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Chapter 6

Blood Testis Barrier: How Does the Seminiferous Epithelium Feeds the Developing Germ Cells?

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Abstract

The testes of mammals are paired organs that essentially perform two functions: steroid biosynthesis and production of spermatozoa. They consist in two compartments encased by the tunica albuginea: the seminiferous tubules, the functional units of the testes, and the intervening interstitial space. Within the seminiferous tubules, the seminiferous epithelium is compartmentalized by adjacent Sertoli cells (SCs) via specialized junctions creating the blood-testis barrier (BTB) where post-meiotic germ cell development takes place in a specialized microenvironment. The fully functional BTB consists in three components: an anatomical/physical barrier to restrict entry of molecules into the adluminal compartment of the seminiferous tubules; an immunological barrier (inside and outside the tubules), that limits the movement of immune cells and regulates the level of cytokines in the seminiferous epithelium; a physiological barrier (transporters and channels at the basolateral and apical membranes), that are highly dynamic to encounter the needs of germ and SCs. Together, these components are essential to the function of BTB in testes and the special microenvironment created is responsible for proper development of the germ cells into fully functional sperm.

Developing germ cells are unable to use glucose for their energy metabolism and preferentially use lactate, used by spermatocytes and spermatids, as a substrate for ATP production. Sertoli cells on the seminiferous epithelium produce the lactate utilized by the developing germ cells. Carbohydrate metabolism of SCs has been shown to present

some unique features. These cells can metabolize various substrates (e.g.: glucose, fatty acids, ketone bodies), but preferentially metabolize glucose being the majority of it converted to lactate and not oxidized via the citric acid cycle. The reasons why SCs preferentially export lactate for germ cells are not entirely understood. However, there are evidences of an anti-apoptotic effect of lactate on germ cells suggesting that it plays a crucial role in spermatogenesis.

Several biochemical mechanisms might contribute to the modulation of lactate secretion by SCs, among which (a) the transport of glucose through the plasma membrane, mediated by a set of homologous glycoprotein molecules (GLUTs); (b) the interconversion of pyruvate to lactate by lactate dehydrogenase (LDH); and (c) the release of lactate from SCs, mediated by the action of the monocarboxylate transporters family (MCTs).

The understanding of the functioning and regulation of these processes are crucial steps to identify key mechanisms associated with seminiferous epithelium (dys)function, and their influence over male (in)fertility.

Introduction

The blood testis barrier (BTB) is a central structural element in testicular physiology. This term has been developed since 1970, and the first studies were performed based in the following evidences: radioactive tracers and dyes when injected in animals, stained most tissues, with the exceptions of the brain and seminiferous tubules of the testis [1-3]. On other hand, it was demonstrated that there were significant differences in fluids composition and proteins obtained from the rete testis, seminiferous tubule lumen, and the testicular lymph and blood plasma [4, 5].

Blood testis barrier is one of the tightest blood-tissue barriers in mammalian tissues [6-8], creating an adequate microenvironment for proper development of germ cells and conferring cell polarity [8]. Blood testis barrier divides the seminiferous epithelium in two compartments: outside the barrier is the basal compartment where spermatogonial renewal occurs and inside the barrier is the apical or adluminal compartment where meiosis, spermiogenesis and spermiation take place (Figure 1). When BTB is dysfunctional, germ cell differentiation is arrested [9]. The generation and maintenance of BTB is assured by somatic Sertoli cells (SCs) [10] and its molecular composition has long been a matter of debate [5, 8, 11-13]. The ultrastructure of BTB is composed by co-existing specialized junctions between adjacent SCs near the basement membrane, which include tight junctions, basal ectoplasmic specializations, basal tubulobulbar complex gap junctions and desmosome-like junctions [12, 13], and anchoring junctions, such as apical ectoplasmic specializations, apical tubulobulbar complex gap junctions, desmosome-like junctions and gap junctions between Sertoli cells and germ cells [14].

It is noteworthy that BTB is not closed all time and must allow the developing germ cell migration through the seminiferous epithelium. Despite the complex composition of BTB, this barrier shows a highly dynamic restructuring at specific stages of the spermatogenic cycle, since developing germ cells must cross BTB towards adluminal compartment [15]. Throughout this process, germ cells continue to be tightly anchored to SCs via anchoring junctions [16]. It is a well-coordinated process so that even during BTB “opening and closing” the immunological privilege is maintained. Recently, Cheng and colleagues [13]

have suggested that probably gap and desmosome-like junctions provide the cross-talk to coordinate the function of multiple junctions at the BTB to effectively safeguard the immunological barrier function. All events that comprise junction disassembly and reassembly are likely involving a complex network of signaling cascade and rapid turnover of junction-associated molecules [8].

Blood testis barrier is more than specialized junctional complexes, it is an interaction of the three components that comprise the fully functional BTB: anatomical/physical, physiological and immunological. The anatomical/physical barrier is composed, as referred, by the SCs basolateral membrane, apical membrane and the tight junctional complex between these cells [17]. Each of these elements contributes for the selective passage of water, electrolytes, ions, nutrients, amino acids, hormones, paracrine and autocrine factors from the outside of the BTB into the adluminal compartment. A good example is the follicle-stimulating hormone (FSH), which is essential for the maintenance of adult spermatogenesis [18], that does not pass through BTB readily [19, 20]. While anatomical barrier restricts passage of molecules into the lumen, physiological barrier reflects the permeability of both basolateral and apical membranes in which several specific transporters are responsible for the movement of molecules in or out of the lumen [17, 21].

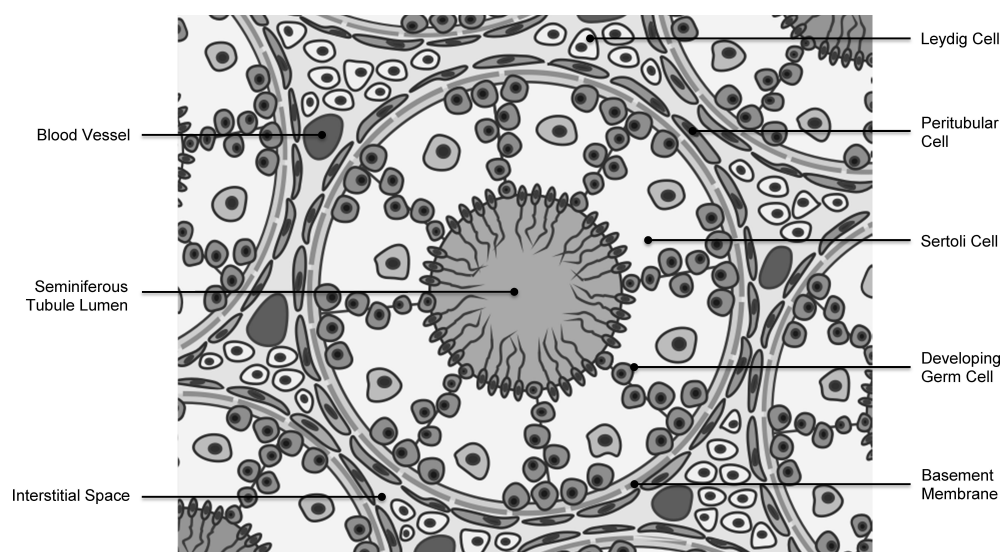


Figure 1. Schematic drawing illustration of the seminiferous tubule and the blood testis barrier (BTB). The BTB is a physical barrier between the blood vessels and the seminiferous tubule lumen and is formed by tight connections between Sertoli cells (SCs). Outside the BTB is the basal compartment where spermatogonial renewal occurs and inside the BTB is the apical compartment where meiosis, spermiogenesis and spermiation take place. At the interstitial space are located the blood vessels and the Leydig cells, which produce testosterone in the presence of luteinizing hormone (LH). The cytoplasmic extensions that enwrap the developing germ cells are responsible for the structural support through a microtubular filament present in the cytoplasm of SCs. This architecture is not static and depends on the stage of the seminiferous tubules. Additionally, the SCs regulate the internal microenvironment of the seminiferous tubule. External to the basement membrane are several layers of modified myofibroblastic cells, termed peritubular cells, responsible for the irregular contractions of the seminiferous tubules, which propel fluid secreted by the SCs. As a result of such particular organization, the establishment of an efficient and regulated BTB is essential to create a special environment for the normal development of a fully efficient sperm.

The flow of molecules is not done in only one direction, but by two, from the outside to inside and from the inside to outside of BTB. Brinster and colleagues [22] injected spermatogonia into the lumen of a single seminiferous tubule either directly or via the rete testis and they observed that injected germ cells can take up a position adjacent to the peritubular tissue and repopulate that area of the tubules with developing germ cells crossing the BTB in opposite direction of the “normal germ cells route”. These observations support the idea that the movement across BTB occurs in both directions. Moreover, the physiological barrier is highly dynamic and the SCs are the responsible for this feature, since they present a dynamic changing in its structure during the spermatogenic events [17]. The last component is the immunological barrier. It has been reported that testes are an immunological privileged site which avoid auto-immune response [17, 23]. The immunological barrier created by BTB isolates the developing germ cells from circulating leukocytes and antibodies [21]. Spermatogonia and preleptotene spermatocytes, which are found in basal compartment, may be a target of host immune cells. Blood testis barrier ceases at rete testis where immune cells and antibodies are able to enter [24]. However, Head and Billingham [25] placed allografts in the interstitial space, a location outside of the BTB, and found an extended survival without immune rejection. Indeed, there are other mechanisms responsible for the maintenance of the testicular immune privilege [23]. The SCs play a key role functioning as “sentinel cells” since they present some particular characteristics of immune cells [26], which are able to trigger an immunosuppressive response [23].

Together, the interaction of the three components of BTB, and all cellular events essentials for its restructuring, play a crucial role in the establishment of a fully functional BTB, which culminates in a special environment within seminiferous tubules required for normal development of spermatogenic process and consequent production of fully functional sperm.

Seminiferous Epithelium

Seminiferous tubules, the functional unities of testes, are highly convoluted structures where spermatogenesis takes place through close association of germ cells with epithelial somatic cells, the SCs [27]. Both ends of each seminiferous tubule open into *tubuli recti* and then connect in the *rete testis* [28]. Seminiferous tubules are lined by a complex stratified epithelium that is composed by two cell types: the SCs and several germ cells types [29]. Sertoli cells occupy a volume of approximately 17–19% in the seminiferous epithelium of adult rats [30] and exhibit numerous cytoplasmic prolongations which allow them to sustain a vast number of developing germ cells [31]. Each SC supports up to 30–50 germ cells at different stages of development [13]. Germ cells form intimate associations with SCs and multiple developing germ cells in different stages may be in contact with one SC (Figure 2). The various generations of germ cells are distributed in defined cellular associations called stages [32]. These stages represent all changes that germinal cells experiment throughout the spermatogenic event and the sequence of these cellular associations constitute the cycle of seminiferous epithelium [33]. In the rat, there are 14 stages to be considered whereas in the mouse there are only 12 stages and in man there are only 6 stages [32].

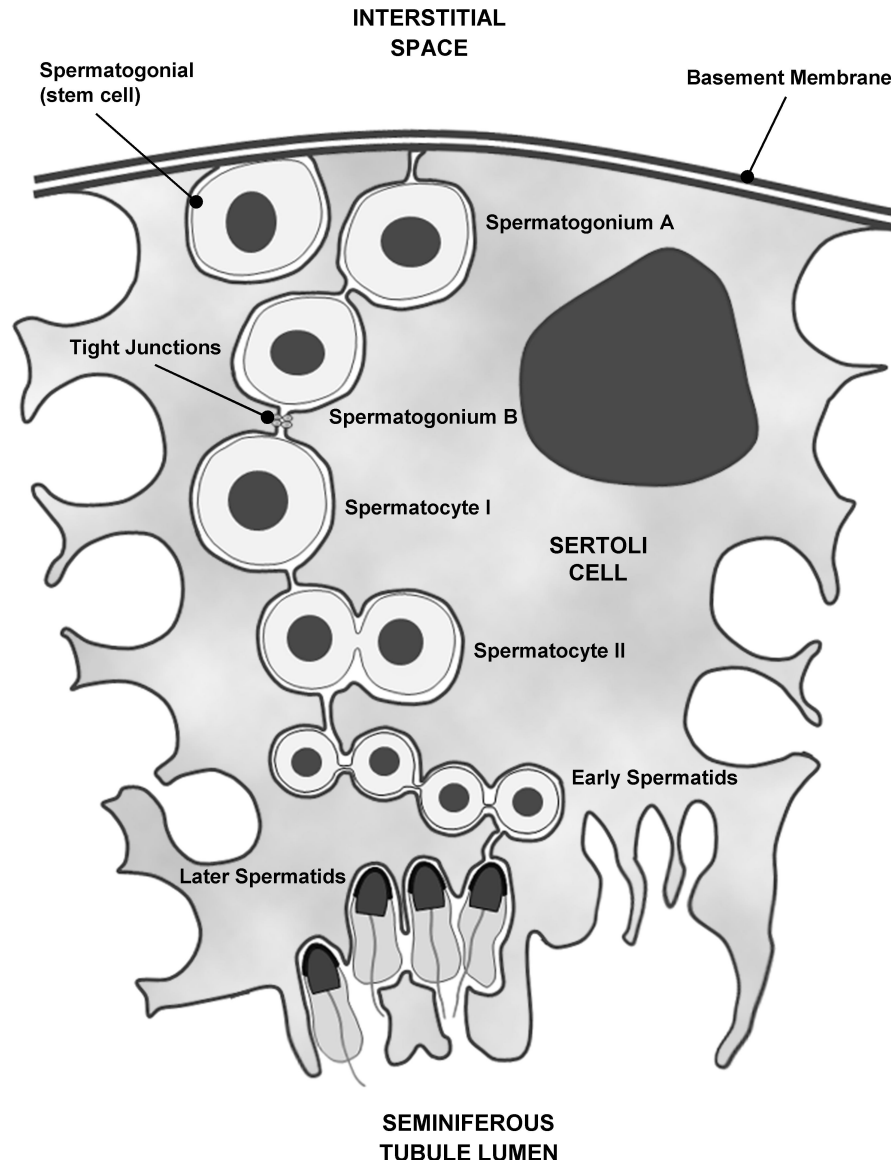


Figure 2. Schematic drawing illustration of spermatogenesis that occurs within the seminiferous epithelium. The seminiferous epithelium is composed by Sertoli cells (SCs) and germ cells at different stages of their development. The process by which male gametes (spermatozoa) are formed in the seminiferous tubules of the testis is called spermatogenesis and is a complex process of cellular transformation that produces male haploid germ cells from diploid spermatogonial stem cells. This process is usually described by 'stages' or 'phases', which progress through precisely timed and highly organized cycles. These cycles of spermatogenesis are essential for continuous sperm production, which is dependent upon several intrinsic (Sertoli and germ cells), extrinsic (hormonal, among others), as well as species-specific factors. The supporting SCs adhere to the basement membrane where spermatogonia are also adherent. Then, spermatogonia type A divide and develop into spermatogonia type B, which enter meiotic prophase and differentiate into primary spermatocytes that separate the homologous pairs of chromosomes in meiosis I (reduction division) to form the haploid secondary spermatocytes. The meiosis II yields four equal-sized spermatids that migrate toward the lumen where fully formed spermatozoa are finally released.

Sertoli cells are the main structural element of the seminiferous epithelium residing on basement membrane, acquiring a columnar shape that extend towards the lumen of the tubules [23, 32]. These branched cells were originally described by Enrico Sertoli in 1865 [34, 35]. More than one century thereafter, Griswold [36] highlighted the evidences establishing the crucial role of SCs in spermatogenesis: 1) It has never been observed testes containing germ cells without SCs; 2) function and efficiency of SCs might be a limitative factor for germ cells; 3) in higher vertebrates, reproductive hormones such as FSH and testosterone, that are essential for normal spermatogenesis, act on SCs and not on germ cells

Often called as “nurse cells”, SCs play distinct functions. In fetal life, SCs play a crucial role in the development of a functional testes and thus determining male phenotype. SCs are the first cells to differentiate in the indifferent fetal gonad and this differentiation results in seminiferous cord formation, prevention of germ cell entry into meiosis [37] by sequestering the germ cells (gonocytes) inside the newly formed seminiferous tubules [36], and differentiation and function of the other somatic cells of the testes [37]. SCs ensure the regression of Müllerian ducts via secretion of anti-Müllerian hormone (AMH), influencing the testis formation in the embryo [36, 38]. During fetal or neonatal life, SCs show a highly proliferative capacity. There are only two periods of life when SCs proliferate in all species: in fetal or neonatal life, and in the peripubertal period [37]. The proliferation of SCs is likely controlled by numerous factors, including intratesticular factors and pituitary hormones, namely FSH that is a mitogenic factor for neonatal SCs [39]. The immature SCs differs extensively from the mature cell with respect to both morphological and biochemical activity [38]. For instance, during proliferative activity of these cells there is a notable production of estrogens, leading to the suggestion that estrogens are involved in this process [32]. The proliferative activity of SCs is extremely important, because the number of SCs in adult testis will determine both testis size and sperm output [36, 37]. As puberty approaches, SCs experiment a radical change both in morphology and functions [37]. The formation of BTB initiates when SCs switches to a mature state [37]. Proliferative activity starts to decline at this time, the SCs become elongated and the nucleus becomes larger and irregularly shaped with a tripartite nucleolus. Abundant smooth and rough endoplasmic reticulum is also present [40]. After BTB is formed, the SCs begin to produce seminiferous tubular fluid (STF) [38].

Fully differentiated SCs must ensure a physical and nutritional support for germ cells, and the structural features of seminiferous epithelium. In regard to the physical support, SCs deposit extracellular matrix components (e.g. collagen and laminin), form specialized junctions and exhibit a well-organized cytoskeleton important for maintenance of seminiferous epithelium [41]. The SCs cytoskeleton consists of actin filaments, intermediate filaments and microtubules [41, 42].

Sertoli cells also secrete specific products that are necessary for germ cell survival. Amongst these products, are included several glycoproteins that can be placed in different categories based on their biochemical properties [36]. These secreted glycoproteins correspond to 15% of total proteins produced by SCs [41]. Griswold and McLean [27] have summarized the recent knowledge regarding secreted proteins and secreted binding proteins by SCs. The first category of glycoproteins includes the transport or bioprotective proteins that are secreted in relative high abundance and metal ion transport proteins such as transferrin and ceruloplasmin. The second category of secreted proteins includes proteases (e.g. different types of cathepsins, plasminogen activator) and proteases inhibitors (e.g. cystatin C, α_2 -macroglobulin), essentials for tissue remodeling processes that occur during

movement of preleptotene spermatocytes through BTB and spermiation. The third category, referred above, includes the glycoproteins that form the basement membrane between the SCs and the peritubular cells. Finally, SCs secrete a class of regulatory glycoproteins that can be made in very low abundance and still carry out their biochemical roles. These glycoproteins function as growth factors, paracrine or endocrine factors and include products such as AMH, *c kit* ligand and inhibin [27, 36]. In addition, the SCs secrete bioactive peptides such as prodynorphin and nutrients or metabolic intermediates [27, 36]. The transfer of nutritive products, which includes amino acids, carbohydrates, lipids, vitamins, and metal ions [41] from the SCs to the germ cells is possible due to the closest relationship between these two cell types. It is imperative that the germ cells receive an adequate level of energy substrates. Studies on the SCs metabolism have shown that lactate play a major role in germ cells fate [43-45], an issue that will be discussed later.

Paradoxally to this protecting and nourishment facet of these “nurse cells”, the SCs can induce apoptosis of germ cells [46], phagocytose apoptotic spermatogenic cells, and are also responsible for the processes of endocytose and degradation of the residual bodies [47]. This could be probably because the number of spermatogenic cells that SCs can support for maturation is limited and when the number of cells reaches the threshold they should be removed to provide space in the seminiferous epithelium for the development of healthy spermatogenic cells [47]. Failure of functional SCs will result in a massive incapacity to support the development of various germ cells, and therefore in unsuccessful spermatogenic process.

Seminiferous Epithelium Environment

Seminiferous tubular fluid serves as a mean of transport for spermatozoa, and also allows keeping an appropriate microenvironment within tubules required for the normal occurrence of spermatogenesis.

Seminiferous tubular fluid starts to be produced by SCs when they switch to the maturational stage [37, 38, 48]. Its composition is controlled by SCs, as well as, the physico-chemical *milieu* where spermatogenesis occurs. It has been shown that the composition of the fluid within the seminiferous tubules is very stable due to the existence of the BTB [49] supporting the fundamental relevance of the STF composition. This luminal *milieu* is markedly distinct from the plasma and the testicular interstitial fluid and is critical to the occurrence of spermatogenesis [50] since meiosis can only be completed after the fluid secretion has been established [51]. Several reports have attempted to elucidate about the formation and composition of STF but unfortunately some of them lack in information concerning the origin and composition of STF. Recently, the knowledge on the mechanisms involved in the secretion, composition and regulation of SFT has been reviewed [48]. First reports from Tuck and collaborators [52] postulated that SCs were responsible for fluid secretion in the seminiferous tubules. These authors, using a variation of the micropuncture technique, suggested that the luminal fluid is composed by a K^+ -rich solution [52]. Later Jenkins and collaborators [53] had observed the same results in their study. Clulow and Jones [54] determined a most suitable approach for defining the composition of the secretions of the seminiferous epithelium and described a fluid rich in Na^+ and Cl^- content, with a K^+

concentration of at least twice that of blood concentration and indicated that this fluid is the main source of the luminal solutes in the extratesticular ducts.

Another important feature in seminiferous *milieu* is the control of its pH. Recent reports have elucidated the role of membrane transporters, both in controlling the STF pH, as in the control of the intracellular pH of SCs [55, 56]. This parameter is kept by intracellular buffers and the balancing between the elimination and production of protons [57]. Sertoli cells express various types of ion membrane transporters directly involved on the movement of basic and acidic particles across the membrane [58]. The involvement of such transporters in the establishment of the STF is not yet completely disclosed, so it is essential further knowledge on their functioning, regulation and in the mechanisms responsible for determining the osmolarity and pH of STF [48]. Furthermore, in the formation of STF, it should be taken into account the other functions of this fluid: nourishment of germ cells, transport of secretion products and transport of the newly formed sperm towards the epididymis [59].

Metabolic Needs of Germ Cells

The adluminal compartment and intraluminal fluid contains both glucose and lactate, since SCs express specific membrane transporters that mediate the passage of these nutrients [43, 60]. Late developmental germ cells cannot metabolize glucose [61], thus they are dependent on SCs, but surprisingly mature spermatozoa show a high glycolytic activity and mainly metabolize fructose but also glucose existing in luminal fluid [62].

During spermatogenesis, germ cells experiment a series of changes that transform them from spermatogonia into fully functional spermatozoa. The spermatogonial cells, which lie at the basement membrane, proliferate by mitotic divisions giving rise a new population of stem cells (spermatogonia A), which is the guarantee of a germ cell line [63]. Other type of spermatogonia (spermatogonia B), are committed to differentiate and start moving down through seminiferous epithelium. Spermatogonia B also experiment mitosis producing primary spermatocytes, which will cross BTB to adluminal compartment [12]. After morphologic transformations, these cells will complete the first meiotic division given rise to secondary spermatocytes. Then they will experiment second meiosis producing round spermatids. After spermatids formation, cell division stops and starts the spermiogenesis, which originates elongated spermatids. Spermiation is the end step of spermiogenesis in which spermatozoa are released in the tubular lumen [33].

It is very interesting, as discussed below, but even more enigmatic the changing of the nutritional requirement of the germ cells during spermatogenesis. Spermatogonia, which lies in basal compartment of BTB, use glucose as a fuel for ATP production whereas spermatids cannot metabolize glucose, albeit they express all enzymes of the glycolytic pathway [61] and prefer lactate for maintaining their ATP content [61, 62]. Spermatocytes which are intermediate developing germ cells may depend on glycolysis, since the glycolytic activities are higher in spermatocytes than in spermatids [62], however it must not be overlooked the utilization of lactate by these cells at these stages of development, especially those that lies at adluminal compartment [64, 65].

Sertoli Cells Metabolism

As lactate is the preferred energy substrate for spermatocytes and spermatids [64, 66] SCs must ensure its supply to these developing germ cells. For this reason, the mechanisms that regulate SCs metabolism are very relevant in maintaining spermatogenesis and male fertility.

The carbohydrate metabolism in SCs presents some unique characteristics since they actively metabolize glucose but the majority of it is converted in lactate and is not oxidized in the Krebs cycle. Robinson and Fritz [67] were the first to describe glucose metabolism in SCs. These authors described that when in culture, the SCs convert the vast majority of glucose to lactate that is then secreted. Only a small fraction of the pyruvate produced from glucose is used in the Krebs cycle being instead converted to lactate. Later, Grootegoed and collaborators [68] continued those studies and concluded that only 25% of the pyruvate originated from glucose metabolism is oxidized via the Krebs cycle (Figure 3). Those authors described that, in SCs under in vitro conditions, the pentose phosphate pathway is not operating at its maximum rate, and the rate of the oxidative activity of the pentose phosphate pathway is determined by the rate of NADPH oxidation [67, 68]. They also found that exogenous pyruvate is oxidized at very low concentrations during incubation in the presence of glucose [68].

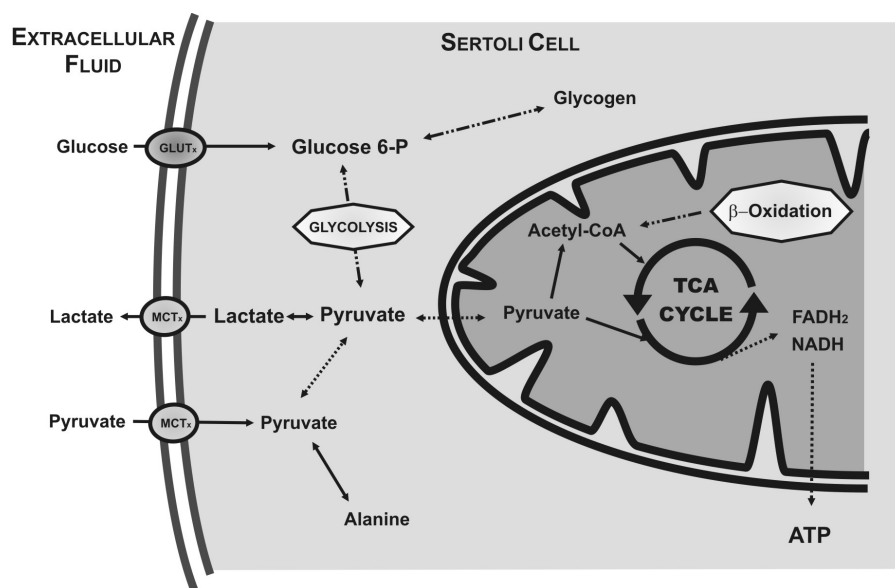


Figure 3. Schematic drawing illustration of Sertoli cell (SC) metabolism. The SCs are capable of consuming a variety of fuels including glucose, lactate, fatty acids, and amino acids. Nevertheless, the SCs actively metabolize glucose, being the majority of it converted into lactate and not oxidized in the TCA cycle. The extracellular lactate and pyruvate are transported via the family of proton-linked plasma membrane transporters that carry molecules having one carboxylate group, the monocarboxylate transporters (MCTs), while glucose is imported via the family of membrane proteins called glucose transporters (GLUTs). Once glucose enters the glycolytic pathway, it is decomposed into pyruvate, which can a) be converted into lactate via lactate dehydrogenase, b) be converted into alanine via alanine transaminase, or c) 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 to form FADH₂ and NADH. The oxidation of these substrates is coupled with ADP phosphorylation via the electron transport chain to form ATP. Abbreviations: TCA, tricarboxylic acid; GLUT, glucose transporter; MCT, monocarboxylate transporter.

Several mechanisms are known to be responsible for the modulation of SCs lactate production. Usually the rate-limiting step for glucose metabolism is the glucose transport of a family of structurally related glycoproteins designated by glucose transporters (GLUTs). Sertoli cells can take up glucose from the external medium [69] because hexose transporters are present on their plasma membranes [70]. Non-metabolizable analogues of glucose, such as 3H-3-O-methyl-D-glucose, can cross the BTB which indicates that glucose can actually be moved into the adluminal compartment, across both pro and antiluminal portion of the SCs plasma membrane [71]. Four glucose transporter isoforms (GLUT1, GLUT2, GLUT3 and GLUT8) have been so far identified in SCs [72-75]. However, GLUT8 is not expected to be involved in glucose transport from the extracellular *milieu* since it has not been identified in the plasma membrane of tissues [76, 77].

Also, lactate dehydrogenase (LDH) plays a crucial role in providing the substrate and later in the interconversion of pyruvate and lactate to developing germ cells. The lactate export and release from SCs by specific monocarboxylate transporters (MCTs) are also responsible for the improvement of lactate supply to germ cells.

The lactate production, in rat SCs, is under the control of FSH [66], epidermal growth factor (EGF) [78], insulin and insulin growth factor-I (IGF-I) [79], paracrine factor P-Mod-S [80], tri-iodothyronine [81], basic fibroblast growth factor (bFGF) [82], cytokines [44] and arachidonic acid [83]. Carnitine has also been described as a metabolic modulator of SCs metabolism. “In vitro” supplementation with carnitine increased the production of lactate and pyruvate, the LDH activity and the hexose transport, supporting that carbohydrate metabolism is increased by carnitine supplementation [84].

Sertoli cells can adapt to conditions of glucose deprivation to ensure the adequate lactate concentration in the microenvironment where germ cell develop and even when glucose levels are low or in the complete absence of glucose, the SCs still produce lactate [85]. Sertoli cells are known to change their metabolism by activating specific signal transduction pathways being the AMP-activated protein kinase (AMPK) a key mediator in cellular energy homeostasis [86]. The AMPK is present in SCs, and its activation increases lactate production with an increase of glucose uptake, increase of GLUT-1 and MCT4 expression [87]. The decrease of glucose levels in SCs medium leads to an activation of AMPK, an increase in glucose uptake, increase in GLUT1 and decrease in GLUT3 expression [85]. Adenosine is described as a sign, possibly emitted by germ cells to SCs, that results in activation of AMPK that can simultaneously promote lactate production and the maintenance of junctional integrity thus allowing the optimal microenvironment for a successful spermatogenesis [88]. In the absence of glucose, production of lactate has been suggested to be a consequence of aminoacids or glycogen metabolism, since it has been described the presence of glycogen and glycogen phosphorylase activity in SCs [89, 90].

Grootegoed and collaborators [68] described that besides glucose, the oxidation of glutamine and leucine can yield much of the required energy by SCs. Others have also described an important role for the oxidation of other aminoacids such as alanine, leucine, and valine [91]. Nevertheless, the SCs have insignificant capacity to oxidize glycine [91] although the creatine synthesized in SCs from glycine and arginine, is crucial for providing energy to the spermatogenic cells [92]. The presence of glucose can modify the metabolism of these aminoacids. Kaiser and collaborators [91] suggested that low amounts of acetyl-CoA arising from glucose can modulate alanine and valine oxidation to CO₂ by competing with the acetyl-CoA, which results from these aminoacids metabolism. Also, the observed stimulation of the

conversion of valine to lipids due to glucose metabolism was suggested to be a result of stimulation of the pentose cycle which is described as an important destination for glucose in SCs [67]. The presence of glutamine inhibited the oxidation of leucine, valine, and alanine but did not alter the conversion of these amino acids to lipids [91] although the incorporation of alanine to proteins was decreased [91]. Sertoli cells also convert leucine via transamination into 4-methyl-2-oxovalerate, spermatocytes and spermatids reduce exogenous 4-methyl- 2-oxovalerate to 2-hydroxy-4-methylvalerate, which is then released by the spermatogenic cells [93].

Sertoli cells are also important for the conversion of essential fatty acids in testis. When labeled fatty acids were injected in testis, they were firstly taken up and metabolized by SCs which proved that these cells have an important role in lipid metabolism [94]. The lipidic remodeling of rat spermatozoa has been shown to occur during their journey through the epididymis [95, 96]. Nevertheless, fatty acid metabolic activity in human testicular cells are low when compared with that found in rats [97], suggesting that human cells have a lower capacity for metabolizing fatty acid substrates. This may be related to the fact that adult rat testis are 0.7% of the body weight compared with approximately 0.04% of the body weight in adult men, and the daily sperm production per gram of testis is 5-6 times greater in rats than in men [98].

Lactate as Fuel for Germ Cell Development

Spermatogenic cells that undergo differentiation-related processes go through changes in their carbohydrate metabolism [62]. It has been suggested that there are specific metabolic signals required to the differentiation process of germ cells [99-101].

Germ cells undergoing meiosis exhibit a change in the type of substrate required for their energy metabolism and the equilibrium between glycolysis and the Krebs cycle shifts in favor of oxidative metabolism in post-meiotic cells [62]. The ratio between glycolysis and Krebs cycle is then greater in spermatocytes than in spermatids although in spermatozoa the equilibrium shifts again favoring glycolysis. Also, respiratory inhibitors reduce pyruvate/lactate consumption by spermatocytes while the spermatids completely degenerate in their presence [102].

Round spermatid energy metabolism is closely dependent on the presence of lactate in the extracellular medium and this lactate is supplied by SCs, as discussed above. That is why the STF is rich in lactate but has low glucose and pyruvate concentration [62]. Spermatids and spermatocytes are located inside the BTB while spermatogonia are outside the barrier. This may explain why spermatogonia might utilize the glucose in the blood as energetic source, but as a large number of germ cells are developing into sperm inside seminiferous tubules, they must have others energy sources.

ATP levels in spermatids decrease in response to glucose metabolism [103]. The ATP is rapidly dephosphorylated when round spermatids are exposed to glucose in the absence of exogenous lactate [104]. Post-meiotic germ cells are unable to use glucose and rather prefer to use lactate as an energy source for their metabolism [105-107]. It has been reported that intratesticular infusion of lactate into the adult cryptorchid rat testis improves

spermatogenesis [108]. Lactate is also responsible for RNA and protein synthesis stimulation in spermatids [106].

The spermatids exclusively use lactate probably due to their lower glycolytic potential whereas spermatocytes have greater glycolytic activity and have a limited dependence on lactate thus being able to use lactate, pyruvate and glucose. There is a metabolic recycling of lactate to glucose-6-P in round spermatids showing that both glycolytic and gluconeogenic pathways are functional in these cells [101]. Spermatozoa have the greatest glycolytic potential and the lowest citric acid cycle (TCA cycle) activity using only glucose or fructose for their energy metabolism [62]. It has been proposed that glyceraldehyde 3-phosphate dehydrogenase (GAPDH) regulates glycolysis in postmeiotic germ cells by being inactive in spermatids and active in spermatozoa [61]. However, the mechanism by which the enzyme becomes activated in sperm still remains unknown, although ATP and other adenine nucleotides might be responsible for that process [61].

The pentose phosphate pathway is activated in germ cells, as indicated by the glucose-6-phosphate activity. This pathway is required for the biosynthesis of nucleotides for RNA and for production of NADPH and ribose 5-phosphate. Also, during their journey through the epididymis, rat spermatozoa undergo considerable lipid remodeling and pachytene spermatocytes are more active in metabolizing the fatty acids than round spermatids [109]. Whether the germ cells are able to synthesize fatty acids from linoleic and α -linolenic acid or whether they must be provided with these fatty acids from the SCs is unknown, but several authors proposed that there is a transport of long polyenes from SCs to the germ cells although it has not been fully proved yet [110-112]. That transport may require a cell-to-cell contact as in vivo the germ cells are in close contact with SCs throughout all steps of development [112].

Conclusion

Formation of competent spermatozoa is a complex multistep process that is initiated in the seminiferous epithelium. One critical feature in this process is the establishment of the BTB that physically and physiologically supports the proliferation and differentiation of germ cells into mature spermatids.

Germ cells have special metabolic needs that render them dependent on the nurturing provided by SCs, the structural element of the seminiferous epithelium. Developing germ cells are unable to use glucose for their energy metabolism and preferentially use lactate. Thus, carbohydrate metabolism in SCs presents some distinctive characteristics as they actively metabolize glucose, but the majority of it is converted to lactate. Moreover, these cells must be capable of modulating their metabolism in order to ensure the adequate lactate concentration in the microenvironment where germ cell develop, even in conditions of glucose limitation. Production of lactate has been suggested to be derived also from aminoacids or glycogen metabolism, although these processes are not entirely understood.

Further knowledge on the functioning and regulation of these biochemical mechanisms is essential for the enlightenment of a process that is central to the propagation of life, spermatogenesis. This will be an important step in understanding infertility linked to BTB dysfunction and the role of cellular metabolism in some pathological conditions.

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References

- [1] Kormano M. Dye permeability and alkaline phosphatase activity of testicular capillaries in the postnatal rat. *Histochemie*. 1967;9(4):327-38.
- [2] Setchell B, Davies R, Gladwell R, Hinton B, Main S, Pilsworth L, et al. The movement of fluid in the seminiferous tubules and rete testis. *Annales de biologie animale, biochimie, biophysique*. 1978;18(2B):623-32.
- [3] Setchell BP, Hinton BT, Jacks F, Davies RV. The restricted penetration of iodinated rat FSH and LH into the seminiferous tubules of the rat testis. *Molecular and Cellular Endocrinology*. 1976 Nov;6(1):59-69.
- [4] Voglmayr JK, Waites GM, Setchell BP. Studies on spermatozoa and fluid collected directly from the testis of the conscious ram. *Nature*. 1966;210(5038):861-63.
- [5] Waites G, Gladwell R. Physiological significance of fluid secretion in the testis and blood-testis barrier. *Physiol. Rev*. 1982;62(2):624-71.
- [6] Su L, Mruk DD, Cheng CY. Drug transporters, the blood-testis barrier and spermatogenesis. *Journal of Endocrinology*. 2011;208(3):207-23.
- [7] Setchell BP. The Functional-Significance of the Blood-Testis Barrier. *Journal of andrology*. 1980;1(1):3-10.
- [8] Wong CH, Cheng CY. The blood-testis barrier: its biology, regulation, and physiological role in spermatogenesis. *Curr. Top Dev. Biol*. 2005;71:263-96.
- [9] Toyama Y, Maekawa M, Yuasa S. Ectoplasmic specializations in the Sertoli cell: new vistas based on genetic defects and testicular toxicology. *Anatomical Science International*. 2003 Mar;78(1):1-16.
- [10] Mazaud-Guittot S, Meugnier E, Pesenti S, Wu X, Vidal H, Gow A, et al. Claudin 11 deficiency in mice results in loss of the Sertoli cell epithelial phenotype in the testis. *Biology of Reproduction*. 2010 Jan;82(1):202-13.
- [11] Lui WY, Cheng CY. Regulation of cell junction dynamics by cytokines in the testis: a molecular and biochemical perspective. *Cytokine Growth Factor Review*. 2007 Jun-Aug;18(3-4):299-311.
- [12] Cheng CY, Mruk DD. An intracellular trafficking pathway in the seminiferous epithelium regulating spermatogenesis: a biochemical and molecular perspective. *Critical Reviews in Biochemistry and Molecular Biology*. 2009 Sep-Oct;44(5):245-63.
- [13] Cheng CY, Wong EW, Yan HH, Mruk DD. Regulation of spermatogenesis in the microenvironment of the seminiferous epithelium: new insights and advances. *Molecular and Cellular Endocrinology*. 2010 Feb 5;315(1-2):49-56.
- [14] Siu MKY, Cheng CY. Extracellular matrix and its role in spermatogenesis. *Molecular Mechanisms in Spermatogenesis*. 2009:74-91.

-
- [15] Russell LD. The blood-testis barrier and its formation relative to spermatocyte maturation in the adult rat: a lanthanum tracer study. *Anatomical Record*. 1978 Jan;190(1):99-111.
 - [16] Yao PL, Lin YC, Richburg JH. Mono-(2-ethylhexyl) phthalate-induced disruption of junctional complexes in the seminiferous epithelium of the rodent testis is mediated by MMP2. *Biology of Reproduction*. 2010;82(3):516-27.
 - [17] Mital P, Hinton BT, Dufour JM. The Blood-Testis and Blood-Epididymis Barriers Are More Than Just Their Tight Junctions. *Biology of Reproduction*. 2011 Jan 5;84(5):851-58.
 - [18] Sharpe R. Follicle-stimulating hormone and spermatogenesis in the adult male. *Journal of Endocrinology*. 1989;121(3):405.
 - [19] Means AR, Huckins C. Coupled events in the early biochemical actions of FSH on the Sertoli cells of the testis. *Current topics in molecular endocrinology*. 1974;1:145-65.
 - [20] Means AR, Dedman JR, Tash JS, Tindall DJ, van Sickle M, Welsh MJ. Regulation of the testis sertoli cell by follicle stimulating hormone. *Annual Reviews of Physiology*. 1980;42:59-70.
 - [21] Setchell BP. Blood-testis barrier, junctional and transport proteins and spermatogenesis. *Adv. Exp. Med. Biol*. 2008;636:212-33.
 - [22] Brinster RL, Zimmermann JW. Spermatogenesis following male germ-cell transplantation. *Proceeding of the National Academy of Science*. 1994 Nov 22;91(24):11298-302.
 - [23] Wang T, Feng X, Han D. Mechanisms of testicular immune privilege. *Frontiers in Biology*. 2011;6(1):19-30.
 - [24] Dym M, Romrell LJ. Intraepithelial lymphocytes in the male reproductive tract of rats and rhesus monkeys. *Journal of Reproduction and Fertility*. 1975 Jan;42(1):1-7.
 - [25] Head JR, Billingham RE. Immune privilege in the testis. II. Evaluation of potential local factors. *Transplantation*. 1985 Sep;40(3):269-75.
 - [26] Nagaosa K, Nakashima C, Kishimoto A, Nakanishi Y. Immune response to bacteria in seminiferous epithelium. *Reproduction*. 2009 May;137(5):879-88.
 - [27] Griswold M, McLean D. The Sertoli cell. In: Neill J, editor. *Knobil and Neill's physiology of reproduction*. San Diego: Elsevier; 2006. p. 949-75.
 - [28] Dym M. The fine structure of monkey Sertoli cells in the transitional zone at the junction of the seminiferous tubules with the tubuli recti. *American Journal Anatomy*. 1974 May;140(1):1-25.
 - [29] Russell L, Ettlin R, Sinha Hikim A, Clegg E. *Histological and histopathological evaluation of the testis*. Clearwater: Cache River Press; 1990.
 - [30] Russell LD, Ren HP, Hikim IS, Schulze W, Hikim APS. A comparative study in twelve mammalian species of volume densities, volumes, and numerical densities of selected testis components, emphasizing those related to the Sertoli cell. *American Journal of Anatomy*. 1990;188(1):21-30.
 - [31] Weber JE, Russell LD, Wong V, Peterson RN. Three-dimensional reconstruction of a rat stage V Sertoli cell: II. Morphometry of Sertoli--Sertoli and Sertoli--germ-cell relationships. *American Journal Anatomy*. 1983 Jun;167(2):163-79.
 - [32] O'Donnell L, Robertson K, Jones M, Simpson E. Estrogen and spermatogenesis. *Endocr. Rev*. 2001;22(3):289-318.

-
- [33] Hess R, de Franca L. Spermatogenesis and cycle of the seminiferous epithelium. *Molecular Mechanisms in Spermatogenesis* Austin, TX: Landes Bioscience/Springer Science. 2008:1–15.
- [34] Sertoli E. Sulla struttura dei canalicoli seminiferi dei testicoli *Arch Sc Med*. 1878;2:107-19.
- [35] Sertoli E. Dell'esistenza di particolari cellule ramificate nei canalicoli seminiferi del testicolo umano. *Morgagni*. 1865;7:31-3.
- [36] Griswold M, editor. The central role of Sertoli cells in spermatogenesis 1998: London: Academic Press.
- [37] Sharpe R, McKinnell C, Kivlin C, Fisher J. Proliferation and functional maturation of Sertoli cells, and their relevance to disorders of testis function in adulthood. *Reproduction*. 2003;125(6):769-84.
- [38] Petersen C, Soder O. The Sertoli cell-a hormonal target and 'super' nurse for germ cells that determines testicular size. *Hormone Research*. 2006;66(4):153-61.
- [39] Gondos B, WE B. Postnatal and pubertal development In: Russell LD GM, editor. The Sertoli Cell. Clearwater, FL: Cache River Press; 1994. p. 116-53.
- [40] Nistal M, Abaurrea MA, Paniagua R. Morphological and histometric study on the human Sertoli cell from birth to the onset of puberty. *Journal of Anatomy*. 1982;134:351-63.
- [41] Mruk DD, Cheng CY. Sertoli-Sertoli and Sertoli-germ cell interactions and their significance in germ cell movement in the seminiferous epithelium during spermatogenesis. *Endocr. Rev*. 2004 Oct;25(5):747-806.
- [42] Vogl AW, Vaid KS, Guttman JA. The Sertoli Cell Cytoskeleton. In: Cheng CY, editor. *Molecular Mechanisms in Spermatogenesis*: Landes Bioscience/ Springer Science + Business Media; 2008. p. 186-211.
- [43] Riera M, Meroni S, Schteingart H, Pellizzari E, Cigorraga S. 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. *Journal of Endocrinology*. 2002;173(2):335-43.
- [44] Riera MF, Meroni SB, Gomez GE, Schteingart HF, Pellizzari EH, Cigorraga SB. Regulation of lactate production by FSH, iL1beta, and TNFalpha in rat Sertoli cells. *General and Comparative Endocrinology*. 2001 Apr;122(1):88-97.
- [45] Erkkila K, Aito H, Aalto K, Pentikainen V, Dunkel L. Lactate inhibits germ cell apoptosis in the human testis. *Molecular human reproduction*. 2002;8(2):109-17.
- [46] Lee J, Richburg JH, Younkin SC, Boekelheide K. The Fas system is a key regulator of germ cell apoptosis in the testis. *Endocrinology*. 1997 May;138(5):2081-8.
- [47] Xiong WP, Wang HK, Wu H, Chen YM, Han DS. Apoptotic spermatogenic cells can be energy sources for Sertoli cells. *Reproduction*. 2009 Mar;137(3):469-79.
- [48] Rato L, Socorro S, Cavaco JE, Oliveira PF. Tubular fluid secretion in the seminiferous epithelium: ion transporters and aquaporins in Sertoli cells. *Journal of Membrane Biology*. 2010 Jul;236(2):215-24.
- [49] Koskimies A, Kormano M. The proteins in fluids from the seminiferous tubules and rete testis of the rat. *Reproduction*. 1973;34(3):433-34.
- [50] Fisher D. New light shed on fluid formation in the seminiferous tubules of the rat. *Journal of Physiology*. 2002 July 15, 2002;542(2):445-52.

-
- [51] Setchell B. The secretion of fluid by the testes of rats, rams and goats with some observations on the effect of age, cryptorchidism and hypophysectomy. *Reproduction*. 1970;23(1):79-85.
- [52] Tuck RR, Setchell BP, Waites GM, Young JA. The composition of fluid collected by micropuncture and catheterization from the seminiferous tubules and rete testis of rats. *Pflugers Arch*. 1970;318(3):225-43.
- [53] Jenkins A, Lechene C, Howards S. Concentrations of seven elements in the intraluminal fluids of the rat seminiferous tubules, rete testis, and epididymis. *Biology of Reproduction*. 1980;23(5):981-87.
- [54] Clulow J, Jones RC. Composition of luminal fluid secreted by the seminiferous tubules and after reabsorption by the extratesticular ducts of the Japanese quail, *Coturnix coturnix japonica*. *Biology of Reproduction*. 2004 Nov;71(5):1508-16.
- [55] Oliveira P, Sousa M, Barros A, Moura T, Rebelo da Costa A. Intracellular pH regulation in human Sertoli cells: role of membrane transporters. *Reproduction*. 2009;137(2):353.
- [56] Oliveira PF, Sousa M, Barros A, Moura T, Rebelo da Costa A. Membrane transporters and cytoplasmatic pH regulation on bovine Sertoli cells. *Journal of Membrane Biology*. 2009a Jan;227(1):49-55.
- [57] Roos A, Boron W. Intracellular pH. *Am. Physiological. Soc*; 1981. p. 296-434.
- [58] Boron W. Regulation of intracellular pH. American Physiological Society. 2004;28(4):160-79.
- [59] Jegou B, Le Gac F, de Kretser D. Seminiferous tubule fluid and interstitial fluid production. I. Effects of age and hormonal regulation in immature rats. *Biology of Reproduction*. 1982;27(3):590-95.
- [60] Brauchi S, Rauch MC, Alfaro IE, Cea C, Concha, II, Benos DJ, et al. Kinetics, molecular basis, and differentiation of L-lactate transport in spermatogenic cells. *American Journal Physiology Cell Physiology*. 2005 Mar;288(3):523-34.
- [61] Boussouar F, Benahmed M. Lactate and energy metabolism in male germ cells. *TRENDS in Endocrinology and Metabolism*. 2004;15(7):345-50.
- [62] Bajpai M, Gupta G, Setty B. Changes in carbohydrate metabolism of testicular germ cells during meiosis in the rat. *European journal of endocrinology*. 1998;138(3):322-27.
- [63] Tokas J, Mukhopadhyay C, and, Verma A. Self Renewal of Spermatogonial Stem Cells: the Most Promising Multipotent Cells - A Review. *Veterinary World*. 2011;4:234-40.
- [64] Jutte N, Grootegoed J, Rommerts F, Van der Molen H. Exogenous lactate is essential for metabolic activities in isolated rat spermatocytes and spermatids. *Reproduction*. 1981;62(2):399-405.
- [65] Jutte N, Jansen R, Grootegoed J, Rommerts F, Clausen O, Van der Molen H. Regulation of survival of rat pachytene spermatocytes by lactate supply from Sertoli cells. *Reproduction*. 1982;65(2):431-38.
- [66] Mita M, Price JM, Hall PF. Stimulation by follicle-stimulating hormone of synthesis of lactate by Sertoli cells from rat testis. *Endocrinology*. 1982 May;110(5):1535-41.
- [67] Robinson R, Fritz IB. Metabolism of glucose by Sertoli cells in culture. *Biology of Reproduction*. 1981;24(5):1032.
- [68] Grootegoed J, Oonk R, Jansen R, Van der Molen H. Metabolism of radiolabelled energy-yielding substrates by rat Sertoli cells. *Reproduction*. 1986;77(1):109.

-
- [69] Hall PF, Mita M. Influence of follicle-stimulating hormone on glucose transport by cultured Sertoli cells. *Biol. Reprod.* 1984 Dec;31(5):863-9.
- [70] Angulo C, Rauch MC, Droppelmann A, Reyes AM, Slebe JC, Delgado-Lopez F, et al. Hexose transporter expression and function in mammalian spermatozoa: cellular localization and transport of hexoses and vitamin C. *J. Cell Biochem.* 1998 Nov 1;71(2):189-203.
- [71] Turner TT, D'Addario DA, Howards SS. The transepithelial movement of 3H-3-O-methyl-D-glucose in the hamster seminiferous and cauda epididymidal tubules. *Fertil. Steril.* 1983 Oct;40(4):530-5.
- [72] Carosa E, Radico C, Giansante N, Rossi S, D'Adamo F, Di Stasi SM, et al. Ontogenetic profile and thyroid hormone regulation of type-1 and type-8 glucose transporters in rat Sertoli cells. *Int. J. Androl.* 2005 Apr;28(2):99-106.
- [73] Galardo MN, Riera MF, Pellizzari EH, Chemes HE, Venara MC, Cigorruga SB, et al. Regulation of expression of Sertoli cell glucose transporters 1 and 3 by FSH, IL1 beta, and bFGF at two different time-points in pubertal development. *Cell Tissue Res.* 2008 Nov;334(2):295-304.
- [74] Ulisse S, Jannini EA, Pepe M, De Matteis S, D'Armiento M. Thyroid hormone stimulates glucose transport and GLUT1 mRNA in rat Sertoli cells. *Mol. Cell Endocrinol.* 1992 Sep;87(1-3):131-7.
- [75] Kokk K, Verajankorva E, Wu XK, Tapfer H, Poldoja E, Pollanen P. Immunohistochemical detection of glucose transporters class I subfamily in the mouse, rat and human testis. *Medicina* (Kaunas, Lithuania). 2004;40(2):156.
- [76] Piroli GG, Grillo CA, Hoskin EK, Znamensky V, Katz EB, Milner TA, et al. Peripheral glucose administration stimulates the translocation of GLUT8 glucose transporter to the endoplasmic reticulum in the rat hippocampus. *J. Comp. Neurol.* 2002 Oct 14;452(2):103-14.
- [77] Reagan LP, Gorovits N, Hoskin EK, Alves SE, Katz EB, Grillo CA, et al. Localization and regulation of GLUTx1 glucose transporter in the hippocampus of streptozotocin diabetic rats. *Proc. Natl. Acad. Sci. U S A.* 2001 Feb 27;98(5):2820-5.
- [78] Mallea LE, Machado AJ, Navaroli F, Rommerts FF. Epidermal growth factor stimulates lactate production and inhibits aromatization in cultured Sertoli cells from immature rats. *Int. J. Androl.* 1986 Jun;9(3):201-8.
- [79] Oonk RB, Jansen R, Grootegoed JA. Differential effects of follicle-stimulating hormone, insulin, and insulin-like growth factor I on hexose uptake and lactate production by rat Sertoli cells. *J. Cell Physiol.* 1989 Apr;139(1):210-8.
- [80] Mullaney BP, Rosselli M, Skinner MK. Developmental regulation of Sertoli cell lactate production by hormones and the testicular paracrine factor, PModS. *Mol. Cell Endocrinol.* 1994 Aug;104(1):67-73.
- [81] Palmero S, Prati M, Bolla F, Fugassa E. Tri-iodothyronine directly affects rat Sertoli cell proliferation and differentiation. *J. Endocrinol.* 1995 May;145(2):355-62.
- [82] Schteingart HF, Meroni SB, Canepa DF, Pellizzari EH, Cigorruga SB. Effects of basic fibroblast growth factor and nerve growth factor on lactate production, gamma-glutamyl transpeptidase and aromatase activities in cultured Sertoli cells. *Eur. J. Endocrinol.* 1999 Nov;141(5):539-45.

-
- [83] Meroni SB, Riera MF, Pellizzari EH, Schteingart HF, Cigorraga SB. Possible role of arachidonic acid in the regulation of lactate production in rat Sertoli cells. *Int. J. Androl.* 2003 Oct;26(5):310-7.
- [84] Palmero S, Bottazzi C, Costa M, Leone M, Fugassa E. Metabolic effects of L-carnitine on prepubertal rat Sertoli cells. *Horm. Metab. Res.* 2000 Mar;32(3):87-90.
- [85] Riera MF, Galardo MN, Pellizzari EH, Meroni SB, Cigorraga SB. Molecular mechanisms involved in Sertoli cell adaptation to glucose deprivation. *Am. J. Physiol. Endocrinol. Metab.* 2009 Oct;297(4):E907-14.
- [86] Hardie DG. Minireview: the AMP-activated protein kinase cascade: the key sensor of cellular energy status. *Endocrinology.* 2003 Dec;144(12):5179-83.
- [87] Galardo MN, Riera MF, Pellizzari EH, Cigorraga SB, Meroni SB. 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 Oct;39(4):279-88.
- [88] Galardo MN, Riera MF, Pellizzari EH, Sobarzo C, Scarcelli R, Denduchis B, et al. Adenosine regulates Sertoli cell function by activating AMPK. *Mol. Cell Endocrinol.* 2010 Dec 15;330(1-2):49-58.
- [89] Leiderman B, Mancini RE. Glycogen content in the rat testis from postnatal to adult ages. *Endocrinology.* 1969 Sep;85(3):607-9.
- [90] Slaughter GR, Means AR. Follicle-stimulating hormone activation of glycogen phosphorylase in the Sertoli cell-enriched rat testis. *Endocrinology.* 1983 Oct;113(4):1476-85.
- [91] Kaiser GR, Monteiro SC, Gelain DP, Souza LF, Perry ML, Bernard EA. Metabolism of amino acids by cultured rat Sertoli cells. *Metabolism.* 2005 Apr;54(4):515-21.
- [92] Moore NP, Gray TJ, Timbrell JA. Creatine metabolism in the seminiferous epithelium of rats. I. Creatine synthesis by isolated and cultured cells. *J. Reprod. Fertil.* 1998 Mar;112(2):325-30.
- [93] Grootegeod JA, Jansen R, van der Molen HJ. Intercellular pathway of leucine catabolism in rat spermatogenic epithelium. *Biochem. J.* 1985 Mar 15;226(3):889-92.
- [94] Beckman JK, Coniglio JG. The metabolism of polyunsaturated fatty acids in rat Sertoli and germinal cells. *Lipids.* 1980 Jun;15(6):389-94.
- [95] Hall JC, Hadley J, Doman T. Correlation between changes in rat sperm membrane lipids, protein, and the membrane physical state during epididymal maturation. *J. Androl.* 1991 Jan-Feb;12(1):76-87.
- [96] Aveldano MI, Rotstein NP, Vermouth NT. Lipid remodelling during epididymal maturation of rat spermatozoa. Enrichment in plasmalogen lipids containing long-chain polyenoic fatty acids of the n-9 series. *Biochem. J.* 1992 Apr 1;283 (Pt 1):235-41.
- [97] Retterstol K, Haugen TB, Woldseth B, Christophersen BO. A comparative study of the metabolism of n-9, n-6 and n-3 fatty acids in testicular cells from immature rat. *Biochim. Biophys. Acta.* 1998 May 20;1392(1):59-72.
- [98] Sharpe R. Regulation of spermatogenesis. In: Knobil E, Neill J, editors. *The physiology of reproduction*. New York: Raven Press Ltd, ; 1994. p. 1363-434.
- [99] Herrera E, Salas K, Lagos N, Benos DJ, Reyes JG. Energy metabolism and its linkage to intracellular Ca^{2+} and pH regulation in rat spermatogenic cells. *Biol. Cell.* 2000 Sep;92(6):429-40.

-
- [100] Reyes JG, Herrera E, Lobos L, Salas K, Lagos N, Jorquera RA, et al. Dynamics of intracellular calcium induced by lactate and glucose in rat pachytene spermatocytes and round spermatids. *Reproduction*. 2002 May;123(5):701-10.
- [101] Yanez AJ, Bustamante X, Bertinat R, Werner E, Rauch MC, Concha, II, et al. Expression of key substrate cycle enzymes in rat spermatogenic cells: fructose 1,6 bisphosphatase and 6 phosphofructose 1-kinase. *J. Cell Physiol*. 2007 Sep;212(3):807-16.
- [102] Grootegoed JA, Jansen R, Van der Molen HJ. The role of glucose, pyruvate and lactate in ATP production by rat spermatocytes and spermatids. *Biochim. Biophys. Acta*. 1984 Nov 26;767(2):248-56.
- [103] 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 Dec 10;257(23):13945-50.
- [104] Grootegoed JA, Jansen R, van der Molen HJ. Effect of glucose on ATP dephosphorylation in rat spermatids. *J. Reprod. Fertil*. 1986 May;77(1):99-107.
- [105] Mita M, Hall PF. Metabolism of round spermatids from rats: lactate as the preferred substrate. *Biology of Reproduction*. 1982;26(3):445.
- [106] Jutte NH, Grootegoed JA, Rommerts FF, van der Molen HJ. Exogenous lactate is essential for metabolic activities in isolated rat spermatocytes and spermatids. *J. Reprod. Fertil*. 1981 Jul;62(2):399-405.
- [107] Nakamura M, Okinaga S, Arai K. Metabolism of round spermatids: evidence that lactate is preferred substrate. *Am. J. Physiol*. 1984 Aug;247(2 Pt 1):E234-42.
- [108] Courtens JL, Ploen L. Improvement of spermatogenesis in adult cryptorchid rat testis by intratesticular infusion of lactate. *Biol Reprod*. 1999 Jul;61(1):154-61.
- [109] Retterstol K, Haugen TB, Tran TN, Christophersen BO. Studies on the metabolism of essential fatty acids in isolated human testicular cells. *Reproduction*. 2001 Jun;121(6):881-7.
- [110] Lynch KM, Jr., Scott WW. Lipid distribution in the Sertoli cell and Leydig cell of the rat testis as related to experimental alterations of the pituitary-gonad system. *Endocrinology*. 1951 Jul;49(1):8-14.
- [111] Beckman JK, Coniglio JG. A comparative study of the lipid composition of isolated rat Sertoli and germinal cells. *Lipids*. 1979 Mar;14(3):262-7.
- [112] Retterstol K, Tran TN, Haugen TB, Christophersen BO. Metabolism of very long chain polyunsaturated fatty acids in isolated rat germ cells. *Lipids*. 2001 Jun;36(6):601-6.