

# Diabetes, insulin-mediated glucose metabolism and Sertoli/blood-testis barrier function

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**Keywords:** blood-testis barrier, diabetes mellitus, Sertoli cells, glucose metabolism, male fertility

**Abbreviations:** BTB, blood-testis barrier; DHT, 5 $\alpha$ -dihydrotestosterone; DM, diabetes mellitus; E2, 17 $\beta$ -estradiol; ED, erectile dysfunction; FSH, follicle-stimulating hormone; GLUTs, glucose transporters; GLUT1, glucose transporter 1; GLUT3, glucose transporter 3; GLUT8, glucose transporter 8; LDHA, lactate dehydrogenase A; LH, luteinizing hormone; MCT4, monocarboxylate transporter 4; SCs, Sertoli cells; T1D, Type 1 diabetes mellitus; T2D, type 2 diabetes mellitus

Blood testis barrier (BTB) is one of the tightest blood-barriers controlling the entry of substances into the intratubular fluid. Diabetes mellitus (DM) is an epidemic metabolic disease concurrent with falling fertility rates, which provokes severe detrimental BTB alterations. It induces testicular alterations, disrupting the metabolic cooperation between the cellular constituents of BTB, with dramatic consequences on sperm quality and fertility. As Sertoli cells are involved in the regulation of spermatogenesis, providing nutritional support for germ cells, any metabolic alteration in these cells derived from DM may be responsible for spermatogenesis disruption, playing a crucial role in fertility/subfertility associated with this pathology. These cells have a glucose sensing machinery that reacts to hormonal fluctuations and several mechanisms to counteract hyper/hypoglycemic events. The role of DM on Sertoli/BTB glucose metabolism dynamics and the metabolic molecular mechanisms through which DM and insulin deregulation alter its functioning, affecting male reproductive potential will be discussed.

## Introduction

Spermatogenesis is a complex event that is dependent on blood-to-germ cells transport of glucose and other metabolic intermediates. However, this blood-to-germ cells communication is hindered by the presence of junctions that control the substances movement between these adjacent but metabolically separate compartments. This barrier is known as blood-testis barrier (BTB, also called Sertoli cell barrier)<sup>1,2</sup> and, as it happens in other blood tissue barriers of the body, the free exchange of substances between the two distinct environments it creates (interstitial and adluminal compartments) is under tight control. In fact, the BTB is one of the tightest blood-tissue barrier<sup>3</sup>

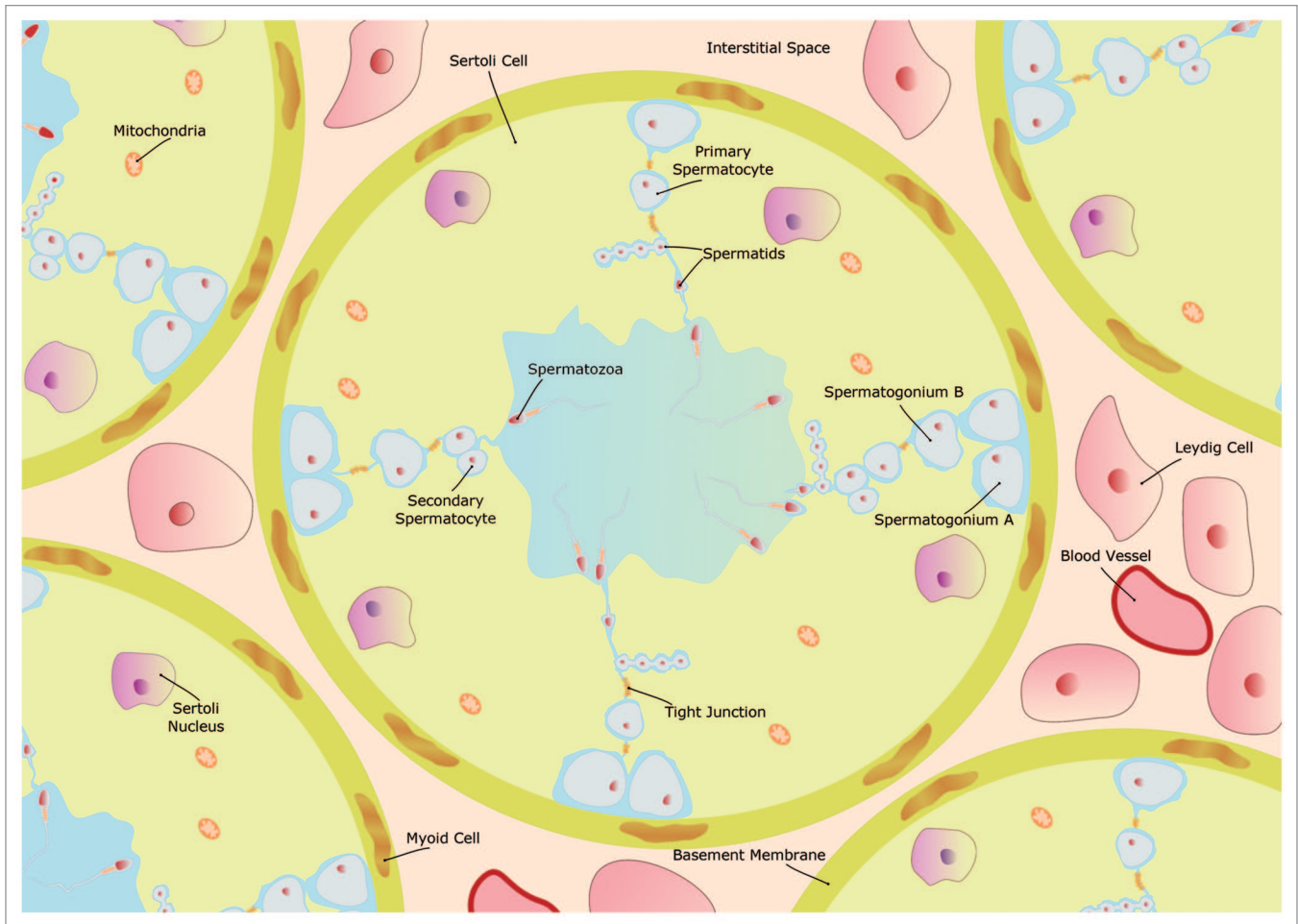
and physically divides the seminiferous epithelium into basal and apical compartments, where different stages of germ cell development occur (Fig. 1). For instance, the spermatids maturation into mature spermatozoa (spermiogenesis) and the release of mature spermatozoa (spermiation) occur in the apical compartment where post-meiotic germ cells are located. Moreover, BTB suffers a restructuring, to facilitate the transit of primary preleptone spermatocytes while differentiating into leptotene and zygotene spermatocytes, at stages VIII–IX of the seminiferous epithelial cycle.<sup>4</sup> Sertoli cells (SCs), the main component of BTB, are polarized epithelial cells that interact with each other through the establishment of tight junctions.<sup>5</sup> They can alter their morphology, via adaptations of their apical and lateral portions, during the cycles of the seminiferous epithelium.<sup>6</sup> These alterations are poorly understood although it is clear that BTB controls the access of plasma substances to the seminiferous epithelium adluminal compartment, maintaining different levels of substances between tubular luminal fluid and lymph or plasma. BTB alterations can be of diverse nature, including histological and/or biochemical modifications, and be a response to pathological conditions that may affect the main barrier functions: transport and permeability. Besides, the membrane fluidity and the cells surface charges regulate the transport rates across the barrier. Thus, any pathological condition that alters the normal functioning of these processes may compromise the male fertility potential.

One of the most prevalent chronic diseases in western societies is diabetes mellitus (DM). DM is described as a metabolic disorder resulting from defective insulin secretion, resistance to insulin, or both, and is rapidly rising worldwide.<sup>7,8</sup> There are several types of DM, but the most important are type 1 diabetes mellitus (T1D) and type 2 diabetes mellitus (T2D). The processes responsible for both types are very distinct: T1D results from an absolute deficiency of insulin due to an autoimmune destruction of the pancreatic  $\beta$  cells, while T2D is characterized by impaired insulin secretion and increased insulin resistance. Both, T1D and T2D, are characterized by a hyperglycemic state that largely contributes to the progression of major vascular and endothelial alterations. Moreover, DM is also responsible for several comorbidities and complications such as dyslipidemia,

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**Figure 1.** Schematic illustration of the seminiferous tubule and the Sertoli/blood testis barrier (BTB). The BTB is a physical barrier between the interstitial space and the seminiferous tubule lumen, formed by Sertoli cells (SCs) and the tight connections between these cells. Outside the BTB is the basal compartment, where spermatogonial renewal occurs, and inside the BTB is the adluminal compartment, where meiosis, spermiogenesis and spermiation take place. Blood vessels and the Leydig cells, which produce testosterone, are located in the interstitial space. Adjacent to the basement membrane are several layers of modified myofibroblastic cells, termed peritubular cells. As a result of such particular organization, the establishment of a functional BTB is essential to create a special environment for the normal development of a fully efficient sperm.

hyperinsulinemia and hypoglycemia that cannot be disregarded when studying DM-related effects. As a metabolic disorder, DM induces structural and functional alterations in cells, tissues and organs and BTB is no exception. DM pandemic has grown rapidly not only in the Western world, but also in developing countries. It is predicted that the disease will have epidemic proportions within the next decades.<sup>9</sup> Because of the increasing number of individuals developing DM at an early age, it is expected that they will experience more years of disease burden and a higher probability of developing serious DM-related complications. These complications will threaten life expectancy and reduce quality of life. Moreover, the individuals may have lower productivity during the prime years of their lives.<sup>10</sup> Importantly, the increase incidence of DM has been associated with failing birth rates and fertility.<sup>11,12</sup> Although the exact mechanisms by which DM affects male reproductive health remain obscure, this pathology has a significant impact on male reproductive function at multiple levels (as a result of dysfunctional spermatogenesis

per se or on its endocrine control, or by impairing penile erection and ejaculation).<sup>13-15</sup> Therefore, male infertility may become more widespread as DM rates rise.

### Functional, Structural and Metabolic Organization of the Sertoli/Blood-Testis Barrier

In mammals, the BTB is a testis-specific structure composed by the Sertoli cell body and tight junctions, basal ectoplasmic specialization, basal tubulobulbar complex, desmosome-like junctions and gap junctions present alongside in the seminiferous epithelium. The BTB physically divides the seminiferous epithelium into basal and apical (or adluminal) compartments (Fig. 1). One of the most important components of BTB is the SC that is also one of the most important testicular cell types, as SCs number determines the testis size.<sup>16</sup> Tight junctions are formed between the adjacent SCs, so that the passage of substances is under strict control and nothing larger than 1 kDa

passes from the basal to the adluminal side of the seminiferous tubule.<sup>17</sup> These tight junctions between SCs are crucial to define the apical and basal spaces in the seminiferous tubules. Among the several proteins involved in these processes (for an extensive review, see refs. 2 and 18–20), occludin and claudins play a crucial role being responsible for the functionality and dynamics of these BTB tight junctions.<sup>21–24</sup> Its presence and regulation contribute to BTB functionality and is essential for BTB stability and selective passage of substances through the germinal epithelium.<sup>22,25,26</sup> In sum, apart from the existence of an interstitial compartment containing interstitial cells, such as Leydig cells and testicular macrophages, and endothelial cells that serve as a first selective place to defend the testis against exogenous pathogens and environmental toxicants, such as heavy metals and xenobiotics (for a review, see refs. 27 and 28), the establishment of the BTB contributes to the creation of two distinct environments: (1) the basal compartment consisting of a tunica propria with peritubular cells and basement membrane and germ cells in the first step of spermatogenesis and (2) the apical (or luminal) compartment, containing mature spermatozoa and spermatids.

The SCs are the main testicular cells present and responsible for the establishment of the BTB. They are responsible for water transport from the interstitial space into the lumen. This is a critical function of SCs as this fluid movement serves as vehicle for moving sperm from the testis to the epididymis.<sup>29</sup> Moreover, SCs also control seminiferous epithelium fluid pH<sup>30,31</sup> by secreting an iso-osmotic fluid through several membrane transporters.<sup>32</sup> Their structure is very complex, with several cupshaped protrusions surrounding the various germ cell types that are distributed within the seminiferous epithelium.<sup>33</sup> Each SC is in contact with multiple germ cells and intimate associations are established. There are two types of Sertoli-germ cell anchoring junctions: desmosome-like junctions and ectoplasmic specializations (ESs) and both anchoring junction types are known to mediate stable adhesion throughout spermatogenesis (for an extensive review, see ref. 34). This unique association between SCs and germ cells is the basis of the seminiferous epithelium cycle and each particular association is referred as a stage. The number of spermatogenesis stages in a particular mammalian species is defined by the number of morphologically recognizable associations established between SCs and germ cells within the testis.<sup>35</sup> The fully differentiated SCs are often described as “nurse cells” because they are responsible for the physical and nutritional support of germ cell. They exhibit a well-organized cytoskeleton and deposit extracellular matrix components such as collagen and laminin.<sup>36</sup> SCs also secrete anti-müllerian hormone (AMH), the *c* kit ligand and inhibin, among other specific products that are necessary for germ cell development and survival, in addition to secreting a series of peptides, nutrients and several metabolic intermediates.<sup>37,38</sup> The metabolic cooperation between SC and developing germ cells involves the transference of various metabolic products, such as amino acids, carbohydrates, lipids, vitamins and metal ions.<sup>36</sup> In fact, the close relationship between these somatic and germinative cell types is imperative for developing germ cells to receive an adequate level of energy substrates.<sup>39–42</sup> Among the

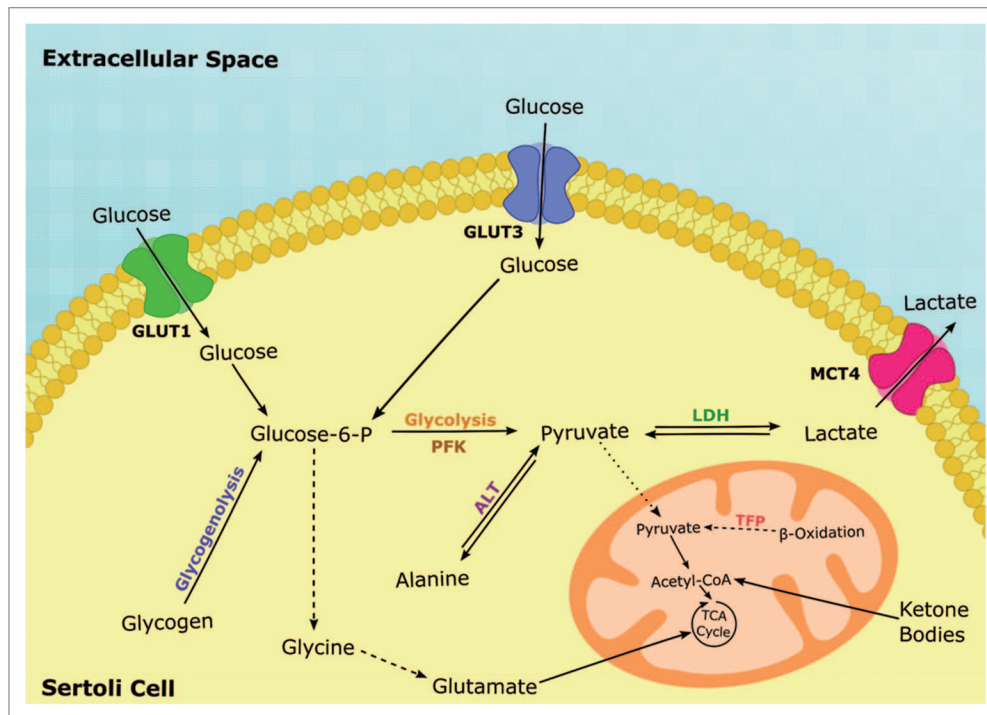
several factors and metabolic substances secreted by SCs, lactate plays a crucial role in the development of germ cells and therefore in the spermatogenic process.<sup>43</sup>

Like in other blood-tissue barriers, the glucose transport through the BTB is under strict control. Furthermore, the glucose metabolism in BTB has some unique characteristics that proven to be essential for a normal spermatogenesis. Glucose must cross the BTB and be metabolized or delivered to the several intra-barrier testicular cells and in the seminiferous epithelium. As discussed above, the SCs have functions that go far beyond the physical support of germ cells. They are responsible for lactate production from extracellular glucose, that is then exported to be metabolized by the developing germ cells.<sup>44</sup> During this process, glucose has to permeate BTB through an energy independent process. This is achieved via facilitated diffusion mediated by glucose transporters (GLUTs) and is dependent of the GLUTs redistribution in plasma membrane and of GLUTs total levels.<sup>45</sup> It has been reported that in SCs, glucose transporter 1 (GLUT1) and glucose transporter 3 (GLUT3) play a synergistic role in maintaining glucose uptake to assure lactate production.<sup>46–48</sup> Recently, it has also been reported that glucose uptake and lactate production by SCs are under hormonal regulation.<sup>40,48–50</sup> Therefore, the metabolic cooperation between testicular cells and the selective passage of metabolic substrates through BTB is exerted by several distinct and specialized mechanisms. The Sertoli cell barrier or BTB has a specific glucose sensing machinery that is under strict hormonal control, especially by sex hormones and FSH. These hormones receptors are located in SCs and are very sensitive to extracellular glucose levels (Fig. 2).<sup>51–54</sup>

The structural organization of the BTB is very complex and therefore has a great influence in the functional status of the overall testicular metabolism. Future studies are needed to explore the mechanisms by which BTB disruption can alter the metabolic cooperation between the different testicular cell types, namely SCs and developing germ cells since this process is dependent of BTB maintenance and organization. Several substances and pathological conditions, such as DM, are known to alter BTB permeability, potentially compromising spermatogenesis.

### Glucose and Lactate Transporters in Sertoli/Blood Testis Barrier

As discussed, the male reproductive health is highly dependent of glucose uptake and metabolism by testicular cells. Glucides are polar molecules. Although they can cross the lipidic bilayers by simple diffusion, they do it in a very inefficient manner. Therefore the cells take up glucose through carriers. There are two families of glucose transporters: the sodium dependent glucose transporters (SGLTs), also known as solute carrier family 5 (SLC5) and the GLUTs, also known as solute carrier family 2 (SLC2).<sup>55–57</sup> These families are composed with a different number of transporters. The SGLTs family is composed by six different active transporters (SGLT1 to 6) while the GLUTs family is composed by 14 glucose transporter isoforms (GLUT1 to 14).<sup>57</sup> GLUTs family can be divided into three subfamilies, namely



**Figure 2.** Schematic illustration of Sertoli cells (SCs) main metabolic pathways. The SCs are capable of consuming a variety of fuels including glucose, lactate, fatty acids and amino acids. Nevertheless, SCs actively metabolize glucose being the majority of it converted into lactate and not oxidized in the TCA cycle. The extracellular lactate and pyruvate are transported via the members of the family of proton-linked plasma membrane transporters that carry molecules having one carboxylate group, the monocarboxylate transporters (MCT4), while glucose is imported via specific members of the family of membrane proteins called glucose transporters (GLUT1 and GLUT3). Once glucose enters the glycolytic pathway, it is decomposed to pyruvate which can (1) be converted into lactate via lactate dehydrogenase, (2) be converted into alanine via alanine transaminase or (3) be transported to the mitochondrial matrix, oxidized and decarboxylated by the pyruvate dehydrogenase forming the two carbon intermediate Acetyl-CoA which can enter the TCA cycle. The oxidation of these substrates is coupled with ADP phosphorylation via the electron transport chain to form ATP. TCA, tricarboxylic acid; GLUT, glucose transporter; MCT, monocarboxylate transporter; ALT, alanine transaminase; LDH, lactate dehydrogenase; PFK, phosphofructokinase; TFP, trifunctional protein.

class I (GLUT1-4), class II (GLUT5, 7, 9, 11) and class III (GLUT6, 8, 10, 12 and H<sup>+</sup>-coupled myo-inositol transporter). Several GLUTs are expressed in testicular cells and in spermatozoa (for extensive review see ref. 55). Positive immunoreaction for GLUT1, GLUT2 and GLUT3 was found in several testicular cells, including peritubular myoid cells.<sup>58</sup> In SCs, the main component of BTB, it has been described at least four glucose transporters isoforms: GLUT1, GLUT2, GLUT3 and GLUT8 (Table 1).<sup>46,47,59</sup> The presence of GLUT4 was also investigated and no positive immunoreaction was not detected in any testicular cell type, inclusive in peritubular myoid cells and SCs.<sup>58</sup> Yet not all the glucose transporters identified are expected to contribute in the same manner to glucose transport. For instance, in the hippocampus, GLUT1 and GLUT3 are reported to be the most important in glucose uptake from the extracellular medium, since GLUT8 was not localized in the plasmatic membrane.<sup>60,61</sup> Moreover, recent studies with GLUT8 knockout mice suggested that this transporter is mainly involved in the transport and recycling of glucose residues in the lysosomes membranes.<sup>62,63</sup> These mechanisms that allow glucose incorporation are of extreme relevance since the tubular fluid has a low level of this sugar and SCs, which are responsible for the maintenance of this fluid homeostasis,<sup>32</sup> need glucose and other metabolic intermediates to produce

the metabolic substrates required for germ cells development.<sup>64</sup> SCs have some particular features of glucose metabolism as they produce lactate at high rates<sup>49,50,64,65</sup> that is then used for energy production by the developing germ cells, namely pachytene spermatocytes and round spermatids.<sup>43,65,66</sup> The lactate export by SCs occurs through specific monocarboxylate transporters (MCTs). The SLC16A family of MCTs is composed of 14 members but only MCTs 1–4 are linked to lactate transport.<sup>67</sup> These MCTs differ in their transport kinetics and their subcellular distribution determines the lactate shuttle across blood-tissues barriers. Although MCTs 1–4 transport monocarboxylates, they differ in substrate binding affinity and selectivity. For instance, MCT1 and MCT2 possess a high affinity for lactate and are expected to mediate the lactate uptake while MCT4 is involved in lactate export.<sup>68</sup> Indeed, within the BTB, the SCs mainly express MCT4 to release lactate into the intratubular fluid that is subsequently taken up by differentiating germ cells via MCT1 and MCT2 (Table 1).<sup>69-72</sup>

The metabolic cooperation established between SCs and the developing germ cells is highly dependent of GLUTs and MCTs functioning. In several pathological conditions, particularly in metabolic-related diseases, they play a crucial role in the development of the deleterious effects.

**Table 1.** Expression of the glucose transporters (GLUT1, GLUT2, GLUT3, GLUT4 and GLUT8) and monocarboxylate transporters (MCT1, MCT2 and MCT4) in seminiferous tubular cells

Testicular cells	Glucose transporters					Monocarboxylate transporters		
	GLUT1	GLUT2	GLUT3	GLUT4	GLUT8	MCT1	MCT2	MCT4
Sertoli cells	+ <sup>46,47,58,59</sup>	+ <sup>58</sup>	+ <sup>47,58</sup>	- <sup>58</sup>	+ <sup>46</sup>	ND	ND	+ <sup>70-72</sup>
Germ Cells	+ <sup>58</sup>	+ <sup>58</sup>	+ <sup>58</sup>	- <sup>58</sup>	ND	+ <sup>69</sup>	+ <sup>69</sup>	ND
Peritubular cells	+ <sup>58</sup>	+ <sup>58</sup>	+ <sup>58</sup>	- <sup>58</sup>	ND	ND	ND	ND

GLUT1, glucose transporter 1; GLUT2, glucose transporter 2; GLUT3, glucose transporter 3; GLUT4, glucose transporter 4; GLUT8, glucose transporter 8; MCT1, monocarboxylate transporter 1; MCT2, monocarboxylate transporter 2; MCT4, monocarboxylate transporter 4; +, expression detected; -, expression not detected; ND, not determined; superscript numbers are references as indicated in references section.

### Diabetes Induces Important Alterations in Testicular Cells that Modulate Glucose Transport in Sertoli/Blood-Testis Barrier

DM is a metabolic disorder that has been associated with several comorbidities and long-term complications that result from the lesions and multiple processes that are originated in several tissues of the organism in response to the disease.<sup>73,74</sup> The glucose deregulation is an important characteristic of DM and some of the most relevant side effects associated with this pathology are related with the hyper- and hypoglycemic events that occur, even if transiently, in diabetic individuals.<sup>75,76</sup> The microvascular disease promoted by DM is a leading cause of blindness, renal failure, cardiovascular complications, increased atherosclerosis and nerve damage.<sup>77-80</sup> Using biopsies from men with DM, it has been reported that testicular capillaries and lymphatic endothelia appeared structurally abnormal due to interstitial “matrix expansion.” Discrete ultrastructural lesions in apical SC cytoplasm were associated with spermatogenic disruption and the described morphological changes in the interstitial compartment suggested microvascular complications.<sup>81</sup> It has also been described that testes from STZ-induced diabetic rats showed a reduction in seminiferous tubular diameter, an increase in the number of empty testicular tubules and also an increase in vascular density.<sup>82,83</sup> Those authors suggested that the reported increased density on testicular vasculature might lead to increased scrotal temperature. Moreover they suggested that the possible increased temperature may be a cause of the observed germ cells apoptotic death. Furthermore, the increased wall thickness of small blood vessels in diabetics might lead to hypoxia-induced cellular damage.<sup>82,83</sup>

The effects of DM in human health are well known and have been object of research for several years. Contrarily, the effects of DM in male reproductive health did not caught much attention from scientists for many years. They were not considered a priority mainly because DM, and particularly T2D, was considered a late-onset disease. Therefore the effects of DM on male reproductive health were not considered as immediate or essential subjects of research. Nowadays this view has been challenged, especially because there is an increasing incidence of DM in young adolescents, that is expected to continue to rise, and the increasing number of DM to epidemic proportions is contemporaneous with failing birth.<sup>84</sup>

Male sexual dysfunctions such as decreased libido and impotence are associated with DM decreasing the male reproductive

health and altering the sexual behavior with dramatic consequences to male fertility.<sup>85</sup> Another important feature that is very prevalent in diabetic men and might contribute to reproductive failure is erectile dysfunction (ED), although it depends on several factors such as the individual age, the disease duration and also the control of blood glucose levels.<sup>86</sup> The ejaculation of diabetic men has also been reported to be affected. In some diabetic men the semen passes backward into the bladder, a condition known as retrograde ejaculation.<sup>87,88</sup> In patients suffering from diabetic autonomic neuropathy, the function of the urethral sphincter is often affected due to loss of innervation, which may be a cause for the reported retrograde ejaculation observed in those individuals.<sup>89</sup> Additionally, DM can cause male subfertility or infertility by disrupting some important mechanisms, inducing abnormal sperm production and/or failure of the reproductive function.<sup>90-92</sup> Although the effects of DM in male reproductive health is a matter of some controversy, several studies reported that sperm parameters, such as semen volume, sperm count, motility and morphology, are also altered in diabetic adolescents and men.<sup>93,94</sup> Interestingly, the semen of diabetic men are reported to present higher fructose and glucose levels<sup>94</sup> although the exact mechanisms of glucose transport through which it happens remain largely unknown.

The effects on male reproductive health are more pronounced when DM animal models are used. In rats it has been reported an increase in the number of damaged seminiferous tubules (e.g., increased seminiferous tubule wall thickness and SCs vacuolization) in the early stages of the disease. Moreover, it has been reported that rats with DM present a decrease in the gonadosomatic index, sperm concentration and motility, as well as decreased levels of serum testosterone and abnormal spermatogenesis.<sup>95-97</sup> The endocrine deregulation caused by DM is well known. Although the absolute values for hormonal fluctuations may differ between studies, it is clear that DM induces a marked reduction in plasma testosterone levels, reaching values decreased more than one half. Moreover, a significant decrease in luteinizing hormone (LH) and follicle-stimulating hormone (FSH) was described in the plasma of diabetic individuals.<sup>98-101</sup> Interestingly, insulin injections can restore FSH to normal levels<sup>98</sup> while the synergistic treatment of insulin with testosterone can restore some alterations in the body and reproductive organ weights, as well as in sperm counts and motility.<sup>100</sup> Besides these alterations in the endocrine system, testes of diabetic rats presented a seminiferous epithelium with abnormal histology and

altered histo-architecture, as well as altered distribution of occludin, a key protein of the BTB tight junctions, which appeared not well organized or totally absent in portions of the seminiferous tubules.<sup>102</sup> Evidences of BTB disruption in diabetic individuals were also described in human biopsies from diabetic individuals, where several morphological changes were detected in testicular cells. Those works reported an increased thickness of seminiferous tubule wall, germ cell depletion and SCs vacuolization and degeneration.<sup>103,104</sup> Although none of these works studied the molecular mechanisms of glucose transport and metabolism, these morphological alterations will be detrimental to SCs functioning. It is expected that blood-to-germ cells substrate are altered, as SCs express several membrane transporters (e.g., GLUTs and MCTs) that are crucial for the transport of substances (e.g., glucose) from the interstitial space (which is in close contact with the systemic circulation, since molecules can freely flow out of the blood stream into this space) into the cell and from the cellular cytoplasm to the adluminal compartment (e.g., lactate). The tight junctions between SCs prevent these molecules from entering the adluminal compartment directly from the interstitial space, while the transporters allow the “cellular passage” of the molecules. Severe alterations in the SCs functioning and morphology will affect the delivery of those molecules to the developing germ cells. Unfortunately, there is a lack of studies focused on glucose transport and metabolism in BTB of diabetic individuals or individuals subjected to diabetic conditions. Nevertheless one must note that, for instance, sex hormones are known as metabolic modulators of SCs metabolism<sup>49,50</sup> and in diabetic individuals there is a severe deregulation of sex hormones levels. Hormonal deregulation induces important alterations in spermatogenesis, as the hormonal control of SCs regulates spermatogenesis.<sup>50,54</sup> Indeed, sex hormones control lactate production by modulating lactate dehydrogenase A (LDHA), monocarboxylate transporter 4 (MCT4) and also GLUTs levels of both rat and human SCs.<sup>49,50</sup> This tight and crucial control is altered by diseases that can promote sex hormone fluctuations, such as DM,<sup>15,105</sup> with dramatic consequences to male fertility.

The mechanisms by which DM alters the metabolic cooperation in testis remain obscure, but there are clear evidences that blood-to-germ cells metabolites transport is altered and thus, spermatogenesis may become impaired. Although there are known morphological and functional alterations in BTB of diabetic male individuals, the exact meaning of those changes in carbohydrates metabolism is still unclear. This is a subject that should deserve special attention in the next few years since spermatogenesis depends of a correct metabolic cooperation between testicular cells.

### **Insulin (De)Regulation and Glucose Transport in the Sertoli/Blood-Testis Barrier**

The management of blood glycaemia in individuals with DM is crucial and it may minimize the development of diabetes-related complications, although it does not entirely eliminate all the detrimental effects associated to this pathology.<sup>106,107</sup> The extent of glycaemic control is dependent of several factors such as the DM type,

the severity and stage of the disease progression among others.<sup>108</sup> Euglycemia is very difficult to attain and maintain with insulin therapy, insulin analogs and sensitizers (e.g., thiazolidinediones), insulin secretagogues (e.g., sulfonylureas) or with other antidiabetic drugs, such as  $\alpha$ -glucosidase inhibitors.<sup>109,110</sup> Additionally, diabetic individuals, especially those with T2D, become progressively more insulin-deficient throughout the years, becoming more vulnerable to the problems that arise from a poor glycaemic control.<sup>111,112</sup> On the other hand, diabetic individuals with intense insulin therapy gradually lose their sensitiveness to small variations in plasma glucose concentration and, as result, both hyperinsulinemia and hypoinsulinemia occur.<sup>113,114</sup> The periods of hypoglycemia/hyperinsulinemia that occur in both T1D and T2D<sup>115,116</sup> are responsible for several problems and compromise the physiological defenses against falling glucose plasma concentration.<sup>117,118</sup> These events are crucial in diabetic individuals and although they are well studied for instance in liver and brain, their effect in BTB has been somewhat disregarded. There are only a few studies focused on insulin signaling and insulin regulation of BTB functioning. Within this barrier, SCs metabolic function in vitro is highly dependent on glucose<sup>53,119</sup> and insulin concentration.<sup>120</sup> Specific insulin receptors have been identified in these cells<sup>121</sup> and it has been reported that insulin increases the rate of lactate production by SCs.<sup>120</sup> More recently, we have studied the effect of insulin deprivation in cultured SCs to elucidate some of the metabolic mechanisms that are modulated by insulin.<sup>48,122</sup> Insulin-deprived SCs presented unique alterations in glucose and acetate metabolism. In the first hours of insulin deprivation, glucose consumption was significantly decreased but after 48 h the insulin-deprived cells presented similar glucose consumption as cells cultured in the presence of insulin. Noteworthy, this identical glucose consumption and consequently lactate production was accompanied by a modulation in GLUTs levels, evidencing that SCs are capable of altering GLUT1 and GLUT3 levels under insulin-deprivation conditions. This may be a crucial mechanism to maintain lactate production, one of the most important SCs functions, within a physiological range in order to ensure adequate concentrations of this metabolite in the microenvironment where germ cell development occurs. The insulin deprivation conditions also downregulated lactate metabolism-associated gene transcript levels, such as LDHA, which is the most predominantly expressed LDH isoform in testis<sup>123</sup> and is responsible for the conversion of pyruvate to lactate.<sup>124</sup> MCT4, which is the main lactate exporter present in SCs was also downregulated.<sup>125</sup> Importantly, SCs produce high amounts of acetate, which has a crucial role in the production of sub-products essential for germ cells high rate of lipids synthesis, that is also regulated by insulin.<sup>122</sup> Insulin-deprived cells completely suppressed acetate production by downregulating Acetyl-CoA hydrolase gene transcript levels, thus suggesting a Krebs cycle stimulation to ensure SCs survival even while compromising lactate production and consequently germ cells development. All these in vitro adaptive mechanisms need further clarifications, but represent the first steps to elucidate the key mechanisms by which insulin deregulation that usually is faced by diabetic individuals can modulate BTB metabolic functioning and thus influence the male diabetic reproductive potential.

## Contribution of Alternative Fuels to Sertoli/BTB Metabolic Cooperation in Diabetic Individuals

As previously discussed, euglycemia is difficult to achieve and maintain in diabetic individuals and as a result they often face hypo- and hypoglycemia episodes resulting from insulin therapy. These hypo- and hyperglycemia episodes are responsible for several deleterious effects such as alterations in proteins and membranes integrity, even if these periods are merely transient.<sup>126-128</sup> Moreover, as discussed above, the BTB is responsible for the selective passage of ions, substances and metabolic intermediates to germ cells. DM alters the overall physiological cellular condition and modifies the metabolic environment surrounding the BTB. Indeed, the BTB cells metabolism is altered, particularly under conditions of prolonged glucose deprivation, as SCs need this substrate for lactate production that is then used by developing germ cells. When BTB faces hyperglycemia, alternative fuels are not expected to play any significant role as glucose is fully accessible and remains the main substrate for SCs. On the other hand, when diabetic individuals are subjected to hypoglycemia, even if transiently, the BTB must adapt its metabolism in order to maintain ATP and lactate production. Although the spermatogenesis maintenance *in vivo* relies mainly on glucose metabolism,<sup>129,130</sup> under undesirable conditions, such as those provoked by DM, SCs can use alternative fuels to maintain lactate production. The main alternative fuels to SCs that can maintain spermatogenesis are monocarboxylic acids, fatty acids and ketone bodies<sup>42,131</sup> (Fig. 2). It has been reported that  $\beta$ -oxidation pathway is used by SCs to produce ATP, especially by using free fatty acids or the recycling lipids of apoptotic spermatogenic cells and residual bodies that are phagocytized and degraded.<sup>132</sup> In fact, lipids metabolism is crucial for spermatogenesis and mice with inactivated genes from lipid metabolism (hormone-sensitive lipase gene) are reported to present a compromised spermatogenesis.<sup>133</sup> The metabolic plasticity exhibit by SCs is intriguing since they can also use unconventional fuels such as palmitate and ketone bodies.<sup>134</sup> Moreover, SCs can also metabolize some aminoacids such as glutamine, alanine, leucine, glycine and valine.<sup>135</sup> Special attention is required when analyzing the contribution of these alternative fuels to spermatogenesis, since BTB is not fully functional at birth and some of the mechanisms that control these fuels metabolism are dependent on the individual's sexual maturity. For instance, higher oxidation of oleate to  $\text{CO}_2$  has been only reported for *in vitro* cultured testicular cells derived from immature animals.<sup>136</sup>

Although it has not been quantified yet, glycogen stores in the BTB secluded environment should not be overlooked. The presence of glycogen and glycogen phosphorylase activity in SCs was reported a long time ago,<sup>137,138</sup> but since then no studies were done to completely elucidate these mechanisms. Glycogen may have an essential role in diabetic conditions. Glycogen storages use can be a valuable compensatory mechanism for the transient hypoglycemic periods. The functional alterations on BTB that arise in the testis regarding the use of alternative substrates in diabetic conditions should also be considered and fully investigated.

## Conclusion

Diabetes mellitus is a metabolic disease associated with subfertility and/or infertility. Nevertheless, not all diabetic men are infertile and there are conflicting results in the literature concerning the real impact of DM in the male reproductive health. The exact role for BTB in glucose dynamics during DM is also unknown, although the BTB plays a crucial role in the maintenance of spermatogenesis that is expected to be compromised by DM. This metabolic disease induces several alterations in the male reproductive tract but there is an urgent need for the elucidation of how individual components of BTB suffer the effect of DM, since these alterations may lead to an increased permeability that may end-up in infertility. Moreover, DM is associated with several comorbidities and thus it is very difficult to isolate the effects of each one when studying the mechanisms related with glucose dynamics through the BTB. SCs, the main cellular components of the BTB, are very sensitive to insulin fluctuations and DM is often associated with either hyper- or hypoinsulinemia. Therefore, the basic molecular mechanisms of glucose and insulin deregulation in SCs metabolism should deserve special attention, as they remain to be disclosed. There are several studies focused on the reproductive alterations of diabetic men or male animals, but the morphological integrity of BTB in diabetic males is still an intriguing issue. Interestingly, some studies also suggest a role for alternative substrates in SCs metabolism during diabetic conditions. The use of other metabolic substrates alternatively to glucose also induces important alterations in BTB permeability and alters the testicular physiology.

In conclusion, from a clinical perspective, the study of the sperm parameters and DNA integrity of diabetic individuals offers a more direct outcome of the disease, as they have a crucial importance for natural and assisted reproduction. However, BTB physiology is essential in the maintenance of the metabolic cooperation between testicular cells and should deserve special attention from researchers in order to identify possible mechanisms by which BTB is compromised in diabetic conditions and point toward possible sites for therapeutic intervention. In the next years this will certainly be a hot topic for those who are interested in DM and male reproductive health.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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