. P. Santana, E. R. James, C. L. Miranda-Furtado et al., "Differential DNA methylation pattern and sperm quality in men with varicocele," *Fertility and Sterility*, vol. 114, no. 4, pp. 770–778, 2020.

[40] V. B. Ucar, B. Nami, H. Acar, and M. Kılınç, "Is methylenetetrahydrofolate reductase (MTHFR) gene A1298C polymorphism related with varicocele risk?" *Andrologia*, vol. 47, no. 1, pp. 42–46, 2015.

[41] M. Rashidi, M. Tavalaee, H. Abbasi, M. Nomikos, and M. H. Nasr-Esfahani, "Increased de novo DNA methylation enzymes in sperm of individuals with varicocele," *Cell Journal*, vol. 23, no. 4, pp. 389–396, 2021.

[42] Q. Zhang, F. Zhang, H.-H. Gao, and J.-M. Zhang, "Effects of varicocele on DNA methylation pattern of H19 and Snrpn gene in spermatozoa and behavioural characteristics of adult rat offspring," *Andrologia*, vol. 49, no. 1, 2017.

[43] H. T. Dinani, N. Naderi, M. Tavalaee, F. Rabiee, and M. H. Nasr-Esfahani, "Aberrant expression of TET2 accounts for DNA hypomethylation in varicocele," *Cell Journal*, vol. 25, no. 10, pp. 706–716, 2023.