The effect of diabetes on fertility and Epigenetic modification and spermatogenesis

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Abstract

Diabetes is a widespread chronic disease with detrimental effects on male fertility. This study aims to investigate the relationship between diabetes and male fertility, focusing on the process of spermatogenesis and the occurrence of epigenetic modifications. This study was done as a review and library research. For this purpose, the researchers conducted in this field was examined. The results showed that spermatogenesis, the complex process of sperm cell production, is highly sensitive to disturbances in glucose metabolism caused by diabetes. The altered metabolic environment in diabetes can potentially disrupt the development and function of sperm cells. Moreover, emerging evidence suggests that diabetes can induce epigenetic modifications, which are heritable changes in gene activity without altering the DNA sequence. These epigenetic alterations may occur during spermatogenesis, affecting the quality and genetic integrity of sperm cells. Consequently, they can have significant implications for fertility and the health of offspring. Understanding the mechanisms underlying the impact of diabetes on male fertility is crucial for developing effective diagnostic and therapeutic strategies. Further research is needed to elucidate the precise molecular pathways involved in the interplay between diabetes and spermatogenesis. Additionally, investigating the epigenetic alterations associated with diabetes can provide valuable insights into the transgenerational effects on fertility and offspring health. In conclusion, diabetes exerts considerable influence on male fertility through its effects on spermatogenesis and the occurrence of epigenetic modifications. This study highlights the importance of comprehensive management and care for individuals with diabetes to mitigate the adverse effects on male reproductive health and ensure the well-being of future generations.

Keywords: “Diabetes”, “Male fertility”, “Sperm cells”, “Glucose metabolism”.

Introduction

Diabetes is a destructive disease that is divided into two types: Type 1 diabetes and Type 2 diabetes. Type 1 diabetes is caused by an autoimmune reaction against beta cells. Approximately 5% of diabetic individuals worldwide have Type 1 diabetes [1]. Type 2 diabetes affects 95% of diabetic patients. This type of diabetes often occurs in adulthood and is associated with genetic predisposition, inflammatory components, and environmental determinants such as obesity, poor dietary habits, and low physical activity. Diabetes affects various tissues, including the reproductive glands (testes). This impact can lead to reduced fertility and infertility [2]. Generally, about 15 to 20% of couples are infertile. Additionally, 30 to 40% of infertility cases are related to male factors. The health of sperm DNA is one of the factors that directly affects sperm quality and quantity. Furthermore, apart from familial and genetic backgrounds, it is possible that a portion of the 13 to 16% of young couples affected by infertility issues may suffer from hyperglycemia [3]. Additionally, metabolic imbalance, oxidative stress, and hormonal disorders caused by hyperglycemia can be considered as factors contributing to infertility. On the other hand, epigenetics refers to factors that create changes in genetic activity without altering the sequence. Research on DNA suggests that epigenetic changes may play a role in spermatogenesis and potentially impact fertility. Moreover, diabetes can directly or indirectly influence epigenetic changes [1]. These changes can particularly affect germ cells and consequently have an impact on fertility. Due to the limited studies conducted so far on the effects of diabetes on fertility, epigenetic changes, and spermatogenesis, and considering the importance of this subject, recent research has investigated this topic.

Theoretical foundations of research

Diabetes is a metabolic disorder in which the body loses its ability to utilize carbohydrates and fats effectively. This condition occurs due to a disruption in insulin secretion or insulin resistance, leading to increased blood glucose levels (hyperglycemia) and the excretion of glucose in the urine (glucosuria). Diabetes has been recognized for a long time and is associated with specific symptoms such as polyuria (increased urination), polydipsia (excessive thirst), polyphagia (increased appetite), and weight loss [2]. It is a significant global health issue today, with an estimated 246 million people worldwide affected by diabetes, according to the International Diabetes Federation. The prevalence of diabetes is rapidly increasing, with projections suggesting that the number of affected individuals will rise to 380 million by 2025 [1].

Diabetes is associated with short-term complications such as hypoglycemia and long-term complications including cardiovascular diseases, neuropathy, nephropathy, and retinopathy. Moreover, diabetes doubles the risk of individuals developing depression, with approximately one in every five diabetic patients affected by depression [2]. Beta cells in the islets of Langerhans, responsible for insulin secretion, are sensitive to the level of glucose present in the blood and adjust their secretion in response to its increase or decrease. In addition to responding to changes in glucose levels, beta cells also secrete insulin in response to changes in amino acids and fatty acids in the blood [3]. Insulin secretion is regulated and modulated by specific neuronal signals, hormones, and pharmacological factors. In individuals with type 2 diabetes, insulin is either not produced or produced in insufficient amounts to adequately control blood glucose levels. Diabetes is classified into two types: type 1 and type 2 [1]. The cause of type 1 diabetes is primarily autoimmune destruction of beta cells. Type 2 diabetes has more complex causes and arises due to the resistance of muscle and fat tissues to insulin. In the advanced stages of type 2 diabetes, multiple stages of the apoptosis cycle (programmed cell death) are activated, leading to the death of beta cells. As a result, insulin deficiency occurs, resembling a state very similar to type 1 diabetes. Therefore, in diabetic patients, blood glucose levels increase, and daily insulin injections are necessary, similar to individuals with type 1 diabetes [4].

Types of Diabetes

There are three main types of diabetes: type 1, type 2, and gestational diabetes (diabetes while pregnant).

- Type 1 Diabetes

Type 1 diabetes is thought to be caused by an autoimmune reaction (the body attacks itself by mistake). This reaction stops your body from making insulin. Approximately 5-10% of the people who have diabetes have type 1. Type 1 diabetes can be diagnosed at any age, and symptoms often develop quickly. If you have type 1 diabetes, you'll need to take insulin every day to survive. Currently, no one knows how to prevent type 1 diabetes.

- Type 2 Diabetes

With type 2 diabetes, your body doesn't use insulin well and can't keep blood sugar at normal levels. About 90-95% of people with diabetes have type 2. It develops over many years and is usually diagnosed in adults (but more and more in children, teens, and young adults). You may not notice any symptoms, so it's important to get your blood sugar tested if you're at risk. Type 2 diabetes can be prevented or delayed with healthy lifestyle changes, such as [5]:

- ✓ Losing weight.
- ✓ Eating healthy food.
- ✓ Being active.
- ✓ Gestational Diabetes.

Gestational diabetes develops in pregnant women who have never had diabetes. If you have gestational diabetes, your baby could be at higher risk for health problems. Gestational diabetes usually goes away after your baby is born.

However, it increases your risk for type 2 diabetes later in life. Your baby is more likely to have obesity as a child or teen and develop type 2 diabetes later in life [1].

Common tests to diagnose diabetes

The following tests are used for the diagnosis of diabetes [3]:

A fasting plasma glucose test measures your blood glucose after you have gone at least 8 hours without eating. This test is used to detect diabetes or prediabetes.

An oral glucose tolerance test measures your blood sugar after you have gone at least eight hours without eating and two hours after you drink a glucose-containing beverage. This test can be used to diagnose diabetes or prediabetes [4].

In a random plasma glucose test, your doctor checks your blood sugar without regard to when you ate your last meal. This test, along with an assessment of symptoms, is used to diagnose diabetes, but not prediabetes.

A hemoglobin A1c (HbA1c) test can be done without fasting, and can be used to diagnose or confirm either prediabetes or diabetes [2].

Positive test results should be confirmed by repeating the fasting plasma glucose test or the oral glucose tolerance test on a different day. When first diagnosed with diabetes, your doctor may suggest a zinc transporter 8 autoantibody (ZnT8Ab) test. This blood test -- along with other information and test results -- can help determine if a person has type 1 diabetes and not another type. The goal of having the ZnT8Ab test is a prompt and accurate diagnosis and that can lead to timely treatment [2].

The relationship between diabetes and fertility

Diabetes can be a factor that affects infertility in couples. Additionally, diabetes can lead to other problems such as disorders in the uterus and fallopian tubes, as well as difficulties in sexual function and intimacy, which can contribute to infertility. Let's explore the impact of diabetes on fertility [1]:

- Impact of diabetes on ovarian function and hormone production in women: Diabetes can have a direct and indirect impact on the functioning of the ovaries and the production of sex hormones in women. Disturbances in blood glucose and insulin levels in the body may disrupt the balance of sex hormones. This can result in irregular ovulation, lack of egg production, or failure in egg release, ultimately affecting fertility.
- Impact of diabetes on sperm quality and sexual function in men: Diabetes can also have a direct and indirect impact on sperm quality and sexual function in men. Imbalances in blood glucose and insulin levels can lead to tissue damage, oxidative stress, and hormonal disturbances, which can affect sperm quality and sexual function. This can result in reduced sperm count and motility, posing challenges to fertility [2].

Factors Affecting Fertility in Individuals with Diabetes

Several factors can impact the fertility of individuals with diabetes. Below are some of these factors: Type of diabetes and duration of the condition: The type of diabetes and the duration of the condition can significantly affect fertility. For example, studies have shown that women with type 2 diabetes may experience ovarian issues such as polycystic ovary syndrome (PCOS), which can lead to infertility. Moreover, the duration of diabetes can also have an impact, as long-term blood glucose control can directly affect fertility [2].

Blood glucose control and HbA1c levels: In examining the impact of diabetes on fertility, maintaining proper blood glucose control and HbA1c levels is crucial for fertility management. Stable blood glucose levels within the normal range, as well as adequate control of HbA1c (which reflects blood glucose control over a few months), can reduce pregnancy-related risks in women with diabetes and improve fertility [4].

Side effects of diabetes medications: When considering the impact of diabetes on infertility, it's important to note that certain medications used to control diabetes may have side effects on fertility. For example, some glucose-lowering medications may have a negative impact on ovulation, egg production, or sperm quality, thereby affecting fertility.

Influence of psychological and emotional factors on fertility in individuals with diabetes: Psychological and emotional factors can play a significant role in the fertility of individuals with diabetes. Stress, anxiety, and depression can be factors that negatively affect fertility. This can impact fertility through their influence on the hormonal system, ovaries, sperm quality, sexual function, and other factors related to fertility [1].

The Role of Epigenetics in Male Infertility

One of the causes of male infertility is epigenetic disorders, which have recently gained importance compared to other factors. Recent technological advancements have enabled the study of the role of epigenetics in male infertility. Epigenetic changes refer to a set of factors that affect gene expression but do not alter the DNA sequence. Figure 1 illustrates the main mechanisms of epigenetics.

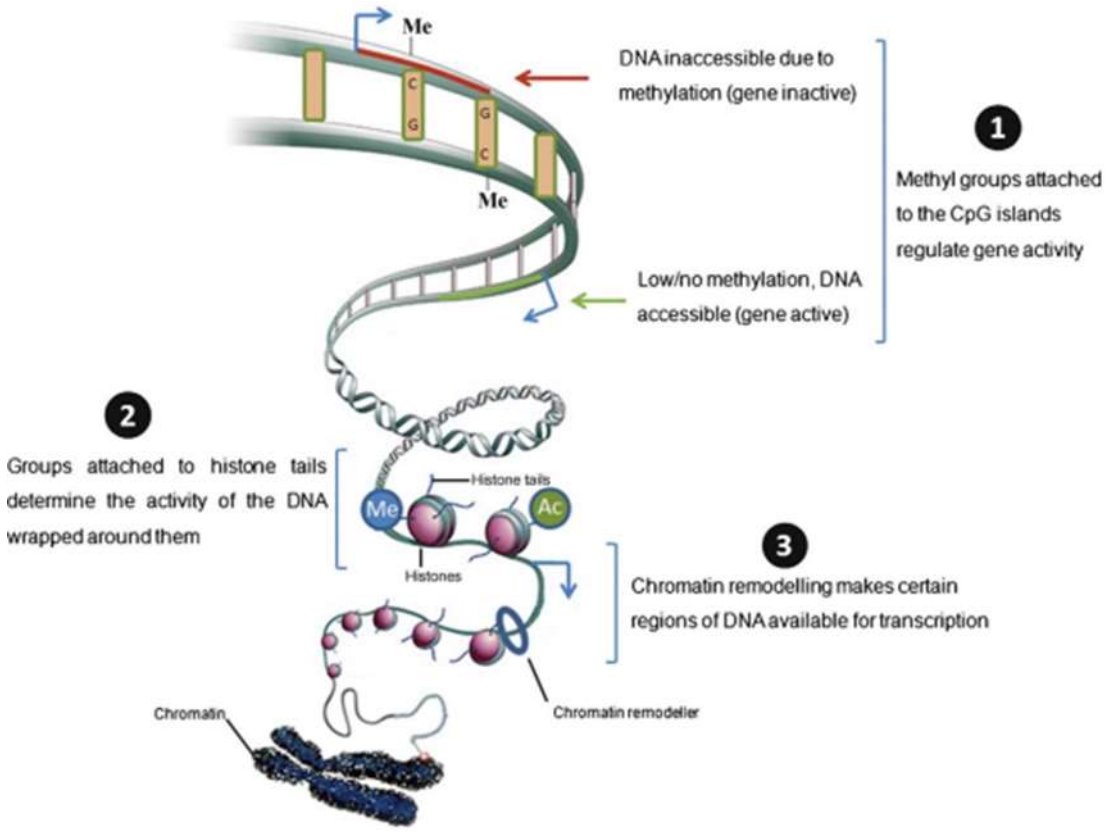

Figure 1- Major epigenetic mechanisms affecting gene activity

Hypermethylation and hypomethylation

Hypermethylation and hypomethylation are the most important mechanisms among other epigenetic factors, involving DNA [6]. Numerous human genes contain CpG islands near their promoter regions. Hypermethylation of these regions leads to gene suppression, while hypomethylation results in gene expression. Many genes are involved in regulating this process, making them good candidates for study [1]. Epigenetic infertility in men is associated with these mechanisms (Table 1).

Table 1- List of the genes/proteins important for epigenetic modifications.

Genes/proteins	Function	Reference
MTHFR	Maintains the pool of methyl donors	[7]
DNMT1, DNMT3A, DNMT3B	DNA methylation	[8, 9]
DNMT3L	Required for DNMT3A2 activity	[10, 11]
SWI/SNF, ISWI	Chromatin remodeling	[12,13]
Jhdm2a	Chromatin remodeling	[14, 15]
Suv39h1	Histone methylation	[15]
G9a	Histone methylation	[16]
LSD1-domain proteins, JmjC-domain proteins	Histone demethylation	[13]
HATs	Histone acetylation	[17]
MYST	Histone acetylation	[14]
HDACs	Histone deacetylation	[17]
SIRT1	Histone deacetylation	[18]
MUTp	Histone phosphorylation	[19]
NHK-1	Histone phosphorylation	[20]
MSK1, MSK2	Histone phosphorylation	[21]
PKA	Histone phosphorylation	[22]
HR6B	Histone ubiquitylation	[23]
E1 SUMO-activating enzyme 1, E1 SUMO-activating enzyme 2, UBC9	Histone sumoylation	[24]
CTCF	Interacts with differential methylated regions	[25, 26]

BORIS	Interacts with demethylases	[25, 26]

Histone modifications

Changes in histones following translation are highly significant for proper cellular functioning. The N-terminal region of histones is crucial for proper cellular processes such as mitosis and spermatogenesis. The N-terminal region contains amino acids that can undergo methylation, acetylation, phosphorylation, and ubiquitination. The information exchange that occurs through these modifications is referred to as the histone code. Some common modifications include acetylation, monomethylation, dimethylation, and trimethylation of lysines, as well as phosphorylation of serines [4]. Enzymes such as histone acetyltransferases, histone methyltransferases, histone kinases, histone deacetylases, histone demethylases, and histone phosphatases are involved in these modifications. Depending on the type of modification, chromatin can be classified into heterochromatin and euchromatin [27]. Heterochromatin regions are less active due to their highly condensed state, while euchromatin regions are less condensed and therefore transcriptionally active. Heterochromatin itself can be divided into two categories: constitutive heterochromatin, which cannot be converted into euchromatin, and facultative heterochromatin, which can be converted into euchromatin. Various histone modifications have been described in both euchromatin and heterochromatin regions (Table 2).

Table 2- List of aberrant epigenetic modification reported in male infertility

Genes/proteins	Aberration and male infertility	Reference
MTHFR	DNA hypermethylation results in poor semen quality and infertility	[28]
PAX8, NTF3, SFN, HRAS	DNA hypermethylation associates with poor sperm concentration, motility and morphology	[29]
JHM2DA	Knockout results in loose packaging of DNA and may cause infertility	[13]
IGF2, H19	Low methylation associates with low sperm concentration	[31]
RASGRF1	Hypermethylation at the imprinted locus associates with poor semen parameters	[29]
GTL2	Hypermethylation at the imprinted locus associates with poor semen parameters	[32]
PLAG1, D1RAS3, MEST	Hypermethylation at the imprinted loci associates with poor semen parameters	[29]

Chromatin remodeling

Chromatin remodeling complexes can alter the structure or position of nucleosomes on DNA using the energy derived from ATP hydrolysis. Through this mechanism, genes can become more accessible for transcription or completely inaccessible.

Epigenetics in assisted reproductive technologies

As described previously, the DNA of spermatozoa is differentially methylated at several maternal and paternal imprinting regions, as well as exhibits unique global methylation patterns. Reprogramming of the epigenome and imprinted loci during gametogenesis and peri-implantation stages is very crucial for maintaining proper pattern of inheritance, particularly at imprinted loci [25, 26]. There is a concern that assisted reproductive technologies such as intracytoplasmic sperm injection (ICSI) and round spermatid injection (ROSI) may increase the incidence of imprinting disorders and adversely affect embryonic development by using immature spermatozoa that may not have proper imprints or global methylation established [29]. Deregulation of imprinted regions has been associated with the onset of Angelman syndrome in the cases undergoing intracytoplasmic sperm injection [1]. Deregulation of Igf2 imprinted loci has been previously associated with malformed offspring in mice, characterized by retardation [29] and Beckwith-Wiedemann syndrome [2]. However, long term studies on imprinting disorders in assisted reproductive techniques have denied an association between the two [4]. The evidence describing ART procedures as increasing the frequency of imprinting disorders such as Prader-Willi Syndrome, Beckwith-Wiedemann Syndrome and Angelman Syndrome has remained contradictory. Doornbos et al., Manning et al. and others contend that use of ART does not increase the risk of imprinting disorders [5], while Manipalviratn et al., Shiota et al. and others conclude that it indeed does [33]. Large longitudinal studies must be conducted to examine this connection more thoroughly. Evidence has been provided, however, that ROSI is linked to abnormal zygotic epigenetic regulation. Indeed, a study by Kishigami et al. demonstrated that injection with round spermatids versus mature spermatozoon results in distinguishable methylation patterns of the paternal zygotic genome (Okada et al, 2007). Data from this study show that zygotic genomes derived from round spermatids are remethylated after initial demethylation before completion of the first mitosis [2]. This inability to prevent global DNA remethylation does indeed lead to abnormal genome-wide DNA methylation in the paternal zygotic genome [33]. Moreover, unlike spermatozoa, round spermatids exhibit

H3K9 trimethylation, which is preserved through the first mitosis of the zygote [5]. The authors believe these differences in epigenetic patterns may account for the lower success rates of ROSI [4].

Impact of assisted reproductive technology on epigenetic profile

Studies in humans and mice have shown that oocytes induced by hormonal stimulation and embryos created through assisted reproductive technologies (ART) exhibit differences in gene expression and DNA methylation patterns. Several recent studies have also indicated a higher prevalence of epigenetic abnormalities among children born through ART compared to naturally conceived children. This increased prevalence has been reported for Angelman syndrome with an odds ratio of 6 to 12, and for Beckwith-Wiedemann syndrome with an odds ratio of 6 to 17 [1]. An interesting observation in both of these syndromes is that they involve abnormalities in the maternal allele imprinting. Among these children, loss of methylation in a differentially methylated region of maternal origin in 11q15 is eight times more frequent for Angelman syndrome, and 1.9 times more frequent for Beckwith-Wiedemann syndrome in 11q15. It is worth noting that environmental conditions can also contribute to these types of changes in the human embryo [2].

For many years, the role of epigenetic factors in infertility and the impact of assisted reproductive technologies on the epigenetic profile of embryos were not widely recognized. However, recent studies have shown that epigenetic factors are one of the important and measurable factors in male infertility, and thus these factors should be further investigated and incorporated into diagnostic methods for male infertility [33]. On the other hand, it is now evident that artificial ovarian stimulation, which is a stage of infertility treatment, is associated with epigenetic alterations in paternal and maternal alleles, but it is still unclear whether epigenetic abnormalities themselves contribute to infertility or if they are a consequence of the artificial ovarian stimulation. Therefore, further studies are needed to determine the precise prevalence of epigenetic abnormalities among children conceived through ART and to investigate the impact of ART on the epigenetic pattern of embryos [4].

Impact of parental diabetes on male germ cell epigenetic regulation

Epigenetic patterning starts in the germ line and is essential for normal embryo and postnatal development.62 Epigenetic modifications during germ cell development are postulated to play roles in gene expression, meiosis, genomic integrity, and genomic imprinting.63 Epigenetics refers to a collection of mechanisms and phenomena that define the phenotype of a cell without affecting the genotype. Epigenetic states can be modified by environmental factors, which may contribute to the development of abnormal phenotypes [29]. Epidemiological evidence increasingly suggests that environmental exposures early in development have a role in susceptibility to disease in later life [28]. A growing body of data, from animal as well as human studies, has established that the molecular basis of programming involves altered DNA methylation. In molecular terms, it represents chromatin modifications including DNA methylation, histone modifications, remodeling of nucleosomes and the higher-order chromatin reorganization, and noncoding RNAs. Epigenetic modifications are essential during spermatogenesis (Figure 2).

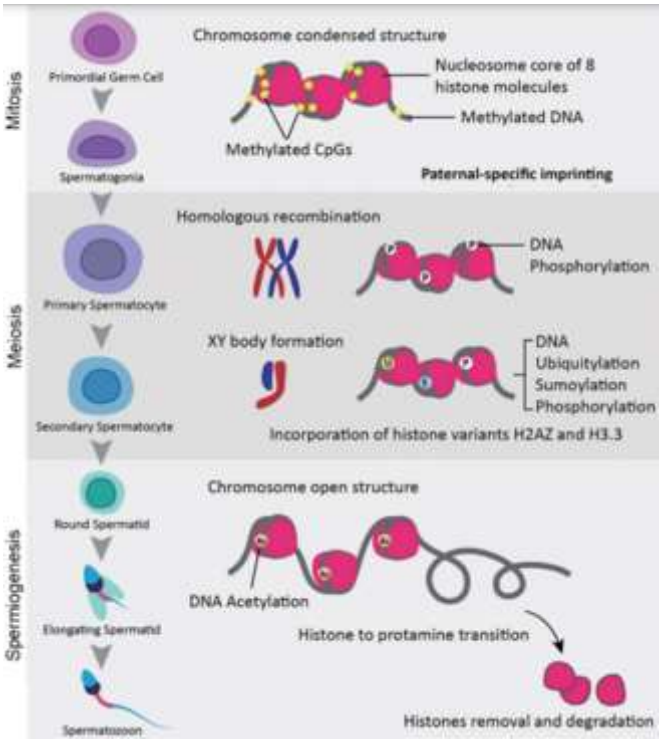

Figure 2- Epigenetic modifications occurring during spermatogenesis.

Streptozotocin induced diabetes model

In animals, the male reproductive milieu (testis and epididymal sperm) of streptozotocin (STZ)-induced diabetic mice is subjected to significant OS during the early diabetic phase and may apparently contribute to the development of testicular dysfunction, thus leading to altered steroidogenesis and impaired spermatogenesis [2]. In insulin-dependent diabetes, leydig cell function and testosterone production decrease due to the absence of the stimulatory effect of insulin on these cells and an insulin-dependent decrease in FSH, which, in turn, reduces LH levels. Sperm output and fertility are reduced because of a decrease in FSH caused by a reduction in insulin [2]. Hyperglycemia has an adverse effect on sperm concentration and motility via the changes in energy production and free radical management [4]. Furthermore, rats treated with STZ for 1 month, showed some metabolic adaptations, such as the increase in the efficiency of mitochondrial ATP production, in order to circumvent the deleterious effects promoted by the disease [2]. Male diabetes may cause male subfertility by altering steroidogenesis, sperm motility, and GLUT expression. Fertilization rates were distinctly lower in the Akita group, and the STZ-injected male group compared with the normal group [5]. Furthermore, in fertilized zygotes, embryo developmental rates to the blastocyst stage in two diabetic models were lower than that in controls [29]. Mallidis et al. described the changes in the testicular metabolome after the induction by STZ in an experimental model of type 1 diabetes and identified the perturbations in several important metabolites. Specifically, diabetic mice showed the decreased carnitine, creatine, and choline and the increased lactate, alanine, and myo-inositol [6]. Epidermal growth factor deficiency is a potential cause for the pathogenesis of oligozoospermia in diabetic mice. STZ-diabetes also notably reduced the epididymal tissue concentrations of testosterone, androgen-binding protein, sialic acid, and glycerylphosphorylcholine, suggesting its adverse effects on the secretory activity and the concentrating capacity of epididymal epithelium [5]. Impaired cauda epididymidal sperm motility and fertility in STZ-diabetic rats implied the defective sperm maturation. Insulin replacement prevented these changes either partially or completely [2]. From the above findings, it is evident that STZ-diabetes has an adverse effect on sperm maturation, which may be caused by the decrease in the bioavailability of testosterone and epididymal secretory products. Some scientists indicated that the germ cell abnormalities observed in the hyperglycemic group could be interpreted as the primary effect of STZ, not the hyperglycemia [34]. Some researchers demonstrated that STZ-induced DM may influence the male fertility potential via affecting sperm parameters and DNA integrity in mice. However, diabetes has no detrimental effect on histone-protamines replacement during the testicular phase of sperm chromatin packaging [4].

Literture review

Cescon et al (2020) considered the environmental impact on male (in) fertility via epigenetic route. In this scenario, evidence now suggests that epigenetics shapes endocrine functions, linking genetics and environment. During fertilization, spermatozoa share with the oocyte their epigenome, along with their haploid genome, in order to orchestrate embryo development. The epigenetic signature of spermatozoa is the result of a dynamic modulation of the epigenetic marks occurring, firstly, in the testis—during germ cell progression—then, along the epididymis, where spermatozoa still receive molecules, conveyed by epididymosomes. Paternal lifestyle, including nutrition and exposure to hazardous substances, alters the phenotype of the next generations, through the remodeling of a sperm epigenetic blueprint that dynamically reacts to a wide range of environmental and lifestyle stressors. With that in mind, this review will summarize and discuss insights into germline epigenetic plasticity caused by environmental stimuli and diet and how spermatozoa may be carriers of induced epimutations across generations through a mechanism known as paternal transgenerational epigenetic inheritance [33].

Rotondo et al (2021) studied the epigenetics of male infertility: the role of DNA methylation. The findings support the hypothesis that sperm DNA methylation is associated with sperm alterations and infertility. Several genes have been found to be differentially methylated in relation to impaired spermatogenesis and/or reproductive dysfunction. Particularly, DNA methylation defects of MEST and H19 within imprinted genes and MTHFR within non-imprinted genes have been repeatedly linked with male infertility. A deep knowledge of sperm DNA methylation status in association with reduced reproductive potential could improve the development of novel diagnostic tools for this disease. Further studies are needed to better elucidate the mechanisms affecting methylation in sperm and their impact on male infertility [2].

Jazayeri et al (2022) explored Underestimated aspects in male infertility: epigenetics is a new approach in men with obesity or diabetes. The results show Many molecular mechanisms involved in male infertility. Destructive effects of infertility on the next generations are not well understood. Approximately 60-75% of male infertility cases have idiopathic causes, and there is a need for additional investigations other than routine examinations. Molecular factors that surround DNA, which are mitotically stable and independently regulate genome activity of DNA sequences, are known as epigenetics. The known epigenetic mechanisms are DNA methylation, histone modifications and non-coding RNAs. Prevalence of metabolic diseases has been increased dramatically because of changes in lifestyle and the current levels of inactivity. Metabolic disorders, such as obesity and diabetes, are prevalent reasons for male infertility; despite the association between metabolic diseases and male infertility, few studies have been conducted on the effects of epigenetic alterations associated with these diseases and sperm abnormalities. Diabetes can affect the reproductive system and testicular function at multiple levels; however, there are very few molecular and epigenetic

studies related to sperm from males with diabetes. On the other hand, obesity has similar conditions, while male obesity is linked to notable alterations in the sperm molecular architecture affecting both function and embryo quality. Therefore, in this review article, we presented new and developed technologies to study different patterns of epigenetic changes, and explained the exact mechanisms of epigenetic changes linked to metabolic diseases and their relationship with male infertility [1].

Jazayeri et al (2022) examined Epigenetic modifications at DMRs of imprinting genes in sperm of type 2 diabetic men. This study has been performed to test the hypothesis of the relationship between T2DM and the epigenetic profile of the sperm, as well as sperm quality indices. This research included 42 individuals referred to the infertility clinic of Royan Institute, Iran in 2019–2021. The study subjects were assigned to three groups: normozoospermic non-diabetic (control), normozoospermic diabetic (DN) and non-normozoospermic diabetic (D.Non-N). Sperm DNA fragmentation was evaluated using the sperm chromatin structure assay technique. The global methylation level was examined using 5-methyl cytosine antibody and the methylation status in differentially methylated regions of H19, MEST, and SNRPN was assessed using the methylation-sensitive high-resolution melting technique. The results showed that the sperm global methylation in spermatozoa of D.Non-N group was significantly reduced compared with the other two groups ($P < 0.05$). The MEST and H19 genes were hypomethylated in the spermatozoa of D.Non-N individuals, but the difference level was not significant for MEST. The SNRPN gene was significantly hypermethylated in these individuals ($P < 0.05$). The results of this study suggest that T2DM alters the methylation profile and epigenetic programming in spermatozoa of humans and that these methylation changes may ultimately influence the fertility status of men with diabetes [27].

Guerra-Carvalho et al (2022) explored the molecular mechanisms regulating spermatogenesis in vertebrates: Environmental, metabolic, and epigenetic factor effects. In animal production, there are many barriers breeders and researchers have to overcome to develop new practices to improve reproductive potential and hasten sexual maturation of the commercially viable species, while maintaining meat quality and sustainability. With the utilization of molecular biology techniques, there have been relevant advances in the knowledge of spermatogenesis, especially in mammals, resulting in new possibilities to control male fertility and the selection of desirable characteristics. Most of these discoveries have not been implemented in animal production. In this review, recent studies are highlighted on the molecular pathways involved in spermatogenesis in the context of animal production. There is also exploration of the interaction between environmental factors and spermatogenesis and how this knowledge may revolutionize animal production techniques. Furthermore, new insights are described about the inheritance of desired characteristics in mammals and there is a review of nefarious actions of pollutants, nutrition, and metabolism on reproductive potential in subsequent generations. Even though there are these advances in knowledge base, results from recent studies indicate there are previously unrecognized environmental effects on spermatogenesis. The molecular mechanisms underlying this interaction are not well understood. Research in spermatogenesis, therefore, remains pivotal as a pillar of animal production sustainability [5].

Ryu et al (2022) examined abnormal histone replacement following BPA exposure affects spermatogenesis and fertility sequentially. The results demonstrated that the mRNA levels of the histone family and PRMs were significantly altered by BPA exposure in testes and spermatozoa. Subsequently, core histone proteins, the PRM1/PRM2 ratio, directly linked to male fertility, and transition proteins were significantly reduced. Furthermore, the results discovered that BPA significantly caused abnormal histone-to-protamine replacement during spermiogenesis by increased histone variants-related to histone-to-PRM transition. The levels of histone H3 modification in the testes and DNA methylation in spermatozoa were significantly increased. Consequently, sperm concentration/motility/hyperactivation, fertilization, and early embryonic development were adversely affected as a consequence of altered signaling proteins following BPA exposure [4].

Minas et al (2024) carried out Effects of diabetes-induced hyperglycemia on epigenetic modifications and DNA packaging and methylation during spermatogenesis. They expressed the impact of diabetes on various organs failure including testis has been highlighted during the last decades. If on one hand diabetes-induced hyperglycemia has a key role in induced damages; on the other hand, glucose deprivation plays a key role in inducing male infertility. Indeed, glucose metabolism during spermatogenesis has been highlighted due to post-meiotic germ cells drastic dependence on glucose-derived metabolites, especially lactate. In fact, hyperglycemia-induced spermatogenesis arrest has been demonstrated in various studies. Moreover, various sperm maturation processes related to sperm function such as motility are directly depending on glucose metabolism in Sertoli cells. It has been demonstrated that diabetes-induced hyperglycemia adversely impacts sperm morphology, motility and DNA integrity, leading to infertility. However, fertility quality is another important factor to be considered. Diabetes-induced hyperglycemia is not only impacting sperm functions, but also affecting sperm epigenome. DNA packing process and epigenetics modifications occur during spermatogenesis process, determining next generation genetic quality transmitted through sperm. Critical damages may occur due to under- or downregulation of key proteins during spermatogenesis. Consequently, unpacked DNA is more exposed to oxidative stress, leading to intensive DNA damages. Moreover, epigenetic dysregulation occurred during spermatogenesis may impact embryo quality and be transmitted to next generations, increasing offspring genetic issues. Herein we discuss the mechanisms by which diabetes-induced hyperglycemia can affect epigenetic modifications and DNA packaging and methylation during spermatogenesis thus promoting long-lasting effects to the next generation [3].

Conclusion

Diabetes is a chronic and prevalent disease worldwide that has wide-ranging and significant impacts on individuals' health. It creates challenges in controlling blood glucose levels and can lead to cardiovascular, neurological, renal, and visual complications. However, one aspect that has received less attention is the impact of diabetes on male fertility. Fertility is a vital aspect of population health, referring to a couple's ability to reproduce and conceive. However, diabetes can directly and indirectly affect male fertility. Unfortunately, the link between diabetes and male fertility is not fully understood and remains a subject that requires further investigation and research. One aspect that has garnered attention in the context of diabetes and male fertility is the impact of diabetes on the process of spermatogenesis. Sperm cells are produced in a complex and delicate process within the testicles and require proper nutrition and an optimal environment for growth and development. Considering that diabetes has a significant impact on glucose metabolism and cellular function, its effects on the process of spermatogenesis may be observed.

Furthermore, research has shown that diabetes can induce epigenetic modifications in cells. Epigenetics refers to changes in gene activity that occur without altering the DNA sequence and can be transmitted from one generation to another. These epigenetic modifications may occur during the process of spermatogenesis and have significant implications for fertility and the health of offspring.

Given the importance of fertility for society and couples affected by diabetes, a thorough examination of the impact of this disease on male fertility and a better understanding of the biological and molecular mechanisms underlying this relationship are warranted. There fore, the purpose of this study is investigating the impact of diabetes on male fertility and epigenetic modifications in the process of spermatogenesis.

Generally, the results of this study have shown that glucose metabolism is highly important for sperm cells, and both type 1 and type 2 diabetes can have detrimental effects on male fertility, particularly on sperm quality. These effects include reduced sperm motility, sperm DNA damage, and changes in seminal fluid composition. Additionally, diabetes may induce epigenetic modifications during the process of sperm production, and these modifications can be transmitted through the male germline to future generations, thereby increasing the risk of diabetes in offspring.

Therefore, this study demonstrates a significant association between diabetes and male fertility, epigenetic changes, and sperm production. Diabetes can have negative impacts on sperm quality and the health of offspring. Investigating the intergenerational effects and inheritance of diabetes on male fertility and the health of offspring is highly important. Studying the epigenetic effects and alterations in the process of sperm production can provide valuable insights into the biological and molecular factors underlying these associations, and multi-generational animal models can be utilized in this area of research.

Considering that epigenetic factors have been implicated in the development of this type of infertility, it is necessary to consider this aspect when investigating infertile individuals. Furthermore, due to the reversibility of epigenetic patterns, correcting these factors is often easier than genetic corrections in individuals. However, further research is needed to fully understand these issues. Multi-generational animal models resulting from parental hyperglycemia and intergenerational epigenetic inheritance can be utilized to investigate the intergenerational effects and inheritance of diabetes.

References

[1] Jazayeri, M., Alizadeh, A., Gilani, M. A. S., Eftekhari-Yazdi, P., Sharafi, M., & Shahverdi, A. (2022). Underestimated aspects in male infertility: epigenetics is a new approach in men with obesity or diabetes: a review. *International Journal of Fertility & Sterility*, *16*(3), 132.

[2] Rotondo, J. C., Lanzillotti, C., Mazziotta, C., Tognon, M., & Martini, F. (2021). Epigenetics of male infertility: the role of DNA methylation. Frontiers in Cell and Developmental Biology, 9, 689624.

[3] Minas, A., Camargo, M., Alves, M. G., & Bertolla, R. P. (2024). Effects of diabetes-induced hyperglycemia on epigenetic modifications and DNA packaging and methylation during spermatogenesis; A narrative review. *Iranian Journal of Basic Medical Sciences*, *27*(1), 3.

[4] Ryu, D. Y., Pang, W. K., Adegoke, E. O., Rahman, M. S., Park, Y. J., & Pang, M. G. (2022). Abnormal histone replacement following BPA exposure affects spermatogenesis and fertility sequentially. Environment International, 170, 107617.

[5] Guerra-Carvalho, B., Carrageta, D. F., Crisóstomo, L., Carvalho, R. A., Alves, M. G., & Oliveira, P. F. (2022). Molecular mechanisms regulating spermatogenesis in vertebrates: Environmental, metabolic, and epigenetic factor effects. *Animal Reproduction Science*, *246*, 106896.

[6] V.L. Simões, M.G. Alves, A.D. Martins, T.D. Dias, L. Rato, S. Socorro, P.F. Oliveira, Regulation of apoptotic signalling pathways by 5α-dihydrotestosterone and 17β-estradiol in immature rat Sertoli cells, J. Steroid Biochem. Mol. Biol. 135 (2013) 15–23.

[7] A. Bird, P. Tate, X. Nan, J. Campoy, R. Meehan, S. Cross, S. Tweedie, J. Charlton, D.

[8] H. Lei, S.P. Oh, M. Okano, R. Juttermann, K.A. Goss, R. Jaenisch, E. Li, De novo DNA cytosine methyltransferase activities in mouse embryonic stem cells, Development 122 (1996) 3195–3205.
[9] M. Okano, D.W. Bell, D.A. Haber, E. Li, DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development, Cell 99 (1999) 247–257.
[10] K. Nimura, C. Ishida, H. Koriyama, K. Hata, S. Yamanaka, E. Li, K. Ura, Y. Kaneda, Dnmt3a2 targets endogenous Dnmt3L to ES cell chromatin and induces regional DNA methylation, Genes Cells 11 (2006) 1225–12237.
[11] H.Y. Fan, K.W. Trotter, T.K. Archer, R.E. Kingston, Swapping function of two chromatin remodeling complexes, Mol. Cell 17 (2005) 805–815.
[12] J.A. Yates, T. Menon, B.A. Thompson, D.A. Bochar, Regulation of HOXA2 gene expression by the ATP-dependent chromatin remodeling enzyme CHD8, FEBS Lett. 584 (2010) 689–693.
[13] Y. Okada, G. Scott, M.K. Ray, Y. Mishina, Y. Zhang, Histone demethylase JHDM2A is critical for Tnp1 and Prm1 transcription and spermatogenesis, Nature 450 (2007) 119–123.
[14] A.H. Peters, D. O'Carroll, H. Scherthan, K. Mechtler, S. Sauer, C. Schofer, K. Weipoltshammer, M. Pagani, M. Lachner, A. Kohlmaier, S. Opravil, M. Doyle, M. Sibilia, T. Jenuwein, Loss of the Suv39h histone methyltransferases impairs mammalian heterochromatin and genome stability, Cell 107 (2001) 323–337.
[15] S. Ait-Si-Ali, V. Guasconi, L. Fritsch, H. Yahi, R. Sekhri, I. Naguibneva, P. Robin, F. Cabon, A. Polesskaya, A. Harel-Bellan, A Suv39h-dependent mechanism for silencing S-phase genes in differentiating but not in cycling cells, EMBO J. 23 (2004) 605–615.
[16] M. Tachibana, M. Nozaki, N. Takeda, Y. Shinkai, Functional dynamics of H3K9 methylation during meiotic prophase progression, EMBO J. 26 (2007) 3346– 3359.
[17] T. Thomas, M.P. Dixon, A.J. Kueh, A.K. Voss, Mof (MYST1 or KAT8) is essential for progression of embryonic development past the blastocyst stage and required for normal chromatin architecture, Mol. Cell. Biol. 28 (2008) 5093–5105.
[18] J. Yi, J. Luo, SIRT1 and p53, effect on cancer, senescence and beyond, Biochim. Biophys. Acta 1804 (2010) 1684–1689.
[19] J.A. Casas-Mollano, B.R. Jeong, J. Xu, H. Moriyama, H. Cerutti, The MUT9p kinase phosphorylates histone H3 threonine 3 and is necessary for heritable epigenetic silencing in chlamydomonas, Proc. Natl. Acad. Sci., U.S.A. 105 (2008) 6486–6491.
[20] H. Aihara, T. Nakagawa, K. Yasui, T. Ohta, S. Hirose, N. Dhomae, K. Takio, M. Kaneko, Y. Takeshima, M. Muramatsu, T. Ito, Nucleosomal histone kinase-1 phosphorylates H2A thr 119 during mitosis in the early drosophila embryo, Genes Dev. 18 (2004) 877–888.
[21] A. Soloaga, S. Thomson, G.R. Wiggin, N. Rampersaud, M.H. Dyson, C.A. Hazzalin, L.C. Mahadevan, J.S. Arthur, MSK2 and MSK1 mediate the mitogen- and stressinduced phosphorylation of histone H3 and HMG-14, EMBO J. 22 (2003) 2788– 2797.
[22] D.A. DeManno, J.E. Cottom, M.P. Kline, C.A. Peters, E.T. Maizels, M. HunzickerDunn, Follicle-stimulating hormone promotes histone H3 phosphorylation on serine-10, Mol. Endocrinol. 13 (1999) 91–105.
[23] D. Takai, P.A. Jones, Comprehensive analysis of CpG islands in human chromosomes 21 and 22, Proc. Natl. Acad. Sci., U.S.A. 99 (2002) 3740–3745
[24] K. Biermann, K. Steger, Epigenetics in male germ cells, J. Androl. 28 (2007) 466– 480.
[25] E.M. Klenova, H.C. Morse 3rd, R. Ohlsson, V.V. Lobanenkov, The novel BORIS + CTCF gene family is uniquely involved in the epigenetics of normal biology and cancer, Semin. Cancer Biol. 12 (2002) 399–414.
[26] D.I. Loukinov, E. Pugacheva, S. Vatolin, S.D. Pack, H. Moon, I. Chernukhin, P. Mannan, E. Larsson, C. Kanduri, A.A. Vostrov, H. Cui, E.L. Niemitz, J.E. Rasko, F.M. Docquier, M. Kistler, J.J. Breen, Z. Zhuang, W.W. Quitschke, R. Renkawitz, E.M. Klenova, A.P. Feinberg, R. Ohlsson, H.C. Morse 3rd., V.V. Lobanenkov, BORIS, a novel male germ-line-specific protein associated with epigenetic reprogramming events, shares the same 11-zinc-finger domain with CTCF, the insulator protein involved in reading imprinting marks in the soma, Proc. Natl. Acad. Sci., U.S.A. 99 (2002) 6806–6811.
[27] Jazayeri, M., Eftekhari-Yazdi, P., Gilani, M. A. S., Sharafi, M., & Shahverdi, A. (2022). Epigenetic modifications at DMRs of imprinting genes in sperm of type 2 diabetic men. *Zygote*, *30*(5), 638-647.

[28] D Chan, D.W. Cushnie, O.R. Neaga, A.K. Lawrance, R. Rozen, J.M. Trasler, Strainspecific defects in testicular development and sperm epigenetic patterns in 5,10 methylenetetrahydrofolate reductase-deficient mice, Endocrinology 151 (2010) 3363–3373. N. Khazamipour, M. Noruzinia, P. Fatehmanesh, M. Keyhanee, P. Pujol, MTHFR promoter hypermethylation in testicular biopsies of patients with non-obstructive azoospermia: the role of epigenetics in male infertility, Hum. Reprod. 24 (2009) 2361–2364.

[29] S. Houshdaran, V.K. Cortessis, K. Siegmund, A. Yang, P.W. Laird, R.Z. Sokol, Widespread epigenetic abnormalities suggest a broad DNA methylation erasure defect in abnormal human sperm, PloS One 2 (2007) e1289.

[30] K. Havas, A. Flaus, M. Phelan, R. Kingston, P.A. Wade, D.M. Lilley, T. OwenHughes, Generation of superhelical torsion by ATP-dependent chromatin remodeling activities, Cell 103 (2000) 1133–1142

[31] A. Poplinski, F. Tuttelmann, D. Kanber, B. Horsthemke, J. Gromoll, Idiopathic male infertility is strongly associated with aberrant methylation of MEST and IGF2/ H19 ICR1, Int. J. Androl. 33 (2010) 642–649.

[32] H. Kobayashi, A. Sato, E. Otsu, H. Hiura, C. Tomatsu, T. Utsunomiya, H. Sasaki, N. Yaegashi, T. Arima, Aberrant DNA methylation of imprinted loci in sperm from oligospermic patients, Hum. Mol. Genet. 16 (2007) 2542–2551

[33] Cescon, M., Chianese, R., & Tavares, R. S. (2020). Environmental impact on male (in) fertility via epigenetic route. *Journal of Clinical Medicine*, *9*(8), 2520.

[34] S.S. Hammoud, J. Purwar, C. Pflueger, B.R. Cairns, D.T. Carrell, Alterations in sperm DNA methylation patterns at imprinted loci in two classes of infertility, Fertil. Steril. 94 (2010) 1728–1733.