

Review

Testicular Glycogen Metabolism: An Overlooked Source of Energy for Spermatogenesis?

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Abstract: The incidence of male infertility has been increasing over the years and is now becoming a serious health problem. This trend has been followed by an increase in metabolic diseases, which are known to induce clear alterations in testicular metabolism, although the underlying mechanism remains unclear. Testicular metabolism displays several unique features, with testicular somatic cells being central in providing the conditions needed for spermatogenesis, including its nutritional and hormonal support. In addition to glucose and lactate, the two main energy sources used by the testis, glycogen is also present in testicular cells. Glycogen metabolism is a potential source of glucose to both testicular somatic (namely Sertoli and Leydig cells) and germ cells. Many of the enzymes involved in the pathways of the synthesis and degradation of glycogen were identified in these cells, emphasising the relevance of this complex carbohydrate. Glycogen, however, has other non-canonical functions in testicular cells; besides its role as a source of energy, it is also associated with events such as cellular differentiation and apoptosis. In this review, we address the relevance of testicular glycogen metabolism, focusing on its role in Sertoli and Leydig cells and spermatogenesis. In addition, all the available information on the role of glycogen and related pathways in male infertility cases is discussed. Our discussion highlights that glycogen metabolism has been somewhat overlooked in testis and its contribution to spermatogenesis may be underestimated.

Keywords: glycogen; male infertility; glycogen synthase; glycogen phosphorylase; Sertoli cell; Leydig cell; spermatogenesis



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1. Introduction

The incidence of male infertility has been increasing over the last few decades, particularly due to environmental and lifestyle factors that negatively affect spermatogenesis [1–4]. A study performed between 1990 and 2017, by the Global Burden of Disease, showed that the incidence of male infertility increased annually by 0.3% [5]. It has been estimated that infertility affects 15% of couples worldwide, with the male factor being implicated in half the cases [6,7]. Although 30% to 40% of these cases are of unknown origin (classified as idiopathic infertility) [8], several diseases have been associated with male infertility, including metabolic diseases, particularly obesity and type 2 diabetes mellitus [9].

Metabolic diseases are linked to increased oxidative stress and testicular metabolic dysfunction, which are also known causes of dysfunction in sperm production and male infertility [9]. Obesity in males is associated with oligospermia and azoospermia [10], with

hyperlipidaemia being intimately connected to poorer sperm morphology [11], and type 2 diabetes mellitus to poorer sperm parameters [12,13]. Indeed, a low percentage of motile spermatozoa in the ejaculate is one of the main causes underlying male infertility (with a prevalence of 19%), and this value increases to 63% when combined with other defects in sperm [14]. Decreased sperm motility is observed in the majority of the cases of idiopathic male infertility [15,16].

The metabolism and bioenergetics of testicular cells are known for their unique characteristics. Sertoli cells (SCs) are testicular somatic cells that play an important role in supporting spermatogenesis due to their location in the seminiferous tubules. The seminiferous tubule is compartmentalised into basal and adluminal spaces by junctions between adjacent SCs, creating the blood–testis barrier (BTB) [17]. This barrier offers immunological protection by regulating the infiltration of cytokines into the adluminal space and the movement of substances between the bloodstream and the lumen of the seminiferous tubule [18]. SCs take up nutrients from the bloodstream, particularly glucose, providing nutritional support for the development of male germ cells [19]. SCs are the main energy regulators of spermatogenesis, as they produce the lactate needed for developing germ cells, primarily through the metabolism of glucose taken from circulation. However, the metabolism of these cells is highly plastic, as they can also metabolise lipids and amino acids [20,21]. Leydig cells are the predominant extra-tubular somatic cells, being embedded in the testicular interstitial tissue. They produce androgens, primarily testosterone, in response to luteinising hormone (LH) stimulation by the pituitary [19]. The androgens produced by Leydig cells are important for initiating, maintaining, and regulating spermatogenesis [22].

The energy source that supplies Leydig cells comes primarily from mitochondrial oxidative phosphorylation, but also glycolysis [23]. As for mammalian spermatozoa, they also exhibit a high degree of flexibility concerning their preferred metabolic pathways [24]. Although it is known that spermatozoa need large amounts of adenosine triphosphate (ATP) to maintain their motility and fertilising ability, their main metabolic pathway responsible for ATP production is still under debate, with data showing that both mitochondrial oxidative phosphorylation and glycolysis are active and contribute to human spermatozoa capacitation and motility [25].

Glycogen is one of the most overlooked and understudied energy sources in male reproductive cells. While the liver has the highest concentration of stored glycogen, which can be degraded into glucose and released into the bloodstream to maintain glucose homeostasis, this polymer can also be found in the male reproductive tract. Glycogen is also found in other tissues such as skeletal muscle, brain, kidneys, adipose tissue, heart, and erythrocytes [26]. However, its role in male fertility remains to be fully elucidated. In this review, we discuss the presence and metabolism of glycogen in the male reproductive tract cells, namely in Sertoli and Leydig cells, and also in spermatozoa. In addition, we aim to discuss the potential role of glycogen and related pathways in male infertility scenarios.

2. Glycogen Dynamics—Synthesis and Degradation

Glycogen is a highly branched glucose polymer, with glucose residues connected by α -1,4-glycosidic linkages, while α -1,6-glycosidic bonds create the branch points. It contains also minor amounts of phosphate and glucosamine [27]. Glycogen is used as a source of energy storage, and its production or degradation occurs in response to the energy needs of the organism, with high levels of glucose in the blood leading to the synthesis of glycogen, while lower levels of blood glucose promote its degradation to produce glucose [28].

The elevation of glycemia stimulates the secretion of insulin. Insulin, in turn, promotes not only the uptake of glucose into tissues but also its conversion into lipids and glycogen [29]. Glucose enters cells through glucose transporters (GLUTs). After glucose uptake, it is phosphorylated by hexokinase isoenzymes, producing glucose-6-phosphate. Humans express four hexokinase isoenzymes (I, II, III, and IV). Hexokinases I, II, and III are inhibited by glucose-6-phosphate, while hexokinase IV (glucokinase) is regulated by the glucokinase regulatory protein. The glucose-6-phosphate suffers an isomerisation to

glucose-1-phosphate by phosphoglucomutase isoenzymes (PGM1 to PGM5). UDP-glucose pyrophosphorylase (UGP) catalyses the formation of uridine diphosphate glucose (UDP-glucose) from uridine 5'-triphosphate (UTP) and glucose-1-phosphate. UGP is ubiquitously present in human tissues, with two known isoforms (UGP1 and UGP2). Ultimately, UDP-glucose is the source of glucose residues that are necessary for the initiation and elongation of glycogen synthesis [26,29].

Glycogenin is a glycosyltransferase that catalyses the transference of glucose residues from UDP-glucose, creating a linear glucose polymer of 10 to 20 glucose residues with α -1,4-glycosidic linkages. There are two known isoforms of glycogenin (GYG1 and GYG2) expressed in humans. The GYG1 isoform is present in multiple tissues but has not been described in the liver, while the GYG2 is fundamentally expressed in the liver [30,31]. The formed glucose polymer suffers the combined actions of glycogen synthase (GYS) and the glycogen-branching enzyme (GBE) to form glycogen (Figure 1).

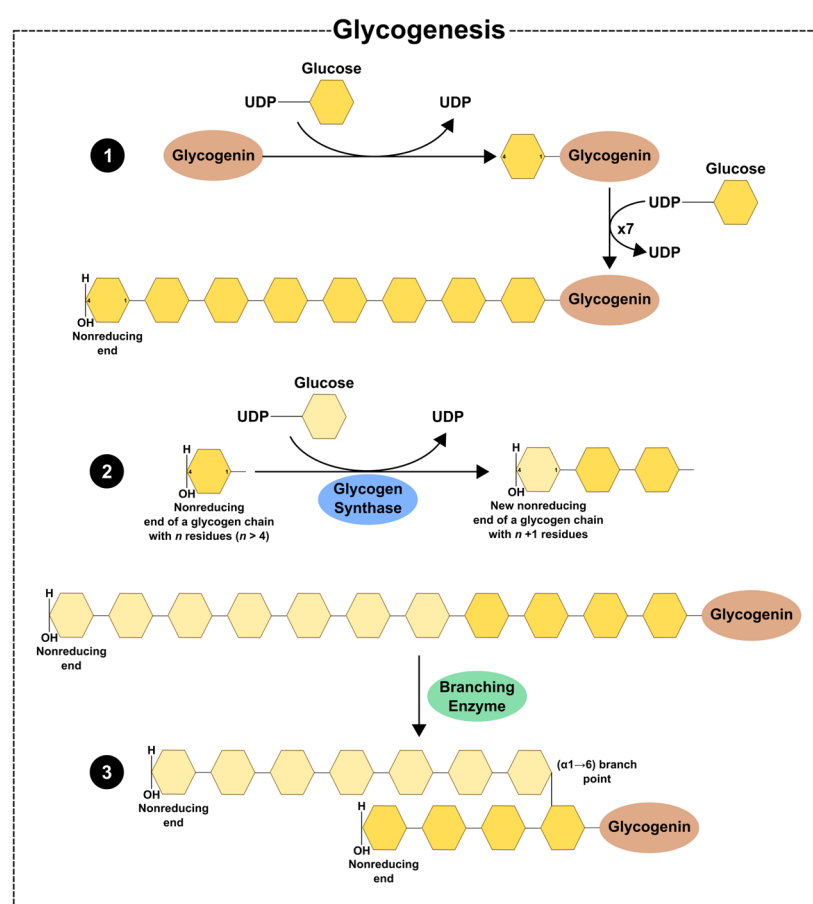


Figure 1. Schematic illustration of glycogen synthesis: (1) glycogenin catalyses the transference of glucose residues from UDP-glucose to itself, forming a linear chain with residues of glucose linked by (α -1 \rightarrow 4) linkages; (2) the linear chain suffers the action of glycogen synthase which creates (α -1 \rightarrow 4) glycosidic linkages; (3) glycogen-branching enzyme creates (α -1 \rightarrow 6) glycosidic linkages, forming a highly branched polymer of glycogen.

GYS is a glycosyltransferase since it catalyses the incorporation of glucose residues from UDP-glucose to the polymer, which links carbon-1 of the donated glucose to carbon-4 of the polymer of glycogen, thus forming α -1,4-glycosidic linkages and releasing UDP. In humans, there are two known isoforms of GYS: GYS1 and GYS2. GYS1 is abundant in skeletal muscle but is also present in other tissues, such as the adipose tissue, kidney, spleen, nervous system, and testis, while GYS2 is tissue-specific [32–34]. These enzymes are activated by phosphatases such as protein phosphatase-1 (PP1), which dephosphorylate their

target proteins, and are stimulated by the allosteric activator glucose 6-phosphate. On the other hand, GYS are inactivated through phosphorylation by kinases such as 5'adenosine monophosphate-activated protein kinase (AMPK) and high levels of glycogen [26,29]. GYS1 is also phosphorylated by glycogen synthase kinase 3 beta (GSK3- β), cAMP-dependent protein kinase A (PKA), phosphorylase kinase, calmodulin-dependent protein kinase II (CAMKII), and casein kinase I and II [33,35,36]. Finally, GBE catalyses the transfer of a glycosyl chain to form a highly branched polymer of glycogen, originating α -1,6-glycosidic linkages [26,29].

Cellular glycogen can be degraded in either lysosomes or the cytosol. Glycogen can be deposited inside the lysosomes, probably due to the action of autophagic vacuoles that encase a piece of cytoplasm and fuse with these organelles to process their content [37]. Glycogen is then hydrolysed by acid α -1,4-glucosidase (GAA), releasing glucose. GAA hydrolyses 1,4-linked α -glucose polymers first, but the mode by which the branch points are untied remains unclear. GAA needs to suffer post-translational processing to act in the degradation of glycogen. After the synthesis of a precursor polypeptide with seven glycosylation sites, allowing the attachment of carbohydrate chains to asparagine residues to form the N-linked glycosylation of GAA, the maturation of GAA occurs due to proteolytic processing in the amino and carboxyl terminus, which results in the formation of two GAA [38]. In the cytosol, glycogen degradation is catalysed by two enzymes—glycogen phosphorylase (PYG) and glycogen debranching enzyme (AGL). PYG catalyses the phosphorolysis of α -1,4-glycosidic bonds, releasing glucose-1-phosphate. This enzyme, however, only acts until four glucose residues remain in the branch before the α -1,6-branch point. Thus, the degradation of glycogen needs the action of AGL to surpass the branch points. This enzyme has two catalytic activities, α -1,4-glucanotransferase and amylo- α -1,6-glucosidase. The first one moves three of the four glucose from the lateral string to another linear strand, while the glucosidase hydrolyses the α -1-6-glycosidic bond of the branch point, releasing glucose and allowing the action of PYG in α -1,4 linkages (Figure 2) [39].

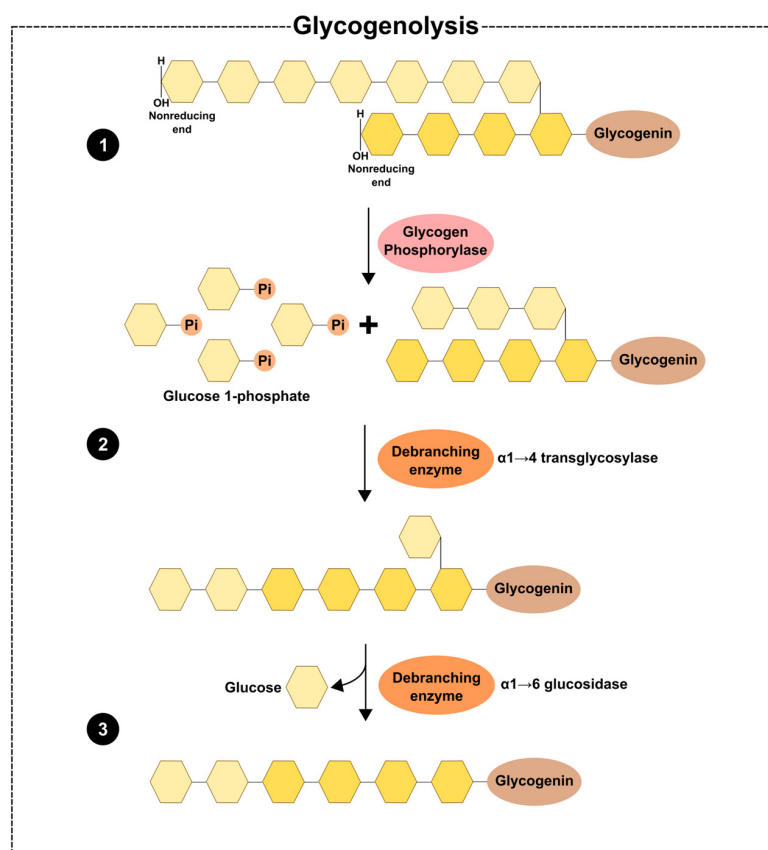


Figure 2. Schematic illustration of the degradation of glycogen in the cytosol: (1) the degradation starts

with the phosphorolysis of the (α -1 \rightarrow 4) glycosidic bonds by glycogen phosphorylase, releasing glucose-1-phosphate, until four glucose residues remain at a branch point. The glycogen debranching enzyme has two activities; (2) the transferase activity catalyses the movement of three residues of glucose from a lateral to a linear chain; (3) the α -1,6-glucosidase activity hydrolyses the (α -1 \rightarrow 6) linkages, releasing glucose.

3. Glycogen in the Testicular Environment

The testes have two important functions: the production of mature sperm (spermatogenesis) and the synthesis of steroid hormones (steroidogenesis). The action of Sertoli, Leydig, and germ cells must be coordinated to accomplish all these events. The metabolic cooperation established within these cells is fundamental to supporting the energy needs for spermatogenesis and steroidogenesis in the testis, although the particular role of glycogen in this event is far from being fully elucidated (Figure 3).

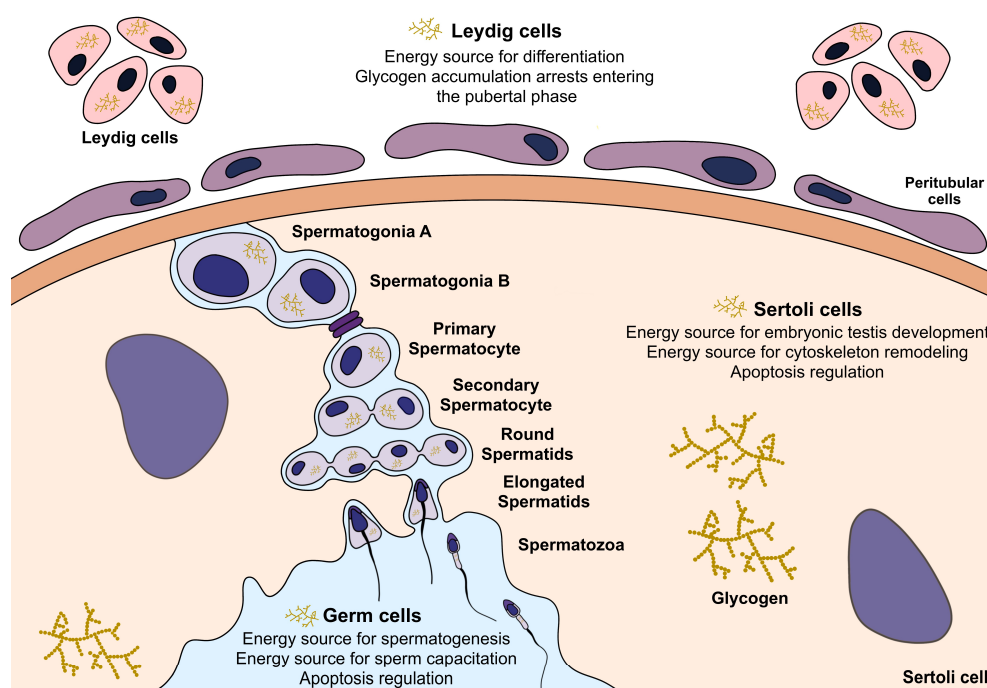


Figure 3. Illustration of effects of glycogen metabolism in testicular somatic and germ cells. In Sertoli cells, glycogen is hypothesised to be a source of energy for embryonic testis development and cytoskeleton remodelling, but also an apoptosis regulator. In other somatic cells, Leydig cells, glycogen metabolism is hypothesised to offer energy for differentiation, but its accumulation arrests their differentiation. Glycogen could also provide energy for spermatogenesis and capacitation and be an apoptosis regulator in germ cells.

3.1. Glycogen Metabolism in Sertoli Cells

In 1865, SCs were described for the first time by Enrico Sertoli [46]. SCs, also known as “nurse cells”, are somatic cells that have fundamental roles in the regulation of spermatogenesis. SCs are responsible for the formation of the BTB, providing structural and nutritional support to the developing germ cells [47,48]. They also create an immunological barrier that regulates the infiltration of cytokines and immune cells in seminiferous tubules and controls the movement of substances between the lumen of the seminiferous tubules and the interstitial compartment [18,49,50].

ATP production in SCs mainly results from the phagocytosis of apoptotic germ cells and residual bodies, through the uptake of lipids that undergo beta-oxidation [51]. Glucose

is canalised into lactate production by SCs, which is preferentially used by germ cells over glucose for ATP production, although the reason behind the preference for this energy substrate remains unclear [48]. Lactate stimulates the synthesis of ribonucleic acid (RNA) and proteins and inhibits apoptosis in germ cells [47,52]. Hence, SC metabolism is of foremost importance for spermatogenesis [53]. SCs present a Warburg-like metabolism and prefer fermentative metabolism to the oxidative metabolism of glucose. As in cancer cells, SCs have a high glycolytic flux for lactate production to be used by developing germ cells and not to sustain high rates of cellular proliferation [53]. Glucose is transported into SCs by GLUTs. The presence of GLUT1, GLUT2, GLUT3, and GLUT4 has been identified in SCs [54–57]. Glucose is metabolised through glycolysis, producing pyruvate. This metabolite can follow three different paths. Pyruvate may be converted to acetyl-CoA and enter the Krebs cycle through the action of the pyruvate dehydrogenase complex. Acetyl-CoA, in turn, can be converted to acetate or used in the Krebs cycle in the mitochondria. Pyruvate may also be converted into alanine through the action of alanine aminotransferase. Most of the pyruvate from the SCs, however, follows a third path—conversion to lactate via the action of lactate dehydrogenase (LDH) [53]. LDH is reported to be regulated by growth factors, cytokines, and sex steroid hormones in SCs [13,58,59]. Interestingly, LDH has been identified to bind and regulate mRNAs [60]. The lactate and acetate produced by SCs are then transported to germ cells by monocarboxylate transporters (MCTs). MCT1 and MCT4 were identified in SCs as being primarily responsible for the release of lactate in the intratubular fluid and used by germ cells [61,62]. MCT4 is an important lactate exporter in cells with high glycolytic capacity, which suggests it has an important role in SCs [61,62].

Interestingly, SCs can continue to produce lactate in the absence of glucose [21]. SCs produce lactate mainly as a product of glycolysis, but also as a product of glycogen degradation (Figure 4). It is estimated that only about 1% of the total glucose that is incorporated into SCs is converted into lipids and glycogen, whereas about 95% is converted to lactate [63]. Compelling evidence suggests that the endogenous sources of energy substrates in SCs, including glycogen, are crucial for the development of germ cells [32]. In 1969, Leiderman and Mancini reported the presence of glycogen in SCs by using the method of periodic acid–Schiff reaction (PAS) in different ages of rats, suggesting that glycogen may have a role in gonadal differentiation [64]. Slaughter and Means identified the presence of PGY in SCs and hypothesised that it had the function of providing energy for cytoskeletal motility. The low presence of glycogen in SCs and the high activity of PYG indicate that it can be used in situations where other energy sources are scarce [65]. Glycogen is also important during embryonic testicular development. In the embryonic stages of mouse sex determination, the differentiation of SCs is promoted via the activation of the sex-determining region Y (SRY), which leads to the activation of SOX9 in XY gonads, resulting in testis formation. Glycogen accumulation begins in the pre-SCs of developing mice, right after the onset of SRY expression. This accumulation is necessary for the activation of SOX9, suggesting that an energy source of glucose is immediately needed for testicular morphogenesis [66]. During postnatal stages, it was reported that less than 2% of incorporated glucose is converted to glycogen [63].

Villarreal-Espindola et al. demonstrated the presence of the muscle isoform of glycogen synthase (GYS1) in SCs, although it was found to have low intrinsic activity. Still, it can synthesise glycogen. These authors also reported that GYS is more active during the first 5 postnatal days in mouse testis [32]. Maldonado et al. observed that GYS1 is localised in the nucleus and cytoplasm of SCs, although it was hypothesised to be mostly inactive because of the low quantity of glucose that is converted into glycogen by SCs. In addition, it was shown that higher quantities of glycogen decreased the activity of GYS1 [67]. The kinases that regulate GYS1 activity, such as GSK3 β , p38 mitogen-activated protein kinases (p38 MAPK), the dual-specificity tyrosine-regulated kinases (DYRK) family, and casein kinase II (CK2), are equally expressed in SCs [68,69]. In other cell types, GYS was identified as a component of ribosomes using proteomic multidimensional protein identification technology, and the authors hypothesised it to have a non-metabolic role due

to the association of this enzyme with translationally active ribosomes, specifically in the phosphorylated form [70]. In support of these results, Maldonado et al. reported that GYS1 in SCs interacts with the molecules of rRNAs and mRNAs, suggesting a potential regulatory role [67]. Studies highlight that GYS is regulated by the malin–laforin complex through polyubiquitination and proteasomal degradation processes [71]. Malin (a ubiquitin ligase) and laforin (a phosphatase complex), which promotes the polyubiquitination of GYS, are expressed in SCs [32]. The malin–laforin complex is important for glycogen homeostasis maintenance [32], as glycogen accumulation is associated with apoptosis [71]. In SCs, glycogen accumulation negatively affects the remodelling of the cytoskeleton and disorganises the germinal epithelium [72,73]. Studies have reported that lithium carbonate, an activator of GYS, causes the desquamation of the male germinal epithelium due to a failure in the integrity of the junctions between SCs and the basal lamina in adult rats [73,74].

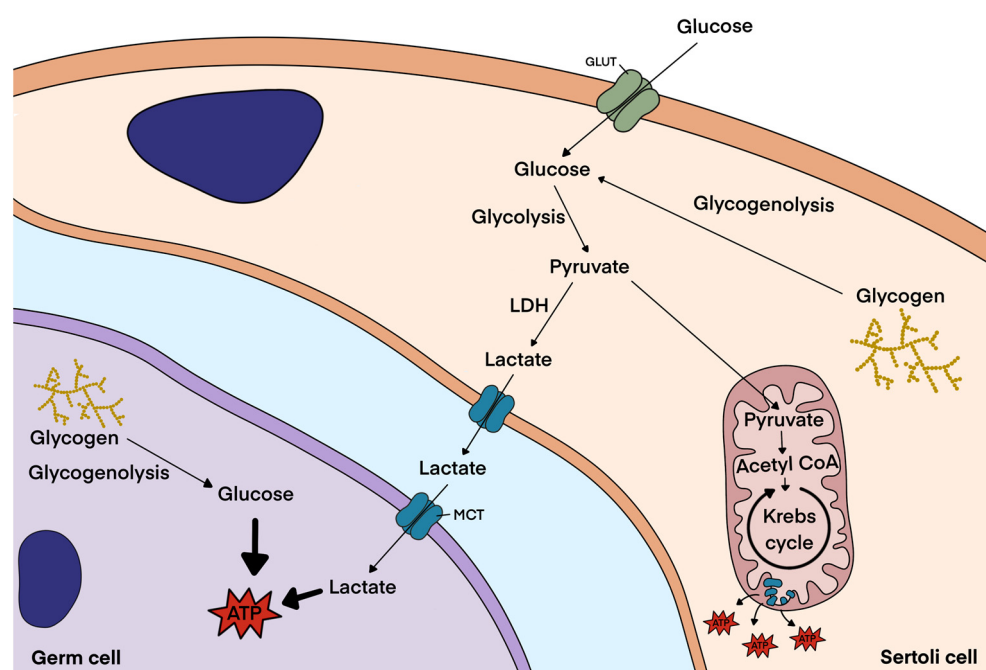


Figure 4. Illustration of the role of glycogen on Sertoli cell and germ cell metabolism. In Sertoli cells, glycogenolysis results in the release of glucose which undergoes glycolysis to produce lactate, the preferential energy source of germ cells. Lactate is transported from Sertoli cells to germ cells by monocarboxylate transporters (MCTs). Pyruvate, resultant from glycolysis, can also be used for energy production through oxidative phosphorylation in the mitochondria. Abbreviations: GLU—glucose transporter, LDH—lactate dehydrogenase, MCT—monocarboxylate transporter.

Glycogen synthesis and degradation are also altered in metabolic dysfunction scenarios. Our group showed that diabetic rats accumulate glycogen in SCs. Our results suggest that SCs use glycogen for germ cell survival in situations of alterations in glucose and insulin homeostasis. In addition, a decrease in glycogen storage occurs when there is a decrease in insulinemia in diabetic rats [13]. Insulin deficiency results in the activation of GSK3, which inhibits GYS1 and keeps the PYG activated, resulting in the inhibition of glycogen synthesis [75]. Taken together, these findings suggest that GYS could play a role in the apoptosis and degeneration of seminiferous tubules, shedding light on its potential role in the crosstalk between male infertility and type 2 diabetes mellitus.

3.2. Glycogen Metabolism in Leydig Cells

In 1850, Franz Leydig described these testicular cells and observed that their location is restricted to the interstitial space, later named Leydig cells [76]. The type of Leydig

cells that differentiate prenatally and postnatally are designed as foetal and adult Leydig cells, respectively. The development of the postnatal cells involves events such as the proliferation of stem Leydig cells, the differentiation of stem Leydig cells to immature Leydig cells, and the subsequent differentiation process into mature adult Leydig cells [77].

Leydig cells are the primary source of androgens [78]. The production of androgens is regulated by the hypothalamic–pituitary–gonadal axis, which not only regulates the secretion of androgens to the systemic circulation but also into the testicular environment. The binding of LH to its receptor on the Leydig cell membrane stimulates the production of testosterone, which is essential for the development of male germ cells [79]. Testosterone also has a role in controlling meiosis in spermatogenesis [80–82], spermiation [83,84], maintenance of BTB [85], and modulating the action of SCs [86].

The synthesis of testosterone (steroidogenesis) is a complex multistep process that starts with cholesterol entering the mitochondria. This process is catalysed through the action of the steroidogenic acute regulatory protein (StAR) and the translocator protein (TSPO) [87,88]. This action is dependent on the binding of LH to its receptor in Leydig cells, activating adenyl cyclase to increase the concentration of cAMP, activating PKA, and a signalling cascade effect that allows the transport of cholesterol to the inside of mitochondria through the StAR protein. After cholesterol enters the mitochondria, it is converted into pregnenolone by the side-chain cleavage cytochrome P450 enzyme (CYP11A1). In the smooth endoplasmic reticulum, pregnenolone is converted into testosterone through the action of 3 β -hydroxysteroid dehydrogenase (HSD3B), 17 α -hydroxylase (CYP17A1), and 17 β -hydroxysteroid dehydrogenase (HSD17B) [79,89].

Androgens have a key role in sexual behaviour and male sex differentiation [90] and are very important for initiating, maintaining, and regulating the process of spermatogenesis [22]. Although there are no functional androgen receptors (ARs) in germ cells, androgens are central to the maintenance of germ cell development. SCs and peritubular myoid cells, in turn, express ARs, which are the pathways for androgen signalling into the developing germ cells [91]. Compelling evidence highlights that Leydig cells also regulate spermatogenesis by producing growth factors such as interleukin 1 α , transforming growth factor β (TGF β), inhibin, insulin-like growth factors 1 (IGF1), and insulin-like peptide 3 (INSL3) [92,93].

During steroidogenesis, the activity of the StAR protein is a limiting step. This protein needs ATP for its activity and imports cholesterol into the mitochondrion [94]. It is hypothesised that the ATP comes from oxidative phosphorylation and cytosolic glycolysis in Leydig cells, but the last pathway produces much less ATP than the first pathway [23]. The dependence of glucose in steroidogenesis was also reported since the inhibition of glucose uptake could induce a decrease in testosterone production [95]. Glucose transporters such as GLUT1, GLUT3, and GLUT8 were identified in rats' Leydig cells, and it was hypothesised that these transporters are essential for steroidogenesis occurrence [96]. A study by Banerjee et al. explored the association of glucose and GLUTs with steroidogenesis. Stimulation with LH increased testosterone production and led to an increase in the expression of GLUT8 and StAR proteins. In addition, a decline in GLUT expression and glucose levels is linked with hypoandrogenism or andropause [97].

The presence of phosphoenolpyruvate carboxykinase, (an enzyme that catalyses the conversion of oxaloacetate to phosphoenolpyruvate with the release of carbon dioxide) and the enzyme glucose-6-phosphatase (involved in the conversion of glucose-6-phosphate into glucose), have been reported in mouse Leydig cells. These enzymes belong to the gluconeogenesis pathway, which may indicate that Leydig cells support a low rate of glucose synthesis. In addition, the importance of these enzymes in supporting steroidogenesis has been reported, because the inhibition of these enzymes decreased steroidogenesis [98].

Besides glucose, glycogen is another energy source available in Leydig cells, and data suggest that it plays a role in cell differentiation and synthesis. Glycogen was found in the Leydig cells of newborn mice, with high levels of glycogen in the form of clusters of beta particles, while in mature Leydig cells, low concentrations of glycogen distributed along

the structure of the endoplasmic reticulum are present. Hence, glycogen distribution in these cells suggests that it may play a role in Leydig cell differentiation by being a source of energy or building material for the synthesis of membranous components by Leydig cells [99]. In fact, a study by Prince showed the presence of glycogen in the cytoplasm of immature human Leydig cells, where higher levels of glycogen were found in cells with the low presence of smooth endoplasmic reticulum [100]. Interestingly, Khalaf et al. reported that the Leydig cells are unable to enter the pubertal phase and remain in the prepubertal phase, containing higher levels of glycogen, in the absence of zinc [101]. Glycogen was also detected in foetal rat Leydig cells [102]. Thus, glycogen metabolism in Leydig cells can not only be a source of energy for steroidogenesis but also play a role in their differentiation and maturation.

3.3. Glycogen Metabolism in Germ Cells and Spermatozoa

Spermatogenesis is a process that involves three events—mitotic spermatogonial proliferation and differentiation, meiotic phase, and spermiogenesis [103]. Spermatogonia are defined as undifferentiated germ cells localised in the basal compartment of the seminiferous tubules and mitotically replicate to give rise to spermatogonia A and spermatogonia B, the latter devoted to differentiating and moving along the seminiferous epithelium [103,104].

Spermatogonia B will differentiate into preleptotene spermatocytes, which undergo a final replication of nuclear DNA and cross the BTB from the basal to the apical compartment. Then, preleptotene spermatocytes differentiate into secondary spermatocytes through meiosis. The second meiotic division produces round spermatocytes. After the formation of spermatids, cell division stops, and spermiogenesis starts to form elongated spermatids. At the end of spermiogenesis, spermatids that were elongated are released into the lumen of the tubule as immature spermatozoa [103].

Germ cells have unique nutritional requirements during spermatogenesis, altering their metabolic profile through their development [105]. The reason behind this divergence is unknown, but it has been assumed to be due to the structure and compartmentalisation of the testis [106]. The testis has been reported as an organ commonly deprived of oxygen [107], which could explain why germ cells utilise different metabolic pathways to produce energy for their various development stages. Germ cells in development rigorously depend on carbohydrate metabolism, utilising both aerobic and anaerobic pathways [105]. The spermatogonia use mostly glucose for ATP production, while spermatocytes and spermatids use lactate supplied by SCs. For this reason, spermatogenesis essentially requires glucose, directly as fuel for spermatogonia, or indirectly for lactate production in spermatocytes and spermatids by Sertoli cells [48].

Glucose is also the preferred source of energy for human sperm capacitation and the acquisition of hyperactivated motility. Capacitation is characterised by a cascade of phosphorylation of proteins involved in signal transduction and the increased oxidation of energy sources. To achieve that, glucose is a source of energy for spermatozoa [108]. Intracellular ionic balance, which is essential for phosphorylation cascade activation and hyperactivated motility, requires active transport, and consequently ATP [109]. The spermatozoa have a high level of functional metabolic flexibility, and their metabolic pathways are complex and finely regulated, whereas any alteration that affects the production of ATP can compromise sperm quality.

Following spermatogonia and sperm requirements of glucose, the presence of GLUTs was identified in human spermatozoa, the first being GLUT5 in the subequatorial region, mid- and principal pieces of human spermatozoa [110,111]. In addition, GLUT1 and GLUT2 were identified in the acrosomal region, principal and end pieces of human spermatozoa [11]. GLUT8 is present in the acrosomal membrane, in the post-acrosomal region, and in the tail [112]. This transporter is highly expressed in the early stages of the development of spermatocytes but in lower levels in mature spermatozoa [113]. The expression of GLUT3 was detected in the midpiece of spermatozoa [114]. Studies with

rats have also shown the presence of GLUT1 and GLUT3 in spermatocytes, spermatids, and spermatozoa. A high expression of GLUT3 was found in spermatids, indicating that GLUTs could participate in the differentiation stage of spermatogenesis [115]. The presence of these glucose transporters indicates that germ cells can use glucose as an energy substrate, but data also showed that glucose is not a good energy substrate for male germ cells [47,116–118]. In addition, the glycolytic pathway is inhibited during the transition of the spermatocytes to spermatids and is later activated in spermatozoa [119].

A study by Bajpai showed that rat spermatocytes have a higher activity of pentose phosphate and glycolytic pathways than spermatids, but spermatids have higher activity in the Krebs cycle than spermatocytes. Higher activity of the glycolytic pathway and lower activity of the Krebs cycle and pentose phosphate pathway were found in spermatozoa. Thus, spermatids use lactate due to their lower glycolytic activity, while spermatocytes can use other substrates, including pyruvate and/or lactate, due to their higher glycolytic activity [105]. Indeed, spermatids incubated with high levels of glucose [10 mM] showed a decrease in ATP levels and an increase in 5'-AMP [120]. Still, both the glycolytic and gluconeogenic pathways may be functional in spermatids due to the metabolic recycling of lactate to glucose-6-phosphate [121]. Spermatozoa, in turn, seem to prefer glucose or fructose as an energy substrate, which may be due to their higher glycolytic activity and lower Krebs cycle activity [122].

Lactate is a major energy substrate produced by SCs and is transported to germ cells through MCTs. MCT1 is expressed in all types of germ cells, while MCT2 was only detected in elongated spermatids of rats [123,124]. Spermatocytes and spermatids use preferentially lactate and not glucose to produce ATP, as it was observed that glucose induces the apoptosis of rat spermatocytes, while lactate could have a protective effect on spermatocytes [124]. Some specific glycolytic enzyme isoforms are predominantly expressed in spermatogenic cells [125], such as LDH-C, an isoform abundantly expressed in spermatids and spermatozoa [126,127], which was also detected in preleptotene spermatocytes [128]. Alterations in the *Ldhc* gene lead to a progressive decline in spermatozoon motility, incapacity to develop hyperactivated motility, and a decrease in ATP levels, resulting in male infertility [129]. Other specific isozymes that are only expressed in spermatogenesis are glyceraldehyde-3-phosphate dehydrogenase-S, which converts glyceraldehyde-3-phosphate to 1,3-diphosphoglycerate [130], and phosphoglycerate kinase-2, which is involved in the synthesis of pyruvate from glyceraldehyde-3-phosphate [131,132].

ATP production in spermatozoa is spatially organised, as glycolysis occurs in the principal piece, while the mitochondria and oxidative phosphorylation are restricted to the midpiece. The source of energy for the acrosome reaction, however, is unclear [125]. One potential source of energy is glycogen. Glycogen was found in boar and dog spermatozoa in both the tail and the head [133,134], and glycogen metabolism in sperm appears to be important for fertilisation. Albarracín et al. reported that glycogen metabolism occurs during the *in vitro* capacitation of dog spermatozoa and also demonstrated that the lactate uptake induces an accumulation of radioactive glycogen, confirming an active gluconeogenic activity [135]. Results from our group suggest the presence of endogenous energy sources that could sustain human spermatozoon motility [109]. The presence of glycogen was also observed in germ cells, with the highest levels in spermatocytes and lower in other germ cells [64].

The presence of enzymes that are involved in gluconeogenesis has also been identified such as fructose-1,6-bisphosphatase, phosphofructose-1 kinase, and aldolase B, suggesting the possibility to store glycogen [121,135,136]. In rat spermatids, it was observed that they can convert lactate to fructose and glucose-6-phosphate which indicates the presence of glycolytic and gluconeogenic activities [121]. The presence of fructose-1,6-bisphosphatase in spermatids and spermatozoa but its absence in spermatocytes suggests the importance of this enzyme in these cells' differentiation [121,136]. Another study also found the presence of phosphorylase, glucose-6-phosphatase, fructose-1,6-bisphosphatase, and amylase in mouse epididymal spermatozoa. Additionally, it has been reported that mouse sper-

matozoa use glycogen to supply glucose for their development, to feed the glycolysis pathway, and, curiously, the level of activity of the glycolysis pathway is higher in the head of spermatozoa than in the tail [137].

The expression and activity of PYG were observed in germ cells, but the isoform that is present was not determined [138,139]. Villarroel-Espíndola et al. reported the presence of the muscle isoform of GYS as responsible for producing glycogen in the testis. The levels of glycogen, however, were found lower in germ cells as compared to SCs [32]. Compelling evidence indirectly suggests that an imbalance in glycogen homeostasis in adult rat testes could induce apoptosis and germ cell degeneration [32,74,140]. The use of phthalate esters, which inhibits the activity of glycogen debranching enzyme, induces a higher increase in the apoptosis of germ cells in adult testes [140]. Another study in human cryptorchid tissue, which showed a strong PAS stain and an abundant fibrosis signal could indicate an indirect connection of glycogen accumulation with testis degeneration [141]. Villarroel-Espíndola et al. reported that the overexpression of protein targeting glycogen (PTG), which is an indirect activator of GYS, promotes an increase in glycogen levels and the presence of a cleaved form of caspase-3, suggesting that the accumulation of glycogen could be a proapoptotic signal in male germ cells principally in spermatogonia and spermatocytes [32].

Taken together, these data suggest that glycogen may be a source of glucose in germ cells in conditions of low substrate conditions, but glycogen accumulation could have a negative effect that leads to the apoptosis of spermatogenic cells. The function and effects of glycogen in germ cells remain unclear. Thus, more studies are needed to understand the impact of glycogen metabolism and accumulation in spermatogenesis.

4. Conclusions and Future Perspectives

Currently, few studies have addressed the potential role of glycogen in the testicular environment. Glycogen is present in the different testicular cells, particularly in Sertoli and Leydig cells, and spermatozoa, but its role is still unclear. Glycogen function appears to be dependent on the type of cell in which it is present. In SCs, glycogen and glycogen-related enzymes were identified and appear to be involved in SCs' metabolism. Interestingly, the accumulation of glycogen could be responsible for the apoptosis and degeneration of seminiferous tubules, leading to male infertility. In Leydig cells, glycogen can act as a source of ATP for steroidogenesis. In addition, glycogen is also important for the differentiation and maturation of Leydig cells. Despite its metabolic function, several other roles for glycogen have been highlighted. Glycogen has a regulatory role in the translation of RNAs, and its accumulation is associated with the induction of proapoptotic cascades in spermatogenic cells. Nevertheless, the data concerning the function of glycogen in the testis are scarce, so more studies are further needed to disclose the role of glycogen in the male reproductive tract. Over the years, the interest in testicular metabolomics has been increasing, and this leads to the possibility of exploring new pathways modulating male fertility. Another interesting idea is that eating habits that affect the metabolism of glycogen could be related to male infertility.

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References

- Jørgensen, N.; Andersen, A.G.; Eustache, F.; Irvine, D.S.; Suominen, J.; Petersen, J.H.; Andersen, A.N.; Auger, J.; Cawood, E.H.H.; Horte, A.; et al. Regional Differences in Semen Quality in Europe. *Hum. Reprod.* **2001**, *16*, 1012–1019. [\[CrossRef\]](#) [\[PubMed\]](#)
- Jørgensen, N.; Carlsen, E.; Nermoen, I.; Punab, M.; Suominen, J.; Andersen, A.G.; Andersson, A.M.; Haugen, T.B.; Horte, A.; Jensen, T.K.; et al. East-West Gradient in Semen Quality in the Nordic-Baltic Area: A Study of Men from the General Population in Denmark, Norway, Estonia and Finland. *Hum. Reprod.* **2002**, *17*, 2199–2208. [\[CrossRef\]](#) [\[PubMed\]](#)
- Fernandez, M.F.; Duran, I.; Olea, N.; Avivar, C.; Vierula, M.; Toppari, J.; Skakkebaek, N.E.; Jørgensen, N. Semen Quality and Reproductive Hormone Levels in Men from Southern Spain. *Int. J. Androl.* **2012**, *35*, 1–10. [\[CrossRef\]](#) [\[PubMed\]](#)
- Nordkap, L.; Joensen, U.N.; Blomberg Jensen, M.; Jørgensen, N. Regional Differences and Temporal Trends in Male Reproductive Health Disorders: Semen Quality May Be a Sensitive Marker of Environmental Exposures. *Mol. Cell. Endocrinol.* **2012**, *355*, 221–230. [\[CrossRef\]](#)
- Sun, H.; Gong, T.-T.; Jiang, Y.-T.; Zhang, S.; Zhao, Y.-H.; Wu, Q.-J. Global, regional, and national prevalence and disability-adjusted life-years for infertility in 195 countries and territories, 1990–2017: Results from a global burden of disease study, 2017. *Aging* **2019**, *11*, 1990–2017. [\[CrossRef\]](#)
- Vander Borgh, M.; Wyns, C. Fertility and infertility: Definition and epidemiology. *Clin. Biochem.* **2018**, *62*, 2–10. [\[CrossRef\]](#)
- Cavallini, G.; Beretta, G. *Clinical Management of Male Infertility*; Springer: London, UK, 2015; pp. 1–187.
- Nieschlag, E.; Behre, H.M.; Nieschlag, S. *Andrology Male Reproductive Health and Dysfunction*; Springer: Berlin/Heidelberg, Germany, 2010; pp. 87–92.
- Rato, L.; Alves, M.G.; Cavaco, J.E.; Oliveira, P.F. High-Energy Diets: A Threat for Male Fertility? *Obes. Rev.* **2014**, *15*, 996–1007.
- Sermondade, N.; Faure, C.; Fezeu, L.; Shayeb, A.G.; Bonde, J.P.; Jensen, T.K.; van Wely, M.; Cao, J.; Martini, A.C.; Eskandar, M.; et al. BMI in relation to sperm count: An updated systematic review and collaborative meta-analysis. *Hum. Reprod. Update* **2013**, *19*, 221–231. [\[CrossRef\]](#)
- Schisterman, E.F.; Mumford, S.L.; Chen, Z.; Browne, R.W.; Barr, D.B.; Kim, S.; Louis, G.M.B. Lipid concentrations and semen quality: The LIFE study. *Andrology* **2014**, *2*, 408–415. [\[CrossRef\]](#)
- Bener, A.; Al-Ansari, A.A.; Ziric, M.; Al-Hamaq, A.O. Is Male Fertility Associated with Type 2 Diabetes Mellitus? *Int. Urol. Nephrol.* **2009**, *41*, 777–784. [\[CrossRef\]](#)
- Rato, L.P.; Alves, M.G.; Dias, T.R.; Cavaco, J.E.; Oliveira, P.F. Testicular Metabolic Reprogramming in Neonatal Streptozotocin-Induced Type 2 Diabetic Rats Impairs Glycolytic Flux and Promotes Glycogen Synthesis. *J. Diabetes Res.* **2015**, *2015*, 973142. [\[CrossRef\]](#) [\[PubMed\]](#)
- Curi, S.M.; Ariagno, J.I.; Chenlo, P.H.; Mendeluk, G.R.; Pugliese, M.N.; Sardi Segovia, L.M.; Repetto, H.E.H.; Blanco, A.M. Asthenozoospermia: Analysis of a Large Population. *Arch. Androl.* **2003**, *49*, 343–349. [\[CrossRef\]](#) [\[PubMed\]](#)
- Xuan, W.; Lamhonwah, A.-M.; Librach, C.; Jarvi, K.; Tein, I. Characterization of organic cation/carnitine transporter family in human sperm. *Biochem. Biophys. Res. Commun.* **2003**, *306*, 121–128. [\[CrossRef\]](#)
- Liu, F.-J.; Liu, X.; Han, J.-L.; Wang, Y.-W.; Jin, S.-H.; Liu, X.-X.; Liu, J.; Wang, W.-T. Aged men share the sperm protein PATE1 defect with young asthenozoospermia patients. *Hum. Reprod.* **2015**, *30*, 861–869. [\[CrossRef\]](#) [\[PubMed\]](#)
- Stanton, P.G. Regulation of the blood-testis barrier. *Semin. Cell Dev. Biol.* **2016**, *59*, 166–173. [\[CrossRef\]](#) [\[PubMed\]](#)
- Kaur, G.; Thompson, L.A.; Dufour, J.M. Sertoli cells—Immunological sentinels of spermatogenesis. *Semin. Cell Dev. Biol.* **2014**, *30*, 36–44. [\[CrossRef\]](#)
- Park, Y.-J.; Pang, M.-G. Mitochondrial Functionality in Male Fertility: From Spermatogenesis to Fertilization. *Antioxidants* **2021**, *10*, 98. [\[CrossRef\]](#)
- Riera, M.F.; Meroni, S.B.; Schteingart, H.F.; Pellizzari, E.H.; Cigorraga, S.B. Regulation of lactate production and glucose transport as well as of glucose transporter 1 and lactate dehydrogenase A mRNA levels by basic fibroblast growth factor in rat Sertoli cells. *J. Endocrinol.* **2002**, *173*, 335–343. [\[CrossRef\]](#)
- Riera, M.F.; Galarido, M.N.; Pellizzari, E.H.; Meroni, S.B.; Cigorraga, S.B. Molecular mechanisms involved in Sertoli cell adaptation to glucose deprivation. *Am. J. Physiol. Metab.* **2009**, *297*, E907–E914. [\[CrossRef\]](#)
- Jarow, J.P.; Zirkin, B.R. The Androgen Microenvironment of the Human Testis and Hormonal Control of Spermatogenesis. *Ann. N. Y. Acad. Sci.* **2005**, *1061*, 208–220. [\[CrossRef\]](#)
- Medar, M.L.J.; Marinkovic, D.Z.; Kojic, Z.; Becin, A.P.; Starovlah, I.M.; Kravic-Stevovic, T.; Andric, S.A.; Kostic, T.S. Dependence of Leydig Cell's Mitochondrial Physiology on Luteinizing Hormone Signaling. *Life* **2020**, *11*, 19. [\[CrossRef\]](#)
- Rodríguez-Gil, J.E.; Bonet, S. Current knowledge on boar sperm metabolism: Comparison with other mammalian species. *Theriogenology* **2016**, *85*, 4–11. [\[CrossRef\]](#) [\[PubMed\]](#)

25. Ruiz-Pesini, E.; Díez-Sánchez, C.; López-Pérez, M.J.; Enríquez, J.A. The Role of the Mitochondrion in Sperm Function: Is There a Place for Oxidative Phosphorylation or Is This a Purely Glycolytic Process? *Curr. Top. Dev. Biol.* **2007**, *77*, 3–19. [[PubMed](#)]
26. Adeva-Andany, M.M.; González-Lucán, M.; Donapetry-García, C.; Fernández-Fernández, C.; Ameneiros-Rodríguez, E. Glycogen metabolism in humans. *BBA Clin.* **2016**, *5*, 85–100. [[CrossRef](#)] [[PubMed](#)]
27. Chikwana, V.M.; Khanna, M.; Baskaran, S.; Tagliabracchi, V.S.; Contreras, C.J.; DePaoli-Roach, A.; Roach, P.J.; Hurley, T.D. Structural basis for 2'-phosphate incorporation into glycogen by glycogen synthase. *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 20976–20981. [[CrossRef](#)]
28. Preiss, J.; Walsh, D.A. *The Comparative Biochemistry of Glycogen and Starch*; Ginsbg, V., Robbins, P., Eds.; John Wiley Sons: New York, NY, USA, 1981; pp. 199–314.
29. Roach, P.J. Glycogen and Its Metabolism. *Curr. Mol. Med.* **2002**, *2*, 101–120. [[CrossRef](#)]
30. Barbetti, F.; Rocchi, M.; Bossolasco, M.; Cordera, R.; Sbraccia, P.; Finelli, P. The Human Skeletal Muscle Glycogenin Gene: CDNA, Tissue Expression, and Chromosomal Localization. *Biochem. Biophys. Res. Commun.* **1996**, *220*, 72–77. [[CrossRef](#)]
31. Mu, J.; Roach, P.J.; Chem, J.B. Characterization of Human Glycogenin-2, a Self-Glucosylating Initiator of Liver Glycogen Metabolism. *J. Biol. Chem.* **1998**, *273*, 34850–34856. [[CrossRef](#)]
32. Villarroel-Espíndola, F.; Maldonado, R.; Mancilla, H.; Stelt, K.V.; Acuña, A.I.; Covarrubias, A.; López, C.; Angulo, C.; Castro, M.A.; Slebe, J.C.; et al. Muscle glycogen synthase isoform is responsible for testicular glycogen synthesis: Glycogen overproduction induces apoptosis in male germ cells. *J. Cell. Biochem.* **2013**, *114*, 1653–1664. [[CrossRef](#)]
33. Halse, R.; Fryer, L.G.D.; McCormack, J.G.; Carling, D.Y.S. Regulation of Glycogen Synthase by Glucose and Glycogen: A Possible Role for Amp-Activated Protein Kinase. *Diabetes* **2003**, *52*, 9–15. [[CrossRef](#)]
34. Nuttall, F.Q.; Gannon, M.C. Allosteric Regulation of Glycogen Synthase in Liver. *J. Biol. Chem.* **1993**, *268*, 13286–13290. [[CrossRef](#)]
35. Palm, D.C.; Rohwer, J.M.; Hofmeyr, J.-H.S. Regulation of glycogen synthase from mammalian skeletal muscle—A unifying view of allosteric and covalent regulation. *FEBS J.* **2012**, *280*, 2–27. [[CrossRef](#)]
36. Jensen, J.; Jebens, E.; Brennevik, E.O.; Ruzzin, J.; Soos, M.A.; Engebretsen, E.M.L.; O'Rahilly, S.; Whitehead, J.P. Muscle glycogen inharmoniously regulates glycogen synthase activity, glucose uptake, and proximal insulin signaling. *Am. J. Physiol. Metab.* **2006**, *290*, E154–E162. [[CrossRef](#)]
37. Kotoulas, O.B.; Kalamidas, S.A.; Kondomerkos, D.J. Glycogen autophagy in glucose homeostasis. *Pathol. Res. Pract.* **2006**, *202*, 631–638. [[CrossRef](#)] [[PubMed](#)]
38. Wisselaar, H.; Kroos, M.; Hermans, M.; van Beeumen, J.; Reuser, A. Structural and functional changes of lysosomal acid alpha-glucosidase during intracellular transport and maturation. *J. Biol. Chem.* **1993**, *268*, 2223–2231. [[CrossRef](#)]
39. Chen, Y.T.; He, J.K.; Ding, J.H.; Brown, B.I. Glycogen debranching enzyme: Purification, antibody characterization, and immunoblot analyses of type III glycogen storage disease. *Am. J. Hum. Genet.* **1987**, *41*, 1002–1015.
40. Burwinkel, B.; Bakker, H.D.; Herschkovitz, E.; Moses, S.W.; Shin, Y.S.; Kilimann, M.W. Mutations in the Liver Glycogen Phosphorylase Gene (PYGL) Underlying Glycogenosis Type VI (Hers Disease). *Am. J. Hum. Genet.* **1998**, *62*, 785–791. [[CrossRef](#)]
41. Kato, K.; Shimizu, A.; Kurobe, N.; Takashi, M.; Koshikawa, T. Human Brain-Type Glycogen Phosphorylase: Quantitative Localization in Human Tissues Determined with an Immunoassay System. *J. Neurochem.* **1989**, *52*, 1425–1432. [[CrossRef](#)]
42. Rath, V.L.; Ammirati, M.; LeMotte, P.K.; Fennell, K.F.; Mansour, M.N.; Danley, D.E.; Hynes, T.R.; Schulte, G.K.; Wasilko, D.J.; Pandit, J. Activation of Human Liver Glycogen Phosphorylase by Alteration of the Secondary Structure and Packing of the Catalytic Core. *Mol. Cell* **2000**, *6*, 139–148. [[CrossRef](#)]
43. Munger, R.; Temler, E.; Jallut, D.; Haesler, E.; Felber, J.-P. Correlations of glycogen synthase and phosphorylase activities with glycogen concentration in human muscle biopsies. Evidence for a double-feedback mechanism regulating glycogen synthesis and breakdown. *Metabolism* **1993**, *42*, 36–43. [[CrossRef](#)]
44. Keppens, S.; Vandekerckhove, A.; Moshage, H.; Yap, S.H.; Aerts, R.; de Wulf, H. Regulation of glycogen phosphorylase activity in isolated human hepatocytes. *Hepatology* **1993**, *17*, 610–614. [[CrossRef](#)] [[PubMed](#)]
45. Cheng, A.; Zhang, M.; Gentry, M.S.; Worby, C.A.; Dixon, J.E.; Saltiel, A.R. A role for AGL ubiquitination in the glycogen storage disorders of Lafora and Cori's disease. *Genes Dev.* **2007**, *21*, 2399–2409. [[CrossRef](#)] [[PubMed](#)]
46. Griswold, M.D. The central role of Sertoli cells in spermatogenesis. *Semin. Cell Dev. Biol.* **1998**, *9*, 411–416. [[CrossRef](#)] [[PubMed](#)]
47. Jutte, N.H.P.M.; Grootegoed, J.A.; Rommerts, F.F.G.; Van Der Molen, H.J. Exogenous lactate is essential for metabolic activities in isolated rat spermatocytes and spermatids. *Reproduction* **1981**, *62*, 399–405. [[CrossRef](#)]
48. Boussouar, F.; Benahmed, M. Lactate and energy metabolism in male germ cells. *Trends Endocrinol. Metab.* **2004**, *15*, 345–350. [[CrossRef](#)]
49. Wong, C.; Cheng, C.Y. The Blood-Testis Barrier: Its Biology, Regulation, and Physiological Role in Spermatogenesis. *Curr. Top. Dev. Biol.* **2005**, *71*, 263–296. [[CrossRef](#)]
50. Mital, P.; Hinton, B.T.; Dufour, J.M. The Blood-Testis and Blood-Epididymis Barriers Are More than Just Their Tight Junctions. *Biol. Reprod.* **2011**, *84*, 851–858. [[CrossRef](#)]
51. Xiong, W.; Wang, H.; Wu, H.; Chen, Y.; Han, D. Apoptotic Spermatogenic Cells Can Be Energy Sources for Sertoli Cells. *Reproduction* **2009**, *137*, 469–479. [[CrossRef](#)]
52. Erkkila, K.; Aito, H.; Aalto, K.; Pentikäinen, V.; Dunkel, L. Lactate inhibits germ cell apoptosis in the human testis. *Mol. Hum. Reprod.* **2002**, *8*, 109–117. [[CrossRef](#)]

53. Oliveira, P.F.; Martins, A.D.; Moreira, A.C.; Cheng, C.Y.; Alves, M.G. The Warburg Effect Revisited-Lesson from the Sertoli Cell. *Med. Res. Rev.* **2014**, *35*, 126–151. [[CrossRef](#)]
54. Kokk, K.; Veräjänkorka, E.; Wu, X.-K.; Tapfer, H.; Pöldoja, E.; Pöllänen, P. Immunohistochemical detection of glucose transporters class I subfamily in the mouse, rat and human testis. *Medicina* **2004**, *40*, 156–160. [[PubMed](#)]
55. Galardo, M.N.; Riera, M.F.; Pellizzari, E.H.; Chemes, H.E.; Venara, M.C.; Cigorraga, S.B.; Meroni, S.B. Regulation of expression of Sertoli cell glucose transporters 1 and 3 by FSH, IL1 β , and bFGF at two different time-points in pubertal development. *Cell Tissue Res.* **2008**, *334*, 295–304. [[CrossRef](#)] [[PubMed](#)]
56. Carosa, E.; Radico, C.; Giansante, N.; Rossi, S.; D’Adamo, F.; Di Stasi, S.M.; Lenzi, A.; Jannini, E.A. Ontogenetic profile and thyroid hormone regulation of type-1 and type-8 glucose transporters in rat Sertoli cells. *Int. J. Androl.* **2005**, *28*, 99–106. [[CrossRef](#)]
57. Ulisse, S.; Jannini, E.A.; Pepe, M.; De Matteis, S.; D’Armiento, M. Thyroid Hormone Stimulates Glucose Transport and GLUT1 mRNA in Rat Sertoli Cells. *Mol. Cell. Endocrinol.* **1992**, *87*, 131–137. [[CrossRef](#)]
58. Xu, B.; Chen, M.; Ji, X.; Yao, M.; Mao, Z.; Zhou, K.; Xia, Y.; Han, X.; Tang, W. Metabolomic profiles reveal key metabolic changes in heat stress-treated mouse Sertoli cells. *Toxicol. Vitro.* **2015**, *29*, 1745–1752. [[CrossRef](#)]
59. Gualtieri, A.F.; Mazzone, G.L.; Rey, R.A.; Schteingart, H.F. FSH and bFGF stimulate the production of glutathione in cultured rat Sertoli cells. *Int. J. Androl.* **2009**, *32*, 218–225. [[CrossRef](#)]
60. Pioli, P.A.; Hamilton, B.J.; Connolly, J.E.; Brewer, G.; Rigby, W.F. Lactate Dehydrogenase Is an AU-rich Element-binding Protein That Directly Interacts with AUF1. *J. Biol. Chem.* **2002**, *277*, 35738–35745. [[CrossRef](#)]
61. Galardo, M.N.; Riera, M.F.; Pellizzari, E.H.; Cigorraga, S.B.; Meroni, S.B. The AMP-activated protein kinase activator, 5-aminoimidazole-4-carboxamide-1- β -D-ribose nucleoside, regulates lactate production in rat Sertoli cells. *J. Mol. Endocrinol.* **2007**, *39*, 279–288. [[CrossRef](#)]
62. Bonen, A. The expression of lactate transporters (MCT1 and MCT4) in heart and muscle. *Eur. J. Appl. Physiol.* **2001**, *86*, 6–11. [[CrossRef](#)]
63. Robinson, R.F.I. Metabolism of Glucose by Sertoli in Culture. *Biol. Reprod.* **1981**, *24*, 1032–1041. [[CrossRef](#)]
64. Leiderman, B.; Mancini, R.E. Glycogen Content in the Rat Testis from Postnatal to Adult Ages. *Endocrinology* **1969**, *85*, 607–609. [[CrossRef](#)] [[PubMed](#)]
65. Slaughter, G.R.; Means, A.R. Follicle-Stimulating Hormone Activation of Glycogen Phosphorylase in the Sertoli Cell-Enriched Rat Testis. *Endocrinology* **1983**, *113*, 1476–1485. [[CrossRef](#)] [[PubMed](#)]
66. Matoba, S.; Hiramatsu, R.; Kanai-Azuma, M.; Tsunekawa, N.; Harikae, K.; Kawakami, H.; Kurohmaru, M.; Kanai, Y. Establishment of testis-specific SOX9 activation requires high-glucose metabolism in mouse sex differentiation. *Dev. Biol.* **2008**, *324*, 76–87. [[CrossRef](#)] [[PubMed](#)]
67. Maldonado, R.; Mancilla, H.; Villarroel-Espíndola, F.; Slebe, F.; Slebe, J.C.; Méndez, R.; Guinovart, J.J.; Concha, I.I. Glycogen Synthase in Sertoli Cells: More Than Glycogenesis? *J. Cell Biochem.* **2016**, *117*, 2597–2607. [[CrossRef](#)] [[PubMed](#)]
68. Guo, T.B.; Chan, K.C.; Hakovirta, H.; Xiao, Y.; Toppari, J.; Mitchell, A.P.; Salameh, W.A. Evidence for a Role of Glycogen Synthase Kinase-3 β in Rodent Spermatogenesis. *J. Androl.* **2003**, *24*, 332–342. [[CrossRef](#)]
69. Singh, T.J.; Huang, K.-P. Glycogen synthase (casein) kinase-1: Tissue distribution and subcellular localization. *FEBS Lett.* **1985**, *190*, 84–88. [[CrossRef](#)]
70. Fuchs, G.; Diges, C.; Kohlstaedt, L.A.; Wehner, K.A.; Sarnow, P. Proteomic Analysis of Ribosomes: Translational Control of mRNA Populations by Glycogen Synthase GYS1. *J. Mol. Biol.* **2011**, *410*, 118–130. [[CrossRef](#)]
71. Vilchez, D.; Ros, S.; Cifuentes, D.; Pujadas, L.; Vallès, J.; García-Fojeda, B.; Criado-García, O.; Sanchez, M.E.F.; Fernandez, I.M.; Domínguez, J.; et al. Mechanism suppressing glycogen synthesis in neurons and its demise in progressive myoclonus epilepsy. *Nat. Neurosci.* **2007**, *10*, 1407–1413. [[CrossRef](#)]
72. Mruk, D.D.; Cheng, C.Y. Tight junctions in the testis: New perspectives. *Philos. Trans. R. Soc. B Biol. Sci.* **2010**, *365*, 1621–1635. [[CrossRef](#)]
73. Yazama, F.; Esaki, M.; Sawada, H. Immunocytochemistry of extracellular matrix components in the rat seminiferous tubule: Electron microscopic localization with improved methodology. *Anat. Rec.* **1997**, *248*, 51–62. [[CrossRef](#)]
74. Thakur, S.C.; Thakur, S.S.; Chaube, S.K.; Singh, S.P. Subchronic Supplementation of Lithium Carbonate Induces Reproductive System Toxicity in Male Rat. *Reprod. Toxicol.* **2003**, *17*, 683–690. [[CrossRef](#)]
75. MacAulay, K.; Woodgett, J.R. Targeting glycogen synthase kinase-3 (GSK-3) in the treatment of Type 2 diabetes. *Expert Opin. Ther. Targets* **2008**, *12*, 1265–1274. [[CrossRef](#)] [[PubMed](#)]
76. Leydig, F. Zur Anatomie der Männlichen Geschlechtsorgane und Analdrüsen der Säugethiere. *Z. Wiss. Zool.* **1850**, *2*, 1–57.
77. Mendis-Handagama, S.M.L.C.; Ariyaratne, H.B.S. Differentiation of the Adult Leydig Cell Population in the Postnatal Testis. *Biol. Reprod.* **2001**, *65*, 660–671. [[CrossRef](#)]
78. Shima, Y.; Miyabayashi, K.; Haraguchi, S.; Arakawa, T.; Otake, H.; Baba, T.; Matsuzaki, S.; Shishido, Y.; Akiyama, H.; Tachibana, T.; et al. Contribution of Leydig and Sertoli Cells to Testosterone Production in Mouse Fetal Testes. *Mol. Endocrinol.* **2013**, *27*, 63–73. [[CrossRef](#)]
79. Wang, Y.; Chen, F.; Ye, L.; Zirkin, B.; Chen, H. Steroidogenesis in Leydig cells: Effects of aging and environmental factors. *Reproduction* **2017**, *154*, R111–R122. [[CrossRef](#)]
80. O’Donnell, L. Testosterone Promotes the Conversion of Round Spermatids between Stages VII and VIII of the Rat Spermatogenic Cycle. *Endocrinology* **1994**, *135*, 2608–2614. [[CrossRef](#)]

81. O'Donnell, L.; McLachlan, R.L.; Wreford, N.G.; de Kretser, D.M.; Robertson, D.M. Testosterone Withdrawal Promotes Stage-Specific Detachment of Round Spermatids from the Rat Seminiferous Epithelium. *Biol. Reprod.* **1996**, *55*, 895–901. [\[CrossRef\]](#)
82. Bartlett, J.M.; Kerr, J.B.; Sharpe, R.M. The Effect of Selective Destruction and Regeneration of Rat Leydig Cells on the Intratesticular Distribution of Testosterone and Morphology of the Seminiferous Epithelium. *J. Androl.* **1986**, *7*, 240–253. [\[CrossRef\]](#)
83. Holdcraft, R.W.; Braun, R.E. Androgen receptor function is required in Sertoli cells for the terminal differentiation of haploid spermatids. *Development* **2004**, *131*, 459–467. [\[CrossRef\]](#)
84. O'Donnell, L.; Pratis, K.; Wagenfeld, A.; Gottwald, U.; Müller, J.; Leder, G.; McLachlan, R.I.; Stanton, P. Transcriptional Profiling of the Hormone-Responsive Stages of Spermatogenesis Reveals Cell-, Stage-, and Hormone-Specific Events. *Endocrinology* **2009**, *150*, 5074–5084. [\[CrossRef\]](#)
85. Pelletier, R.-M. The blood-testis barrier: The junctional permeability, the proteins and the lipids. *Prog. Histochem. Cytochem.* **2011**, *46*, 49–127. [\[CrossRef\]](#) [\[PubMed\]](#)
86. Kopera, I.A.; Bilinska, B.; Cheng, C.Y.; Mruk, D.D. Sertoli-Germ Cell Junctions in the Testis: A Review of Recent Data. *Philos. Trans. R. Soc. B Biol. Sci.* **2010**, *365*, 1593–1605. [\[CrossRef\]](#)
87. Midzak, A.; Akula, N.; Lecanu, L.; Papadopoulos, V. Novel Androstenetriol Interacts with the Mitochondrial Translocator Protein and Controls Steroidogenesis. *J. Biol. Chem.* **2011**, *286*, 9875–9887. [\[CrossRef\]](#) [\[PubMed\]](#)
88. Midzak, A.; Zirkin, B.; Papadopoulos, V. Translocator protein: Pharmacology and steroidogenesis. *Biochem. Soc. Trans.* **2015**, *43*, 572–578. [\[CrossRef\]](#) [\[PubMed\]](#)
89. Aghazadeh, Y.; Zirkin, B.R.; Papadopoulos, V. Pharmacological Regulation of the Cholesterol Transport Machinery in Steroidogenic Cells of the Testis. *Vitam. Horm.* **2015**, *98*, 189–227. [\[CrossRef\]](#)
90. Antonio-Cabrera, E.; Paredes, R.G. Effects of chronic estradiol or testosterone treatment upon sexual behavior in sexually sluggish male rats. *Pharmacol. Biochem. Behav.* **2012**, *101*, 336–341. [\[CrossRef\]](#)
91. O'Shaughnessy, P.J.; Verhoeven, G.; De Gendt, K.; Monteiro, A.; Abel, M.H. Direct Action through the Sertoli Cells Is Essential for Androgen Stimulation of Spermatogenesis. *Endocrinology* **2010**, *151*, 2343–2348. [\[CrossRef\]](#)
92. Haider, S.G. Cell Biology of Leydig Cells in the Testis. *Int. Rev. Cytol.* **2004**, *233*, 181–241. [\[CrossRef\]](#)
93. Svechnikov, K.; Landreh, L.; Weisser, J.; Izzo, G.; Colón, E.; Svechnikova, I.; Söder, O. Origin, Development and Regulation of Human Leydig Cells. *Horm. Res. Paediatr.* **2010**, *73*, 93–101. [\[CrossRef\]](#)
94. King, S.R.; Stocco, D.M. ATP and A mitochondrial electrochemical gradient are required for functional activity of the steroidogenic acute regulatory (star) protein in isolated mitochondria. *Endocr. Res.* **1996**, *22*, 505–514. [\[CrossRef\]](#)
95. Murono, E.P.; Lin, T.; Osterman, J.; Nankin, H.R. Relationship between Inhibition of Interstitial Cell Testosterone Synthesis by Cytochalasin B and Glucose. *Biochem. Biophys. Res. Commun.* **1982**, *104*, 299–306. [\[CrossRef\]](#)
96. Chen, Y.; Nagpal, M.L.; Lin, T. Expression and regulation of glucose transporter 8 in rat Leydig cells. *J. Endocrinol.* **2003**, *179*, 63–72. [\[CrossRef\]](#)
97. Banerjee, A.; Anuradha; Mukherjee, K.; Krishna, A. Testicular glucose and its transporter GLUT 8 as a marker of age-dependent variation and its role in steroidogenesis in mice. *J. Exp. Zool. Part A Ecol. Genet. Physiol.* **2014**, *321*, 490–502. [\[CrossRef\]](#) [\[PubMed\]](#)
98. Ahn, S.W.; Gang, G.T.; Tadi, S.; Nedumaran, B.; Kim, Y.D.; Park, J.H.; Kweon, G.R.; Koo, S.H.; Lee, K.; Ahn, R.S.; et al. Phosphoenolpyruvate Carboxykinase and glucose-6-Phosphatase Are Required for Steroidogenesis in Testicular Leydig Cells. *J. Biol. Chem.* **2012**, *287*, 41875–41887. [\[CrossRef\]](#)
99. Russo, J. Glycogen content during the postnatal differentiation of the Leydig cell in the mouse testis. *Z. Zellforsch. Mikrosk. Anat.* **1970**, *104*, 14–18. [\[CrossRef\]](#)
100. Prince, F.P. Ultrastructure of immature leydig cells in the human prepubertal testis. *Anat. Rec.* **1984**, *209*, 165–176. [\[CrossRef\]](#)
101. Khalaf, G.; Fahmy, H.F.; Saleh, H.A. Effect of a zinc-free diet from weaning through puberty on the structure of the testis in albino rats, with a special focus on the Leydig cells. *Egypt. J. Histol.* **2014**, *37*, 304–315. [\[CrossRef\]](#)
102. Kerr, J.; Knell, C. The fate of fetal Leydig cells during the development of the fetal and postnatal rat testis. *Development* **1988**, *103*, 535–544. [\[CrossRef\]](#) [\[PubMed\]](#)
103. Hess, R.; de Franca, L. *Molecular Mechanisms in Spermatogenesis*; Cheng, C.Y., Ed.; Landes Bioscience: Austin, TX, USA; Springer Science: Berlin/Heidelberg, Germany, 2008.
104. Cheng, C.Y.; Mruk, D.D. An intracellular trafficking pathway in the seminiferous epithelium regulating spermatogenesis: A biochemical and molecular perspective. *Crit. Rev. Biochem. Mol. Biol.* **2009**, *44*, 245–263. [\[CrossRef\]](#)
105. Bajpai, M.; Gupta, G.; Setty, B.S. Changes in carbohydrate metabolism of testicular germ cells during meiosis in the rat. *Eur. J. Endocrinol.* **1998**, *138*, 322–327. [\[CrossRef\]](#) [\[PubMed\]](#)
106. Setchell, B.P. Hormones: What the Testis Really Sees. *Reprod. Fertil. Dev.* **2004**, *6*, 535–545. [\[CrossRef\]](#)
107. Wenger, R.H.; Katschinski, D.M. The hypoxic testis and post-meiotic expression of PAS domain proteins. *Semin. Cell Dev. Biol.* **2005**, *16*, 547–553. [\[CrossRef\]](#)
108. Williams, A.C.; Ford, W.C. The role of glucose in supporting motility and capacitation in human spermatozoa. *J. Androl.* **2001**, *22*, 680–695.
109. Carrageta, D.F.; Guerra-Carvalho, B.; Sousa, M.; Barros, A.; Oliveira, P.F.; Monteiro, M.P.; Alves, M.G. Mitochondrial Activation and Reactive Oxygen-Species Overproduction during Sperm Capacitation are Independent of Glucose Stimuli. *Antioxidants* **2020**, *9*, 750. [\[CrossRef\]](#) [\[PubMed\]](#)

110. Burant, C.F.; Takedatll, J.; Brot, E.; Bellstii, G.I.; Davidsons, N. Fructose Transporter in Human Spermatozoa and Small Intestine Is GLUT5. *J. Biol. Chem.* **1992**, *267*, 14523–14526. [[CrossRef](#)]
111. Angulo, C.; Rauch, C.; Droppelmann, A.; Reyes, A.M.; Slebe, J.C.; Delgado-lo, F.; Guaiquil, V.H.; Vera, J.C. Hexose Transporter Expression and Function in Mammalian Spermatozoa: Cellular Localization and Transport of Hexoses and Vitamin C. *J. Cell Biochem.* **1998**, *71*, 189–203. [[CrossRef](#)]
112. Gómez, O.; Romero, A.; Terrado, J.; Mesonero, J.E.; Romero-Picó, A. Differential expression of glucose transporter GLUT8 during mouse spermatogenesis. *Reproduction* **2006**, *131*, 63–70. [[CrossRef](#)]
113. Roth, R.; Ibberson, M.; Riederer, B.M.; Uldry, M.; Guhl, B.; Thorens, B.; Toxicology, M.I.; Biology, C.; Morphology, B.M.R. Immunolocalization of GLUTX1 in the Testis and to Specific Brain Areas and Vasopressin—Containing Neurons. *Endocrinology* **2002**, *143*, 276–284.
114. Haber, R.S.; Weinstein, S.P.; O’Boyle, E.; Morgello, S. Tissue distribution of the human GLUT3 glucose transporter. *Endocrinology* **1993**, *132*, 2538–2543. [[CrossRef](#)] [[PubMed](#)]
115. Rauch, M.C.; Ocampo, M.E.; Bohle, J.; Amthauer, R.; Yáñez, A.J.; Rodríguez-Gil, J.E.; Slebe, J.C.; Re Yes, J.G.; Concha, I.I. Hexose Transporters GLUT1 and GLUT3 Are Colocalized with Hexokinase I in Caveolae Microdomains of Rat Spermatogenic Cells. *J. Cell. Physiol.* **2006**, *207*, 397–406. [[CrossRef](#)] [[PubMed](#)]
116. Rato, L.; Alves, M.G.; Socorro, S.; Duarte, A.I.; Cavaco, J.E.; Oliveira, P.F. Metabolic regulation is important for spermatogenesis. *Nat. Rev. Urol.* **2012**, *9*, 330–338. [[CrossRef](#)]
117. Mita, M.; Hall, P.F. Metabolism of Round Spermatids from Rats: Lactate as the Preferred Substrate. *Biol. Reprod.* **1982**, *26*, 445–448. [[CrossRef](#)]
118. Mueckler, M.; Thorens, B. The SLC2 (GLUT) family of membrane transporters. *Mol. Asp. Med.* **2013**, *34*, 121–138. [[CrossRef](#)]
119. Hoshi, K.; Tsukikawa, S.; Sato, A. Importance of Ca²⁺, K⁺ and Glucose in the Medium for Sperm Penetration through the Human Zona Pellucida. *Tohoku J. Exp. Med.* **1991**, *165*, 99–104. [[CrossRef](#)] [[PubMed](#)]
120. Nakamura, M.; Fujiwara, A.; Yasumasu, I.; Okinaga, S.; Arai, K. Regulation of glucose metabolism by adenine nucleotides in round spermatids from rat testes. *J. Biol. Chem.* **1982**, *257*, 13945–13950. [[CrossRef](#)]
121. Yáñez, A.J.; Bustamante, X.; Bertinat, R.; Werner, E.; Rauch, M.C.; Concha, I.I.; Reyes, J.G.; Slebe, J.C. Expression of key substrate cycle enzymes in rat spermatogenic cells: Fructose 1,6 biphosphatase and 6 phosphofructose 1-kinase. *J. Cell. Physiol.* **2007**, *212*, 807–816. [[CrossRef](#)]
122. Jones, A.R.; Chantrell, L.A.; Cokinakis, A. Metabolism of glycerol by mature boar spermatozoa. *J. Reprod. Fertil.* **1992**, *94*, 129–134. [[CrossRef](#)]
123. Bustamante-Marin, X.; Quiroga, C.; Lavandero, S.; Reyes, J.G.; Moreno, R.D. Apoptosis, necrosis and autophagy are influenced by metabolic energy sources in cultured rat spermatocytes. *Apoptosis* **2012**, *17*, 539–550. [[CrossRef](#)]
124. Herrera, E.; Salas, K.; Lagos, N.; Benos, D.J.; Reyes, J.G. Energy metabolism and its linkage to intracellular Ca²⁺ and pH regulation in rat spermatogenic cells. *Biol. Cell* **2000**, *92*, 429–440. [[CrossRef](#)]
125. Gómez, M.; Navarro-Sabaté, A.; Manzano, A.; Duran, J.; Obach, M.; Bartrons, R. Switches in 6-phosphofructo-2-kinase isoenzyme expression during rat sperm maturation. *Biochem. Biophys. Res. Commun.* **2009**, *387*, 330–335. [[CrossRef](#)]
126. Li, S.S.-L.; O’Brien, D.A.; Hou, E.W.; Versola, J.; Rockett, D.L.; Eddy, E.M. Differential Activity and Synthesis of Lactate Dehydrogenase Isozymes A (Muscle), B (Heart), and C (Testis) in Mouse Spermatogenic Cells. *Biol. Reprod.* **1989**, *40*, 173–180. [[CrossRef](#)] [[PubMed](#)]
127. Goldberg, E.; Eddy, E.M.; Duan, C.; Odet, F. LDHC: The Ultimate Testis-Specific Gene. *J. Androl.* **2010**, *31*, 86–94. [[CrossRef](#)] [[PubMed](#)]
128. Alcivar, A.A.; Trasler, J.M.; Hake, L.E.; Salehi-Ashtiani, K.; Goldberg, E.; Hecht, N.B. DNA Methylation and Expression of the Genes Coding for Lactate Dehydrogenases A and C during Rodent Spermatogenesis. *Biol. Reprod.* **1991**, *44*, 527–535. [[CrossRef](#)] [[PubMed](#)]
129. Odet, F.; Duan, C.; Willis, W.D.; Goulding, E.H.; Kung, A.; Eddy, E.M.; Goldberg, E. Expression of the Gene for Mouse Lactate Dehydrogenase C (Ldhc) Is Required for Male Fertility. *Biol. Reprod.* **2008**, *79*, 26–34. [[CrossRef](#)]
130. Welch, J.E.; Brown, P.L.; O’Brien, D.A.; Magyar, P.L.; Bunch, D.O.; Mori, C.; Eddy, E.M. Human Glyceraldehyde 3-Phosphate Dehydrogenase-2 Gene Is Expressed Specifically in Spermatogenic Cells. *J. Androl.* **2000**, *21*, 328–338. [[CrossRef](#)]
131. Boer, P.H.; Adra, C.N.; Lau, Y.M.M. The Testis-Specific Phosphoglycerate Kinase Gene Pkg-2 Is a Recruited Retroposon. *Mol. Cell Biol.* **1987**, *7*, 3107–3112.
132. McCarrey, J.R.; Thomas, K. Human testis-specific PGK gene lacks introns and possesses characteristics of a processed gene. *Nature* **1987**, *326*, 501–505. [[CrossRef](#)]
133. Ballester, J.; Fernández-Novell, J.M.; Rutllant, J.; García-Rocha, M.; Jesus Palomo, M.; Mogas, T.; Peña, A.; Rigau, T.; Guinovart, J.J.; Rodríguez-Gil, J.E. Evidence for a Functional Glycogen Metabolism in Mature Mammalian Spermatozoa. *Mol. Reprod. Dev.* **2000**, *56*, 207–219. [[CrossRef](#)]
134. Marin, S.; Chiang, K.; Bassilian, S.; Lee, W.-N.P.; Boros, L.G.; Fernández-Novell, J.M.; Centelles, J.J.; Medrano, A.; Rodriguez-Gil, J.E.; Cascante, M. Metabolic strategy of boar spermatozoa revealed by a metabolomic characterization. *FEBS Lett.* **2003**, *554*, 342–346. [[CrossRef](#)]

135. Albarracín, J.; Fernández-Novell, J.; Ballester, J.; Rauch, M.; Quintero-Moreno, A.; Peña, A.; Mogas, T.; Rigau, T.; Yáñez, A.; Guinovart, J.; et al. Gluconeogenesis-Linked Glycogen Metabolism Is Important in the Achievement of In Vitro Capacitation of Dog Spermatozoa in a Medium without Glucose. *Biol. Reprod.* **2004**, *71*, 1437–1445. [[CrossRef](#)] [[PubMed](#)]
136. Yáñez, A.J.; Nualart, F.; Droppelmann, C.; Bertinat, R.; Brito, M.; Concha, I.I.; Slebe, J.C. Broad expression of fructose-1,6-bisphosphatase and phosphoenolpyruvate carboxykinase provide evidence for gluconeogenesis in human tissues other than liver and kidney. *J. Cell. Physiol.* **2003**, *197*, 189–197. [[CrossRef](#)] [[PubMed](#)]
137. Panse, G.T.; Sheth, A.R. Glycogen Metabolism in Epididymal Spermatozoa of Developing Mice. *Ind. J. Exp. Biol.* **1981**, *19*, 183–185.
138. Datta, K.M.; Dasgupta, J.; Sengupta, T.; De, S. Glycogen metabolism in human fetal testes. *J. Biosci.* **1988**, *13*, 117–121. [[CrossRef](#)]
139. Reddy, K.V.; Geethanjali, N.; Reddy, Y.D.; Reddanna, P.; Govindappa, S. Effect of induced bilateral cryptorchidism on the carbohydrate metabolism of reproductive tissues in albino rats. *Arch. Int. Physiol. Biochim.* **1983**, *91*, 405–410. [[CrossRef](#)]
140. Kuramori, C.; Hase, Y.; Hoshikawa, K.; Watanabe, K.; Nishi, T.; Hishiki, T.; Soga, T.; Nashimoto, A.; Kabe, Y.; Yamaguchi, Y.; et al. Mono-(2-ethylhexyl) phthalate Targets Glycogen Debranching Enzyme and Affects Glycogen Metabolism in Rat Testis. *Toxicol. Sci.* **2009**, *109*, 143–151. [[CrossRef](#)]
141. Arzac, J.P. Glycogen in human testicular biopsy material: Preliminary report. *J. Clin. Endocrinol. Metab.* **1950**, *10*, 1465–1470. [[CrossRef](#)]