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The Role of Diabetes Mellitus in Male Reproductive Function: Review

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Diabetes mellitus (DM) induced long-term damage, dysfunctions and failures of various organs including abnormalities in male reproductive system. A lot of studies in both human and animals show that both type I and type II diabetes can cause male infertility via action at multiple levels including altered spermatogenesis, sperm count and quality, degenerative and apoptotic change in germ cells, impaired glucose metabolism in Sertoli cells compromised testosterone production and secretion and ejaculatory dysfunction. The aim of current review is to analyze the mechanisms of the two types of DM (I and II) and their impact on spermatogenesis on cellular and molecular level, evaluating hyperglycemia as a risk factor for male infertility.

Key words: diabetes mellitus, hyperglycemia, spermatogenesis, germ cells, male infertility

Introduction

Metabolic syndrome involves various abnormalities like obesity, insulin resistance/diabetes, hypertension, hormonal disorders being serious risk factor for male infertility, often associated with compromised hypothalamic-pituitary-gonadal axis. The prevalence of diabetes mellitus (DM) has risen in recent years and it was estimated that 382 million people suffer from this disease [31]. It is a chronic metabolic disorder characterized by chronic hyperglycemia impairing fat and protein metabolism. DM instigates long-term damage, dysfunctions and failures of various organs including abnormalities in male reproductive system. Type I DM is consider as a chronic autoimmune disease with a strong inflammatory component, characterized by loss of the insulin-producing beta cells of the islets of Langerhans in the pancreas, leading to insulin deficiency [26, 53]. Type II DM is characterized by insulin resistance due to compromised functioning of insulin receptors which in some cases might be combined with relatively reduced insulin secretion [18]. A number of lifestyle factors are known to be important to the development of type 2 diabetes, including obesity, lack of physical activity, poor diet, stress, and urbanization [32, 52]. Glucose metabolism is an important factor for normal spermatogenesis and steroidogenesis and disturbance in these processes can cause male

infertility [14, 36]. A lot of studies in both human and animals show that both type I and type II diabetes can cause male infertility via action at multiple levels including altered spermatogenesis, sperm count and quality, degenerative and apoptotic change in germ cells, impaired glucose metabolism in Sertoli cells compromised testosterone production and secretion and ejaculatory dysfunction [2, 21, 35].

Streptozotocin (STZ) or alloxan are used to chemically induce diabetes in rats in order to study the effect and pathogenesis of DM. STZ and alloxan share structural similarity with glucose and thus can be taken via the cell membrane glucose transporter 2 (GLUT2) causing DNA alkylation and eventual β-cells death [7, 40].

The aim of current review is to analyze the mechanisms of the two types of DM (type I and type II) and their impact on spermatogenesis on cellular and molecular level, evaluating hyperglycemia as a risk factor for male infertility.

Type I DM and spermatogenesis

DM type I is associated with male reproductive dysfunction, reduced fertility and poor sperm quality [44]. Mechanisms including changes in hormonal panels, oxidative stress, DNA damage of sperm and abnormal spermatogenesis have been reported in studies on rodent models [45]. These effects might be mediated through hormonal changes in the hypothalamic-pituitary-gonadal axis or through direct influence of insulin on the testis and spermatogenesis. It is still unclear whether the damage is induced by changes in glucose levels or by impairment in hormonal levels due to compromised hypothalamic-pituitary-gonadal axis [39]. The hypothalamus releases GnRH stimulating the pituitary to secrete LH and FSH. LH and FSH act on Sertoli cells (SCs) and Leydig cells (LCs) of the testis, respectively, stimulating spermatogenesis. DM induced changes in the levels of insulin and leptin alters GnRH release from the hypothalamus producing downstream effects on LH and FSH secretion from the pituitary, testosterone secretion from the LCs, qualitative and quantitative characteristics of spermatogenesis. As FSH and LH considered survival factor for germ cell, the reductions of levels of both of hormones triggers apoptotic cascade in germ cells [19].

The effects of DM on the reproductive axis are mediated by brain signaling. Insulin mediates its effect through binding with insulin receptor resulting in signaling cascade. How exactly insulin action in the brain impacts reproductive axis is still unknown. However, studies using a brain-specific insulin receptor knockout showed the connection between insulin signaling in the brain and fertility. Data from neuron-specific insulin receptor knockout mice (NIRKO) displayed a significant reduction in fertility and impaired spermatogenesis. Histological examinations revealed that many of the seminiferous tubules appeared normal but approximately 20% did not have a lumen and had a little or no mature sperm cells. Another study on STZ induced diabetic rats demonstrated significant decrease in diameter of seminiferous tubules [16].

Insulin/IGF1 signaling is implicated in male reproductive system and both are secreted by Leydig and Sertoli cells, suggesting its regulatory role on spermatogenesis. Consistent with this, receptors for IGF1 and/or insulin have been described at all stages of spermatogenesis in a variety of mammals. Gene knockout studies have further demonstrated the importance of the insulin/IGF1 signaling pathway in growth and reproduction. Deletion of Igf1 causes reductions in body weight, testis weight, testosterone concentration, and sperm counts. In the absence of the insulin family of receptors, male sex determination during embryonic development is inhibited [29].

In type I, deficiency of insulin production is linked with circulating levels of leptin, which is an important molecule secreted by the fat cells, responsible for the signals to the hypothalamus and thus for regulation of the reproductive function. Leptin-treated male animals had significantly elevated serum levels of FSH, increased testicular and

seminal vesicle weight, elevated sperm counts compared to controls, suggesting that leptin stimulates reproduction in males and may serve as a permissive signal to reproductive system of normal animals [13]. Results in humans, showed that leptin levels were decreased in newly-diagnosed type 1 diabetes patients [10]. A recent study showed that leptin-therapy in non-obese diabetic mouse restored normal blood glucose levels suggesting that restoration of leptin levels in type I DM might be able to reverse many of the effects of insulin deficiency on reproductive system [62].

It is well known that both leptin and insulin interact with the hypothalamus regulating the output of GnRH. The relationship between LH and insulin has been shown in transgenic mice that lacked brain insulin receptors [17]. Reduction by 60% in the circulating LH was found which indicated dysfunction of hypothalamus-pituitary-gonadal axis and testosterone deficiency. A significant reduction in number of LCs was reported in this type transgenic animals [12]. Also, insulin is an imperative role on control of cell proliferation and metabolism of LCs [7]. In vitro studies with primary pituitary cultures demonstrate induced release of LH in the presence of insulin-like growth factor-1 (IGF-1) and insulin [64]. In type I DM, the absence of the inductive effect of insulin on GnRH-induced LH and FSH release, inhibits testosterone production by LCs of the testis.

Clinical investigation on diabetic patients reported that LH secretion in response to GnRH signals was lower in this patient population than in healthy controls [11]. Similar results by Lopez-Alvarenga et al. concluded that type I DM affected the hypothalamic-pituitary-gonadal axis resulting in decreased LH secretion and hence decreased testosterone production [39]. Data by Maneesh et al. [42] on diabetic and controlled patients found out that men with DM had reduced testosterone, LH and FSH levels [42].

Another study in sixty-nine men with type I diabetes revealed that only 7% had low total testosterone levels. By contrast 20.3% with type I diabetes had low calculated free testosterone, similar to that observed in type II diabetes. Low testosterone levels were independently associated with insulin resistance in men with type I diabetes as well as type II diabetes. Serial measurements also reveal an inverse relationship between changes in testosterone and insulin resistance [30]. Normalization of the plasma testosterone concentration were observed after 4 days of insulin treatment in newly-diagnosed type I diabetic patients [28]. All these data suggest that testosterone level is impaired by type I diabetes which could lead to inhibition of spermatogenesis.

Taken together the data from animal models and clinical studies about decreased LH secretion, reported disturbance of spermatogenesis, especially later stages, associated by decreased testosterone production by LCs.

One of the key enzymes in androgen biosynthesis is the enzyme 3β-hydroxysteroid dehydrogenase (3β-HSD) which is a marker for LC steroidogenesis. Data from STZ-induced diabetic rats suggested an increase in smooth endoplasmic reticulum, mitochondrial and lipid content in LCs, together with decreased 3β-HSD activity and serum testosterone levels [7]. Recent studies revealed that the expression of 3 β-HSD was significantly decreased in LCs from the diabetic mice [37]. It is reported that the number of LH binding sites in LCs from diabetic rats was severely lowered after induction of diabetes, leading to suppression of synthesis and secretion of testosterone associated with altered expression of androgen receptor and IGF-1 [12, 34].

It is well known that germ cells are highly differentiated cells. In order to acquire and maintain motility, as well as to complete capacitation and the following acrosome reaction, the male gametes need a lot of energy. The main sources of energy for sperms are the carbohydrates, such as glucose and fructose. Sugars are rich in –OH groups, thus making them polar molecules and impeding their passage through the membrane lipid bilayer. Membrane proteins, such as sodium-dependent glucose transporters (SDGTs)

and glucose transporters (GLUTs) are necessary for sugar uptake. In mature sperm, GLUTs are important for uptake of sugars used as energy source for motility and fertilization ability. The hyperglycemia typical for diabetes cause downregulation of GLUTs which leads to lower intracellular glucose levels in germ cells [49]. Studies in type I DM mice showed that two members of the GLUT family - GLUT8 and GLUT9b were decreased in testes and GLUT9a was undetectable and insulin treatment for 7 days improves sperm motility and fertility [2].

Sperm cells of men with type I DM have reduced motility and decreased *zona pellucida* binding, as well as structural defects and nuclear and mitochondrial DNA fragmentation [2]. A variety of factors can lead to this increased oxidative stress, generation of free radicals and subsequent DNA damage. However, under pathophysiological condition, such as hyperglycemia in diabetic patients the proteins or lipids become glycated as a result of sugar exposure and they form the so called advanced glycation end products (AGEs) [61]. The number of sperms expressing the receptor for AGEs (RAGE) and increased protein amount, were prominently higher in samples from type I diabetic men [41]. The interaction between RAGE and its AGEs-ligands leads to increased reactive oxygen radicals production which in turn can induce DNA fragmentation [33]. The response to cellular oxidative stress required a complex network of sensors and effectors from multiple signaling pathways and in the center of which is the nuclear enzyme poly(ADP-ribose) (PAR) polymerase-1 (PARP-1) [23]. The latter is covalently linked to poly(ADP-ribose) polymers that serve as a signal and a platform for recruitment of proteins associated with the DNA damage response that promote chromatin remodeling, DNA repair, cell cycle arrest, senescence, or cell death. One such protein is tumor suppressor protein p53 that plays a central role in the induction of germ cell apoptosis [55].

Diabetes associated germ cell apoptosis is mediated by two major apoptosis pathways following reproductive dysfunction: the extrinsic death-receptor pathway (Fas-membrane signaling) and the intrinsic mitochondrial pathway. The regulation of these apoptotic pathways occurred through members of Bcl-2 family (pro- and anti-apoptotic proteins) [19]. DM induced germ cells death occurs in the testis of both type I and II DM. A significant increase in germ cell death in the testis of STZ-induced type I diabetic rats and mice has been documented by several studies [60]. In diabetic males, germ cells apoptosis in stage VII and VIII was also reported probably as a result of reduced LH/FSH and low serum testosterone levels. Multinucleated germ cells or giant cells linked with germ cells apoptosis were found in seminiferous tubules in diabetic rats.

Ultrastructural features of germ cell apoptosis were observed such as intense cytoplasm vacuolization of spermatogonia, apoptotic nuclei with dense nuclear chromatin in spermatogonia and spermatocytes. Deposition of intracytoplasmic dark substances (lypofuscin) in germ cells are most frequently observed ultrastructural changes in testis of diabetic rats. Diabetic animals at 1 year of follow-up presented greater amount of spermatids and spermatozoa with defects in the dense fiber complex or in mitochondrial sheet or axoneme [3, 34, 51, 59].

Sertoli cells (SCs) play a key role in testicular development, orchestrating and regulating proliferation and differentiation of germ cells, Leydig cells and peritubular myoid cells. During puberty, the role of SCs are responsible to support germ-cell differentiation. At this terminally differentiated stage the SCs are considered as non-proliferative, becoming the main component of blood-testis barrier (BTB) [54].

In diabetic rats, remarkable changes were observed in the mitochondria of SCs. These organelles had some abnormalities in their shape and quantity which may reflect their abnormal function [34]. Previous studies also indicated that denatured and decomposed SCs cytoplasm were observed in STZ-induced DM in animal models [55].

BTB is responsible for maintaining different levels of substrates and metabolites between germ cells and blood. This is possible due to specific glucose sensitive machinery that is under strict hormonal control mainly by sex hormones and FSH. This hormones receptors are located in SCs and they are very sensitive to extracellular glucose levels [38]. Glucose passive transport across the BTB is executed via glucose transporters which isoforms GLUT 1, GLUT3 and GLUT8 have been found in SCs [20, 27]. Sertoli cells produce lactate at a high rate as it is the main metabolite used by the pachytene spermatocytes and spermatids to meet their energy requirements [5]. The lactate is exported to the intratubular fluid by proton-coupled membrane monocarboxylate transporters (MCTs), mainly MCT4. Studies suggested that insulin deprivation does not affect significantly glucose consumption of SCs due to compensational increase of the expression of GLUT1 but lactate production rate and the levels of MCT4 were severely reduced [46]. Additionally, SCs produce high amounts of acetate, which is essential for the lipid synthesis in the germ cells [4]. Interestingly, some studies suggest a role for alternative substrates in SCs metabolism during hyperglycemic conditions. The main alternative fuels for the SCs that can maintain spermatogenesis are monocarboxylic acids, fatty acids and ketone bodies [22]. By downregulating gene transcript levels of Acetyl-CoA hydrolase, insulin-deprived SCs completely suppress the acetate production thus ensuring Krebs cycle stimulation. This mechanism ensures the survival of SCs while compromising lactate production and germ cells development.

Different cell markers are used in order to better understand the molecular mechanisms behind the DM-induced changes in spermatogenesis. Vascular endothelial growth factor (VEGF) and nerve growth factor beta (NGF-β) are important neurotrophic factors for male reproductive system. Reduction of VEGF and NGF-β levels were observed in diabetic testes and are associated with increase of apoptosis in diabetic rats. Testicular VEGF and NGF-β could be potential novel biomarkers for diabetes induced testicular damage [56]. Proliferating cell nuclear antigen (PCNA) is well known marker for DNA synthesis. PCNA is expressed in spermatogonia and early phase primary spermatocytes at all stages of seminiferous tubules. PCNA positive cells were strongly detected in non-diabetic in contrast with diabetic animals [3]. Decrease of PCNA in the germ cells of diabetic animals indicates reduction of proliferative activity in spermatogenesis.

Our previous studies showed that testicular angiotensin converting enzyme (tACE) is useful marker for developmental stage of germ cell differentiation and fertility [9]. This isoform is expressed in germ cells during spermiogenesis and is localized in elongating spermatids and spermatozoa. Based on the expression profile of tACE in diabetic rats, these results provided the first evidence that prepubertally testis is more affected by hyperglycemia than adult testis [9]. Epidemiological studies are in concert with these findings. Number of live births in a population-based, retrospective cohort of 2819 men with childhood-onset DM I showed that men with diabetes had a smaller number of live births than controls. Later age at onset of DM was associated with a higher rate of having a first child among men [17, 57].

Morphological and morphometric studies on diabetic rats demonstrated decrease in macro parameters as epididymal and testicular weight, diameter of seminiferous tubules, height of seminiferous epithelium. The morphology of the tubules vary from totally or partially disorganized epithelium with impaired organization of spermatogenic stages. Atrophy of tubules with varying degree of spermatogenesis was detected. Vacuolization of epithelium in many areas have been reported. Our previous comparative studies on DM induced neonatally or prepubertally in rats, showed that testicular morphology was more affected in prepubertally induced DM, compared to neonatally induced DM. Spermatogenesis is not completed and different degree of delay in spermatids development was observed [9].

In clinical studies patients with DM I had a lower percentage of spermatozoa with progressive motility and a higher percentage of spermatozoa with abnormal mitochondrial function than controls. Disruption of spermatozoa mitochondrial function due to ultrastructural mitochondrial alterations may be responsible for the decline in spermatozoa motility observed in DM I patients [6, 47]. Semen analysis of patients with both types I and II DM showed alterations in progressive sperm motility and significantly compromised sperm morphology, while the sperm concentration did not show significant differences [24].

In vitro fertilization using sperm from diabetic men produced far fewer pregnancies compared to healthy men [44]. This suggested that sperms of diabetic men are able to fertilize eggs but DNA damage might occur and prevents competent embryo development. Data from animals confirmed that fertilization rates were significantly lower in STZ-injected male mice. Embryo development rates to the blastocyst stage is lower in diabetic animals when compared with controls [58].

Erectile dysfunction is commonly reported condition in diabetic patients and is defined as inability to achieve and/or maintain an erection. Diabetic males showed a threefold higher probability to develop erectile dysfunction in comparison with non-diabetic men [43]. The pathogenesis behind this condition may include vascular insufficiency and neuropathy which further deteriorates into ejaculation dysfunction and decreased libido. Oxidative stress-mediated neurovascular alterations in diabetic patients is responsible for impaired endothelial function and neuropathy in the *corpus cavernosum* in diabetic men [15]. Erectile dysfunction appears at the early stage in diabetic males and its effects increase with the duration of the disease.

Type II DM and spermatogenesis

Type II diabetes mellitus is one of the most prevalent serious metabolic diseases affecting individuals on modern societies. Recently, a prodromal stage called pre-diabetes has been described and considered a high-risk factor for type II DM. These pathophysiological condition affects male reproductive function in particular testicular metabolism, leading to disturbance in testosterone synthesis [50]. Evidence has shown that 40% of men with type II DM present testosterone deficiency [25] leading to abnormal function of SCs [48]. Expression of inhibin B and androgen receptor, which are produced by mature SCs, are dramatically changed in conditions of DM II. The ultrastructural study of testicular tissue in insulin-resistant rats demonstrated remarkable changes not only in SCs but in LCs as well. It has been reported that, in patients with type II DM, the activities of some mitochondrial enzymes decrease and also some abnormalities in the shapes of these organelles have been noted [1]. Low concentration of glucose in *ad luminal* parts of seminiferous tubules exerts effect on metabolism of developing germ cells located in this region of the tubules.

In comparison with alloxan/STZ induced diabetic rats, severe histological changes have been observed in insulin-resistant diabetic animals, resulting in hypospermatogenesis in over 90% of tubules [8]. Histological samples from DM II mice revealed many seminiferous tubules with significant or partial depletion of germ cells, along with several multinucleated giant cells and vacuoles. Diabetes type II mice displayed a significant elevation of germ cell apoptosis, presumably via receptor-mediated caspase-8 activation. Although a significant increase in testicular cell death was observed, there was no concomitant significant change in testicular weight [63].

Conclusion

The great concern of recent society about dropping the average age at diagnosis of DM, required special attention in tandem with clinical and research priorities. Indeed, more than 90% of these patients are diagnosed before age of 30. The epidemic increase in diabetes, one of the human metabolic disorders demonstrate that glucose metabolism is essential for spermatogenesis and either type I or type II diabetes could have detrimental effects on male fertility. More studies on pathophysiological events in reproduction in DM are needed for elucidation of the precise mechanism involved in this metabolic disease that will contribute to development of new strategies for prevention and treatment of reproductive disorders, following DM.

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