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IDIOPATHIC MALE INFERTILITY CAUSED BY DNA METHYLATION AND GENOMIC IMPRINTING

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ABSTRACT: Epigenetics is the study of changes in organisms caused by genetic control through different factors without alteration of the genetic code itself. Epigenetic inheritance and its under lying molecular mechanisms are the most fascinating areas of current biological and medical research data regarding the epigenetically mediated effects of a man's diet on sperm quality are emerging in the field of human reproductive health. In this regard, epigenetics has become one of the most promising research areas in understanding male infertility. Infertility is the inability to conceive after at least a year of unprotected intercourse which influences about 15% of couples worldwide. The actual cause has not surely revealed yet, hence it is termed as idiopathic male infertility. Many studies have indicated that epigenetic modifications including DNA methylation in imprinted and developmental genes, modifications of histone tail as well as the short non-coding RNAs in spermatozoa may have a role in idiopathic male infertility. The present review aims to assess the significance of DNA methylation in male infertility.

Key Words: Epigenetics, Male infertility, DNA methylation, Histone tail, Non-coding RNAs.

INTRODUCTION

Epigenetic changes can switch genes on or off, and thus can determine which proteins will be expressed and which will not. There are some known mechanisms of how the epigenetic changes impact gene expression where the DNA methylation is one of these, which is a chemical process that adds a methyl group in a region where a cytosine nucleotide is located next to a guanine nucleotide which is linked by a phosphate group. The site of such an addition is called a CpG site. One of the three enzymes known as DNA methyl transferases (DNMTs) methylates CpG sites (Egger *et al.,* 2004; Robertson 2002). DNA methylation has a close association with male infertility. Infertility is a

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heterogeneous complex disorder with multiple genetic as well as other factors such as chemicals, diet and the leading lifestyle that influences about 15% of couples worldwide where male infertility affects approximately 7% of men (Robertson 2002). It is a major public health concern that is significant on psychological, social and economic impact with an equal proportion of males and females affected. In order to identify the relationship between dysregulated DNA methylation and male infertility, the current review evaluates the role of DNA methylation in spermatogenesis. This may provide a basis for the prevention and treatment of male infertility as well as permit the evaluation of the epigenetic quality of sperm. Which may help to reduce the risk of epigenetic diseases in cases where conception is performed by Assisted Reproductive Technology (ART).

The endogenous ten-eleven translocation (TET)-mediated conversion of 5-methylcytosine (5mC) back to the unmethylated state through 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (*5fC*), and 5-carboxylcytosine (*5caC*) intermediates has raised some concerns over the interpretation of the past methylation data produced by bisulfite modification technique, since this technique is unable to distinguish between 5mC and 5hmC, leaving them both as cytosine (Huang *et al.,* 2010). Few new techniques such as oxidative bisulfite sequencing have been developed to make this distinction possible (Yu *et al.,* 2012; Booth *et al.,* 2013)**.** In order to developing the therapeutic strategies for male genital system diseases, the understanding of the mechanisms underlying DNA methylation is particularly important. This review aims to assess the significance of DNA methylation in male infertility.

MATERIAL AND METHODS

To determine the primary cause of the male infertility issue, a thorough assessment of the literature was done. In order to provide an illustration of the Mechanism of DNA methylation through the action of DNA methyltransferases (DNMTs), it was stressed that publications created on the basis of scientific findings and literature.

RESULTS AND DISCUSSION

DNA methylation mechanism: During spermatogenesis, the expression of a gene is crucially regulated by the epigenetic mechanisms which may influence male fertility. Cytosine, a key DNA base, is methylated at the position, typically in the context of CpG (a cytosine nucleotide is followed by a guanine nucleotide with the association of a phosphate group) dinucleotides and these are located on the promoter region just before a gene. The methylation of constitutive heterochromatin and promoter regions is generally associated with the reduced

gene transcription (Fig. I) (Jena *et al.,* 2014; Kuramochi *et al.,* 2014; Schutte *et al.,* 2013; Klaver *et al.,* 2013). Therefore, DNA methylation is a type of epigenetic modification that can effectively promote gene silencing (Fig. I). The methyl group for this chemical modification of the DNA is donated by S-adenosyl-L-methionine (SAM) and the methylation reaction is catalyzed by members of the DNA methyltransferases (DNMTs) family, in mammals which are classified into the following three types: DNMT1, DNMT2 and DNMT3 (Jena *et al.,* 2014; Kuramochi *et al.,* 2014; Klaver *et al.,* 2013). When the cytosine is methylated, then it inhibits the activator to set on the promoter region and leads to the silencing of the particular gene (Takashima *et al.,* 2009).

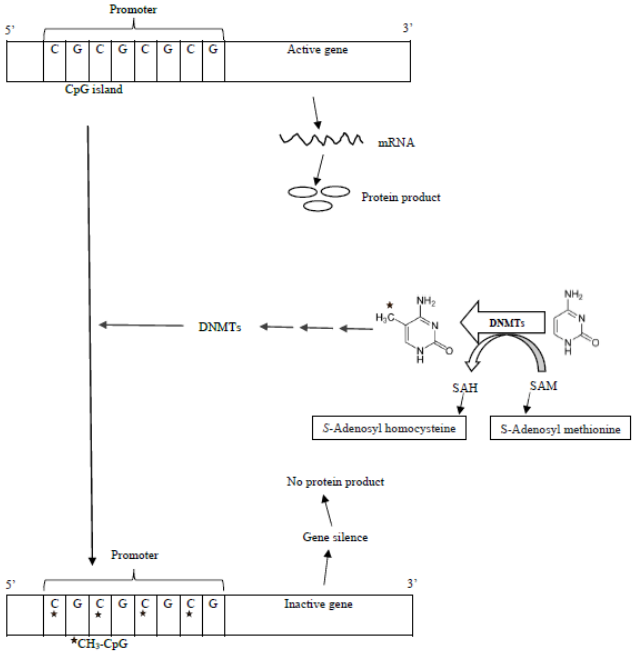

Fig. I. Mechanism of DNA methylation by the action of DNA methyltransferase (DNMTs). Cytosine is converted to 5'- methyl cytosine (*) and typically methylated in the context of CpG dinucleotides which can effectively promote gene silencing (Takashima *et al.,* 2009).

Reprogramming of methylation, genomic imprinting and male infertility: Previous studies have demonstrated that male germ cells in adult mice have a highly distinct epigenetic pattern, characterized by a unique genome wide pattern of DNA methylation (Takashima *et al.,* 2009; Godmann and Lambrot 2009; Houshdaran *et al.,* 2007; Tanaka 2007). The methylation status of testicular DNA is highly distinct, displaying an eight times higher number of hypomethylated loci as compared with somatic tissues (Marques 2010; Minor *et al.,* 2011). During the early stages in development of germ cells, a crucial role is

served to establish an epigenetic state by the alterations in DNA methylation, allowing for transcription to occur at the later phases (Kato *et al.,* 2007; Oakes *et al.,* 2007). Dean, 2005 showed that the primordial germ cells (PGCs) become demethylated at the early stage of development where re-methylation has occurred in pro-spermatogonia and fertilization signals the second stage of methylation reprogramming.

Genomic imprinting is an epigenetic mechanism resulting in altered expression of certain parental genes, while the actual gene sequence remains unchanged (Von and Reik 2015). Genomic imprinting is regulated by DNA methylation which resulting in the inheritance of only one copy-relevant imprinted gene in an embryo where a majority of imprinted genes contain DMRs that varying the methylation between parental and maternal alleles. DNA methylation-mediated genomic imprinting is established during gametogenesis, prior to fertilization (Kato *et al.,* 2007; Oakes *et al.,* 2007).

In male infertility, epigenetic modifications may serve a leading role by regulating male germ cell development and maintenance (Oldereid *et al.,* 2014; Guerrero *et al.,* 2014 and Komiya *et al.,* 2014). As a result of DNA methylation dysregulation, abnormal imprinting may be associated with male infertility. Methylation profiles of CpG sites within ICRs of imprinted genes H19 and SNRPN may potentially serve as epigenomic biomarkers for the assessment of infertility in men with multiple sperm defects (Peng *et al.,* 2018). Male infertility is associated with H19 reduced sperm DNA methylation and to MEST and SNRPN increased methylation which finally leads to the impairment of sperm and infertility arises (Santi *et al.,* 2014). An analysis is of semen from infertile men has shown that 14.4% of anomalous patriarchal methylation occurred in H19 and gene trap locus 2 genes (Khazaie *et al.,* 2014; Barazani *et al.,* 2014). In the case of spermatozoa from the oligozoospermic men, specifically in patients with the <10x106/ml volumes of ejaculation, the emergence of the hypermethylation of several maternal DMRs or the hypomethylation of H19 and intergenic-DMR was intensified (Dada *et al.,* 2012; Pacheco *et al.,* 2011). Methylation was significantly decreased at all CpGs in men with oligoasthenoteratozoospermia (OAT), achieving statistical significance in subgroups with sperm concentrations of less than 10x106/ml. These findings suggest that abnormal DNA methylation mediated genomic imprinting is associated with oligoasthenoteratozoospermia (OAT) and oligozoospermia (Owen and Segars 2009; Berthaut *et al.,* 2011; Gabory and Junien 2013). The underlying mechanisms are unknown in 70% of male infertility cases, even in cases where the causes of male infertility are clear. Therefore, further studies are required in order to elucidate the mechanisms underlying male infertility (Boissonnas *et al.,* 2013; Gunes *et al.,* 2016; Calicchio *et al.,* 2014; Gan *et al.,* 2016).

CONCLUSION

Infertility does not depend only on genetic factors. Epigenetics, DNA methylation, environmental factors as well as different life styles are also responsible. Smoking, exposure to pollution, a lack of physical activity, obesity, stress and an unhealthy diet have negative impacts on fertility in males. DNA methylation has a strong association with male infertility. In order to design the therapeutic strategies for male genital system diseases lead by abnormal sperm DNA methylation, realizing the mechanism of underlying DNA methylation is crucially important. As the studies on the regulatory mechanisms of DNA methylation during spermatogenesis are still in its' initial stages, so comprehensive studies on idiopathic male infertility caused by epigenetics are recommended through this review.

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