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Review

New insights on hormones and factors that modulate Sertoli cell metabolism

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Summary. Sertoli cells (SCs) play a key role in spermatogenesis by providing the physical support for developing germ cells and ensuring them the appropriate nutrients, energy sources, hormones, and growth factors. The control of SCs metabolism has been in the spotlight for reproductive biologists, since it may be crucial to determine germ cells' fate. Indeed, the maintenance of spermatogenesis is highly dependent on the metabolic cooperation established between SCs and germ cells, though this event has been overlooked. It depends on the orchestration of various metabolic pathways and an intricate network of signals. Several factors and/or hormones modulate the metabolic activity of SCs, which are major targets for the hormonal signalling that regulates spermatogenesis. Any alteration in the regulation of these cells' metabolic behaviour may compromise the normal development of spermatogenesis and consequently, male fertility. In this context, SC metabolism arises as a key regulation point for spermatogenesis. Herein, we present an up-to-date overview on the impact of hormones and factors that modulate SC metabolism, with special focus on glycolytic metabolism, highlighting their relevance in determining male reproductive potential.

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Abbreviations. 5α-DHT, 5α-dihydrotestosterone; 7-oxo-DHEA, 7-Oxo-dehydroepiandrosterone; ACC, acetyl-coA carboxylase; AMH, anti-Mullerian hormone; AMPK, 5' adenosine monophosphate-activated protein kinase; AR, androgen receptor; bFGF, basic fibroblast growth factor; BTB, blood-testis barrier; DHEA, dehydroepiandrosterone; DM, diabetes mellitus; E2, 17β-estradiol; EGF, epidermal growth factor; FSH, follicle-stimulating hormone; GLUT1, glucose transporter 1; GLUT2, glucose transporter 2; GLUT3, glucose transporter 3; HPT-axis, hypothalamus-pituitary-testis axis; IL-1, interleukin 1; IL-1α, interleukin 1α; IL-1β, interleukin 1β; LDH, lactate dehydrogenase; LDHA, lactate dehydrogenase A; MAPK, mitogen-activated protein kinase; MCT4, monocarboxylate transporter 4; MCTs, monocarboxylate transporters; P-Mod-S, peritubular modifying substrate; P-Mod-S A, peritubular modifying substrate A; P-Mod-S B, peritubular modifying substrate B; PDC, pyruvate dehydrogenase complex; PDK, pyruvate dehydrogenase kinase; PDP, pyruvate dehydrogenase phosphorylase; PFK, phosphofructokinase; PFKFB, 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase; PFKFB1, 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase isoform 1; PFKFB3; 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase isoform 3; PI3K, phosphatidylinositol 3-kinase; PKB, protein kinase B; PPAR, peroxisome proliferator-activated receptors; PPARα, peroxisome proliferator-activated receptor α; PPARβ/δ, peroxisome proliferator-activated receptor β/δ; PPARγ, peroxisome proliferator-activated receptor γ; PUFAS, polyunsaturated fatty acids; RHOX5, reproductive homeobox 5 of the chromosome X; SC, Sertoli cell; SCs, Sertoli cells; T, testosterone; T2DM, type 2 diabetes mellitus; T3, triiodothyronine; TH, thyroid hormones

Introduction

The two major functions of testes are the synthesis and release of sex steroid hormones, particularly testosterone (T), and the formation of sperm. These functions are finely regulated by the hypothalamus-pituitary-testis axis (HPT-axis). The release of pituitary gonadotropins is stimulated by gonadotropin-releasing hormone. Luteinizing hormone acts on T-producing Leydig cells located in the *interstitium* while follicle stimulating hormone (FSH) acts on Sertoli cells (SCs), within the seminiferous tubules (Griswold, 1998; Walker and Cheng, 2005). The control of spermatogenesis, the process by which immature germ cells undergo meiosis, division, and differentiation to give rise to haploid elongated spermatids, is maintained *via* a chain of complex local interactions involving several testicular cells such as germ, Sertoli, peritubular and Leydig cells (Shubhada et al., 1993; Walker and Cheng, 2005). This process occurs within the seminiferous tubules that consist of a fibrous structure composed of various extracellular matrix proteins (such as laminin, collagen and heparan sulphate proteoglycans), responsible for the tubules structural integrity (Hadley et al., 1985), through a close association between germ cells and SCs. Within the seminiferous tubules, SCs reside on the basement membrane, outside of which are the lymphatic endothelium and the peritubular myoid cells (Dym and Fawcett, 1970). SCs are extremely important for testicular development. They are the first cells to recognizably differentiate in the indifferent fetal gonad and ensure the regression of Mullerian ducts, *via* secretion of anti-Mullerian hormone (AMH) (Sharpe et al., 2003). Moreover, when differentiated, these cells play a key role in spermatogenesis by providing a unique microenvironment, structural support and nourishment of germ cells (Fig. 1) (Catalano et al., 2007).

SCs present a columnar shape, extending from the base of the seminiferous tubule to its lumen and are adhered to the basal lamina. The cytoplasmic extensions of SCs allow them to sustain a vast number of germ cells in development, with each Sertoli cell (SC) supporting

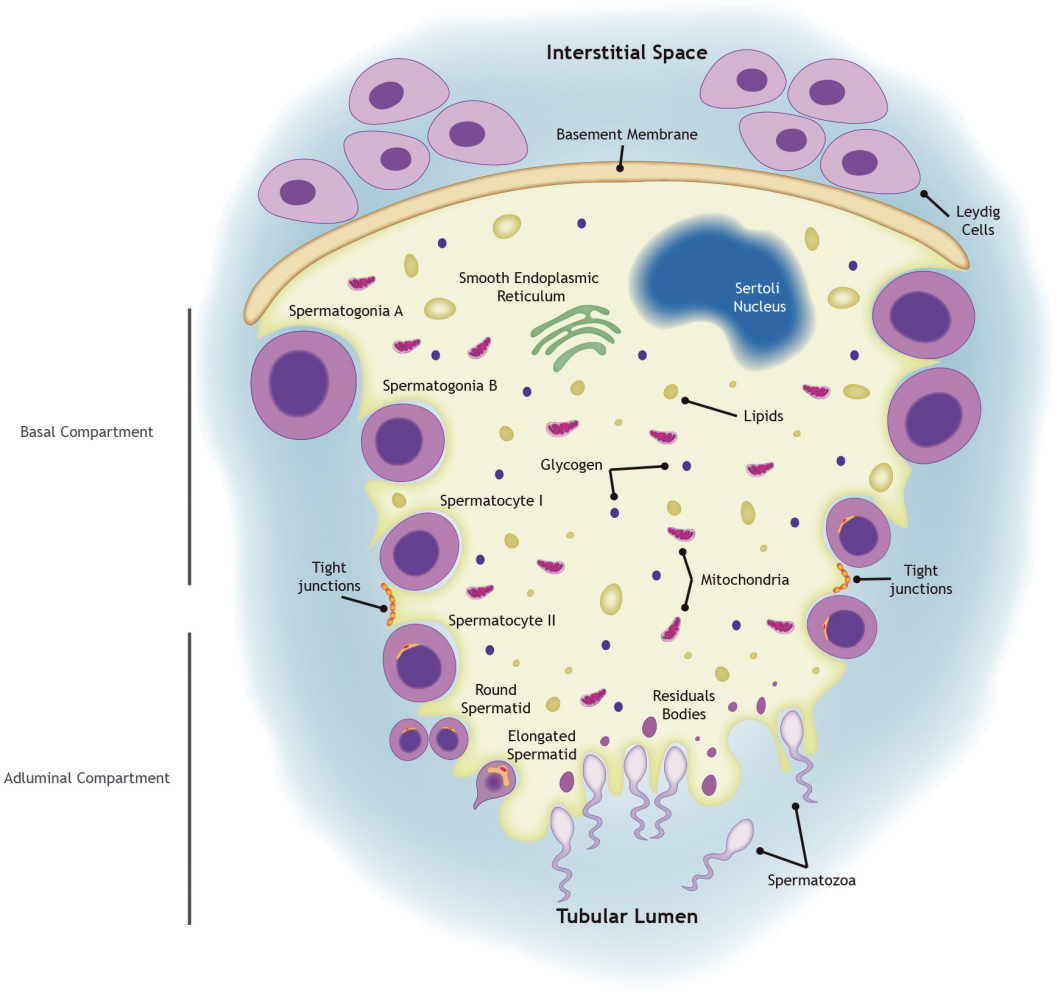

Fig. 1. Schematic illustration of the seminiferous epithelium and Sertoli cells (SCs). The seminiferous epithelium is composed by SCs and different subtypes of developing germ cells. Male gametes (spermatozoa) are formed in the seminiferous tubules of the testis in a complex process, known as spermatogenesis, the cellular division and transformation that produces male haploid germ cells from diploid spermatogonial stem cells. 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 (Spermatocyte I) that separate the homologous pairs of chromosomes in meiosis I (reduction division) to form the haploid secondary spermatocytes (Spermatocyte II). The meiosis II yields four equalized spermatids that migrate toward the lumen where fully formed spermatozoa are finally released.

between 30 to 50 germ cells in various stages of development (Cheng et al., 2010). Hence, the number of SCs will determine the number of germ cells that can be supported through spermato-genesis. This will numerically determine the extent of sperm production, since each SC has a fixed capacity for the number of germ cells that it can support (Sharpe, 1994; Petersen and Soder, 2006). Hence, SCs are pivotal for the regulation of spermatogenesis and the establishment of different rates of sperm production (Walker and Cheng, 2005).

Adjacent SCs establish between them tight junctions, which allows them to create the unique environment within the tubular compartment, where meiotic and post-meiotic steps of spermatogenesis occur. The establishment of this barrier, known as Sertoli/blood-testis barrier (BTB), allows the formation of a specific intratubular fluid dependent on SC function. Thus, these cells control the composition of adluminal compartment and developing germ cells are dependent on the secretion and/or selective passage of nutrients and other factors by SCs. A fully functional BTB allows: 1) a selective passage of molecules and substances into the adluminal compartment of the seminiferous tubules; 2) the prevention of movement of cells from the immune system and the regulation of cytokines levels in the seminiferous epithelium and 3) the maintenance of transporters and channels in the apical and basolateral membranes, which are highly dynamic and responsible for the formation of a specific microenvironment. Yet, this barrier allows the migration of developing germ cells throughout the seminiferous epithelium. The modification of proteins associated to tight junctions (occludins, claudins and JAM proteins (Chiba et al., 2008)) alters the establishment of BTB and its permeability properties, a condition that is observed in several pathological conditions associated to male subfertility/infertility. In fact, during specific stages of the spermatogenic cycle, the BTB is restructured to allow the migration of germ cells in such a well-coordinated process that the immune privilege is not compromised (Cheng et al., 2010). The flow of nutrients, growth factors and other substances to germ line is regulated by SCs. Indeed, the production and secretion of many SC-related proteins and factors involved in germ cell development occur in a stage-dependent manner, illustrating that these cells adapt to the changing needs of developing germ cells (Mruk and Cheng, 2004). They produce several proteins and factors (e.g. androgen-binding protein (Fritz et al., 1976), transferrin (Skinner and Griswold, 1980), plasminogen activator (Marzowski et al., 1985), glycoproteins (O'Brien et al., 1993), sulphoproteins (Elkington and Fritz, 1980), myo-inositol (Robinson and Fritz, 1979) and sertolin (Li et al., 2014)) that play important roles in germ cell development. Finally, SCs are also responsible for the transport of water, which serves as a vehicle for moving sperm from the testis to the epididymis, from the interstitial space into the lumen (Rato et al., 2010). They also control the seminiferous fluid pH (Oliveira et al., 2009). Without the physical and metabolic support of SCs, meiosis, germ-cell differentiation and trans-formation into spermatozoa will not occur (Sharpe, 1994; Lui et al., 2003).

The metabolic control of SCs has emerged as a hot topic in reproductive biology and much has been discussed about the importance of SC metabolism in germ cells' fate. The maintenance of spermatogenesis is highly dependent on the metabolic cooperation established between SCs and germ cells. For many years, this synergistic relationship has been overlooked, but recent studies have highlighted the relevance of these processes on male fertility. In fact, SCs have been suggested as candidates for an *in vitro* model to study male reproductive toxicology (Reis et al., 2015) or even as a target for male contraception (Alves et al., 2014a). Moreover, compelling evidence has suggested that some effects of various diseases affecting male reproductive potential may be due to alterations in the mechanisms underlying the metabolic cooperation established between SCs and germ cells (Alves et al., 2012a, 2013a,b; Alves and Oliveira, 2013; Rato et al., 2014a,b). However, it is difficult to evaluate the contribution of each hormone or factor to those effects. Most of the studies are performed *in vitro* and allow to evaluate the contribution of different metabolic substrates and metabolic pathways on spermatogenesis. Nevertheless, further *in vivo* studies are needed to consolidate these findings (Petersen and Soder, 2006). In this review, we present and discuss the newest evidence on SC energy metabolism (de)regulation and its hormonal control, with special focus on glucose metabolism.

Sertoli cell metabolism in brief

Junctions between adjacent SCs form the BTB (Rato et al., 2011) and SCs are responsible for the selective passage of substances from blood plasma and testicular lymph to the adluminal compartment (Setchell, 1980). Among those substances secreted and exported by SCs to the intratubular fluid, one must highlight the crucial role of glucose metabolites and metabolic intermediates. The SCs produce high quantities of lactate, which acts as an anti-apoptotic factor (Erkkila et al., 2002) and is the preferred substrate for developing germ cells (Fig. 2) (Boussouar and Benahmed, 2004). The high glycolytic flux of SCs, which ends up in the production of high amounts of lactate, is often compared to what occurs in cancer cells and reported as a "Warburg-like" metabolism (Oliveira et al., 2015a). Glucose enters in the SC through glucose transporters, namely glucose transporter 1 (GLUT1) (Carosa et al., 2005), glucose transporter 2 (GLUT2) (Kokk et al., 2003) and glucose transporter 3 (GLUT3) (Galardo et al., 2008), the isoforms already identified in the plasma membrane of SCs. Then, glucose is converted into pyruvate through glycolysis. During glycolysis, phosphofructokinase (PFK) plays a major role since it catalyses a rate-limiting

step of this pathway and its activity is proposed to be related with the energy status of the cell (Morgante et al., 2011). After glycolysis, pyruvate can be: 1) converted into alanine by alanine aminotransferase; 2) converted into lactate by lactate dehydrogenase (LDH) or 3) be directed to the mitochondria and be converted into acetyl-CoA (Fig. 2). The latter may then enter in the Krebs cycle or be converted into acetate by acetyl-CoA hydrolase (Yamashita et al., 2006). Besides lactate, acetate is also produced at high rates by SCs (Oliveira et al., 2012). Acetate is an intermediate for the synthesis of cholesterol and fatty acids (Yoshimoto et al., 2001) and although its role remains to be fully elucidated, it is suggested that it is used to sustain the high rate of lipids needed by developing germ cells (Alves et al., 2012b).

The preferred pathway by SCs is the conversion of

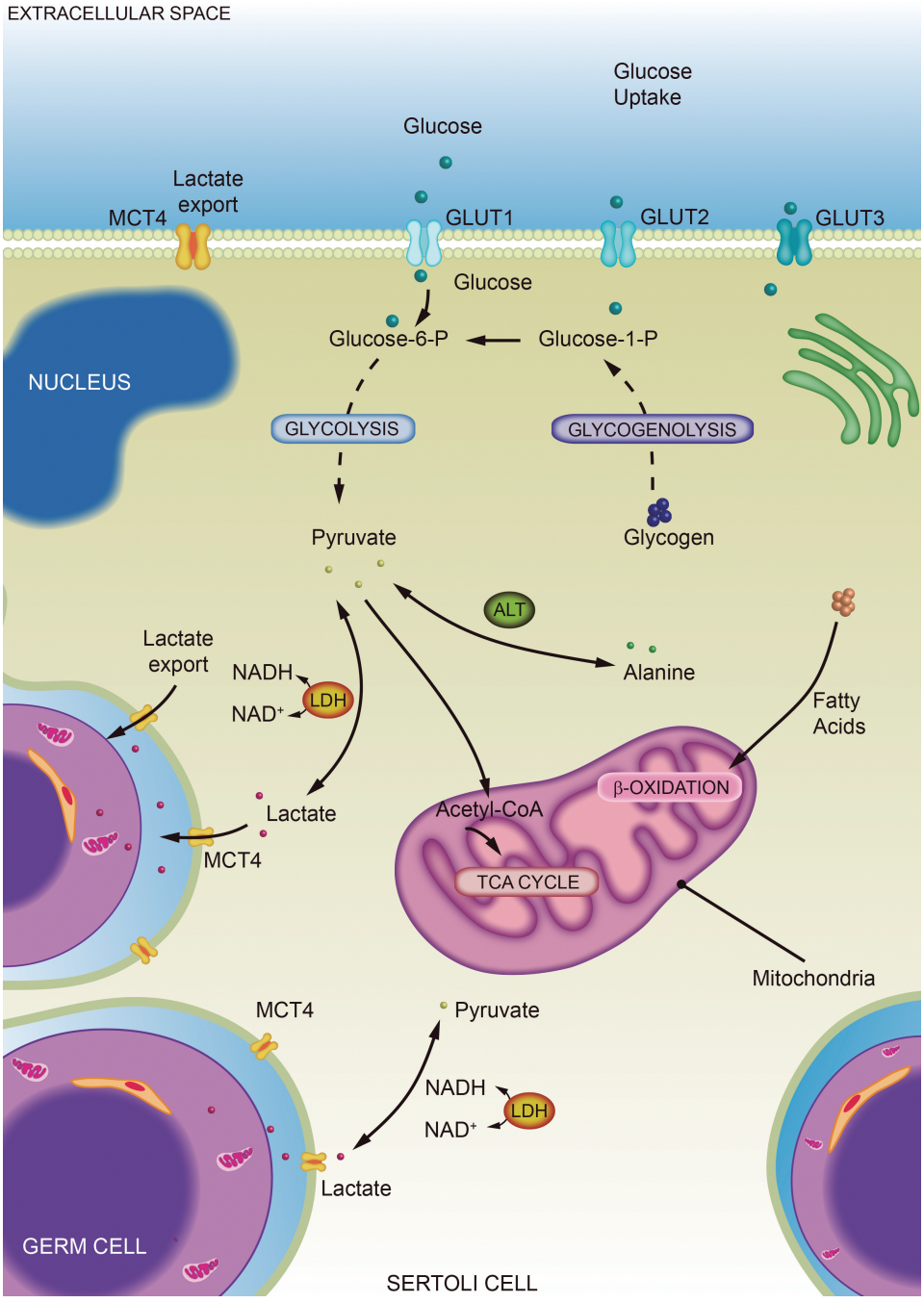

Fig. 2. Schematic illustration of the metabolic cooperation established between the somatic Sertoli cells (SCs) and developing germ cells. The SCs are capable of consuming a variety of fuels (e.g. glucose, lactate and fatty acids). Nevertheless, SCs actively metabolize glucose being the majority of it converted to lactate. The extracellular lactate and pyruvate are transported *via* the family of proton-linked plasma membrane transporters known as 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 to pyruvate which can a) be converted into lactate *via* lactate dehydrogenase (LDH) b) be converted into alanine *via* alanine transaminase or c) be transported to the mitochondrial matrix, oxidized and decarboxylated by pyruvate dehydrogenase, forming Acetyl-CoA, which can enter the TCA cycle. The oxidation of these substrates is coupled with ADP phosphorylation, *via* the electron transport chain to form ATP. Abbreviations: GLUT, glucose transporter; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; MCT, monocarboxylate transporter; TCA, tricarboxylic acid.

pyruvate into lactate. Notably, in the case of glucose or insulin deprivation, SCs are capable of altering their glucose transport system to ensure the appropriate uptake of glucose and, consequently, the adequate levels of lactate production (Riera et al., 2009; Oliveira et al., 2012). Once produced, lactate is exported to the tubular fluid through the monocarboxylate transporter 4 (MCT4) and used by developing germ cells as the main energy source for ATP production.

SCs may use other substrates, besides glucose, as energy sources in order to sustain a high metabolic activity. The branched chain amino acid aminotransferase was already identified in these cells (Kaiser et al., 2005). This enzyme is responsible for the conversion of the branched chain amino acids valine, leucine and isoleucine, to the corresponding branched-chain acids, which provides evidence that SCs are capable of actively metabolize amino acids (Kaiser et al., 2005). Furthermore, SCs accumulate glycogen and present glycogen phosphorylase activity (Leiderman and Mancini, 1969; Slaughter and Means, 1983). Nevertheless, the influence of glycogen metabolism to SCs is probably underestimated. Glycogen is a fuel that can be readily hydrolized when glucose is not available and/or when its metabolism is deficient (Villarroel-Espíndola et al., 2013; Rato et al., 2015b) as observed in diabetic states. It was recently shown that SCs from rats with progressive stages of diabetes reprogram their glycolytic metabolism towards glycogen synthesis (Rato et al., 2015b). This "metabolic shift" was suggested to be a compensatory mechanism to guarantee endogenous energy reserves when SC metabolism is "pressured" by metabolic dysfunctions.

Clearly, SCs show a remarkable metabolic flexibility and use different substrates, a peculiar metabolic feature that is not entirely explained, though it has been attributed to the place and functions that these cells present. The precise contribution of each substrate to SC metabolism remains largely unknown and further studies are necessary to elucidate the real relevance of each for male fertility potential.

Control of carbohydrate metabolism in Sertoli cells

The metabolic cooperation established between SCs and germ cells is regulated by a complex interaction of events that are dependent on the proper functioning of various metabolic pathways. Such molecular events are under the control of factors and hormones that regulate spermatogenesis. Deregulation of these mechanisms may compromise the hormonal control of spermatogenesis and, consequently, male fertility. This is particularly relevant since nowadays men are permanently exposed to a myriad of environmental toxicants. These toxicants can easily reach the testicular environment and may act as endocrine disruptors, interfering with the hormonal control of spermatogenesis and the metabolic cooperation between SCs and developing germ cells. In addition, nutritional disorders, such as overconsumption of high-energy diets, have contributed to the growth of obesity and diabetes epidemics. These metabolic diseases induced by erroneous dietary habits have a negative impact on male fertility, not only through indirect effects mediated by changes in hormonal levels, but also by direct changes in the metabolic cooperation established between testicular cells. In this topic, we will present and discuss the impact of endogenous hormones and factors on SC metabolism and how these cells metabolically adapt under pathological conditions, with special emphasis on diabetes mellitus.

Hormonal regulation of Sertoli cell metabolism

SCs are targeted by gonadotropin FSH and several other hormones, including sex steroids, insulin and leptin (see Table 1). FSH is extremely important for male reproductive potential and particularly for SC physiology. This hormone acts *via* specific G-coupled receptors exclusively located on SCs and stimulates their proliferation, thus contributing to the output of spermatogenic adult male. In mature SCs, FSH regulates their glycolytic metabolism, by increasing glucose uptake (Hall and Mita, 1984) and both pyruvate and lactate production (Jutte et al., 1983; Riera et al., 2001). It was proposed that the high lactate production is due to enhanced LDH activity and increased expression of lactate dehydrogenase A (LDHA) mRNA in SCs (Riera

Table 1. Hormones and endogenous factors that regulate Sertoli cell metabolism and lactate production.

Factor	Protein Levels				Lactate
	GLUT1	GLUT3	MCT4	LDH	production
Follicle-stimulating hormone	+ (a)	/ (a)	nd	+ (b)	+ (a)
Insulin	nd	nd	nd	nd	+ (c)
Insulin growth factor-I	nd	nd	nd	nd	+ (c)
Epidermal growth factor	nd	nd	nd	nd	+ (d)
Paracrine factor P-Mod-S	nd	nd	nd	nd	+ (e)
Triiodothyronine	nd	nd	nd	nd	– (f)
Basic fibroblast growth factor	+ (a, g)	/ (a, g)	nd	+ (g)	+ (a, g)
Cytokines (IL1-β)	+ (a)	/ (a, b)	nd	+ (b)	+ (a)
Arachidonic acid	nd	nd	nd	+ (h)	+ (h)
L-carnitine	+ (i)	nd	nd	+ (j)	+ (i)
AMP-activated protein kinase	+ (k)	– (k)	+ (k)	nd	+ (k)
Leptin	/ (l)	/ (l)	/ (l)	/ (l)	/ (l)
5α-dyhidrotestosterone	nd	– (m)	/ (m, n)	– (m, n)	– (m, n)
DHEA	/ (o)	/ (o)	/ (o)	/ (o)	+ (o)
7-oxo-DHEA	/ (o)	/ (o)	/ (o)	/ (o)	+(o)
17β-estradiol	nd	+ (m)	+ (m)	– (n)	/ (m, n)

+, increase; –, decrease; /, no effect. Abbreviations: DHEA, hidroxicorticosterona; GLUT, glucose transporter; LDH, lactate dehydrogenase; MCT, monocarboxylate transporter; nd, not determined. a- (Galardo et al., 2008); b- (Riera et al., 2001); c- (Oonk et al., 1989); d- (Mallea et al., 1986); e- (Mullaney et al., 1994); f- (Palmero et al., 1995); g- (Riera et al., 2002b); h- (Meroni et al., 2003); i- (Caviglia et al., 2004); j- (Palmero et al., 2000); k- (Galardo et al., 2007); l- (Martins et al., 2015); m- (Oliveira et al., 2011); n- (Rato et al., 2012b); o- (Dias et al., 2015a).

et al., 2001). However, stimulation of the glycolytic pathway was also shown to occur from the interaction between FSH and phosphatidylinositol 3-kinase (PI3K) (Meroni et al., 2002). This molecular mechanism involves the adenylyl cyclase/cAMP pathway, *via* activation of a G-protein. In fact, FSH increases phosphorylated protein kinase B (p-PKB) levels in a PI3K-dependent mechanism (Meroni et al., 2002) promoting the translocation of GLUT1 to plasma membrane (Samih et al., 2000). There are other mechanisms by which FSH stimulates glycolysis. Recently, it was shown that FSH increases the expression of mRNA levels of bifunctional enzyme 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase (PFKFB) isoform 1 (PFKFB1) and 3 (PFKFB3) (Regueira et al., 2015). PFKFB is responsible for the synthesis and degradation of fructose 2,6-biphosphate, which acts as an allosteric activator of PFK. Up-regulation of the expression of different isoforms of PFKFB, in particular PFKFB3, is concurrent with an increased glycolytic flux and high lactate production (Novellasdemunt et al., 2012). The observation that FSH-stimulated lactate production by SCs was inhibited in the presence of a PFKFB3 inhibitor illustrates that FSH is directly involved with this mechanism (Regueira et al., 2015). Apart from the stimulatory effects of FSH on glycolytic enzymes, the high lactate production exhibited by SCs may also result from the reduced conversion of pyruvate to acetyl-coA, due to inhibition of pyruvate dehydrogenase complex (PDC). PDC links glycolysis with Krebs cycle and its activity is tightly regulated by reversible phosphorylation/dephosphorylation reactions (Holness and Sugden, 2003). Phosphorylation of PDC by pyruvate dehydrogenase kinase (PDK) causes inactivation of the complex, which can be reversed by pyruvate dehydrogenase phosphatase (PDP) that removes phosphate from PDC. Regueira and collaborators (Regueira et al., 2015) observed that FSH increased the expression of pyruvate dehydrogenase kinase 3 (PDK3), which is able to phosphorylate PDC. Of note, PDC complex has three specific serine residues that can be phosphorylated and phosphorylation of a single site leads to complete inactivation of this complex (Rardin et al., 2009). So, stimulation of lactate production through inhibition of PDC *via* FSH should not be excluded.

Similarly to FSH, thyroid hormones (TH) are directly involved in testicular development and also in SC proliferation. For instance, triiodothyronine (T3) administration in neonatal animals stopped SC proliferation. It was proposed that TH might regulate this event by mechanisms in which aromatase, 17β-estradiol (E2) and cyclic-dependent kinase inhibitors are involved. However, the involvement of androgens, and probably FSH, in this regulation cannot be excluded, since some studies showed that FSH and T3 induced androgen receptor (AR) expression in immature SCs and suppressed AMH expression (Arambepola et al., 1998a,b). Besides, TH also regulates SC metabolism by stimulating GLUT1 mRNA expression, thus illustrating that TH are able to modulate glucose uptake. In addition, it was observed that administration of physiologic concentrations (1 nM) of T3 in cultured SCs stimulated protein synthesis and lactate production (Palmero et al., 1995), possibly through MCTs, since these transporters showed high affinity for TH (Carosa et al., 2005). However, the mechanisms by which TH control SC metabolism remain to be fully elucidated.

Amongst the peripheral hormones that link cellular metabolism and reproduction, insulin appears as a key regulator of SCs' metabolism. Insulin links whole body metabolism and reproduction *via* HPT-axis and plays an important role within the seminiferous epithelium, since SCs express insulin receptors (Oonk and Grootegoed, 1987). Insulin increases *in vitro* production of pyruvate and lactate (Jutte et al., 1983) probably *via* stimulation of enzymatic activities or by modulation of glucose transport (Oonk et al., 1989). Oliveira and collaborators (2012) showed that glycolytic metabolism of insulin-deprived SCs is seriously impaired, due to a significant decrease in glucose consumption, probably caused by modulation in GLUT1 and GLUT3 transcript levels. Lactate production was also compromised, which was concomitant with a decrease in LDHA and MCT4 expression. However, the effects of insulin deprivation also extend beyond the glycolytic metabolism. As discussed, SCs also produce acetate at high rates and this metabolite is suggested to serve as precursor of lipid synthesis and remodelling for developing germ cells (Oresti et al., 2010). Notably, the production of acetate is totally suppressed under insulin-deprived conditions (Alves et al., 2012b). Insulin deprivation decreases the expression of acetyl-coA hydrolase, which may explain why acetate production is significantly reduced in these conditions (Alves et al., 2012b). These results showed that insulin is a key regulator of SC metabolism, though the mechanisms by which insulin signalling acts in these cells need to be fully elucidated. In this context, Maclean and collaborators (2013) showed that reproductive homeobox 5 of the chromosome X (RHOX5) may act as a key mediator of insulin signalling in SCs. RHOX5 is a transcriptional factor widely expressed in testes, particularly in SCs, being required for insulin signalling, since it directly induces *insulin* 2 gene transcription (Maclean et al., 2013). While direct evidence that insulin promotes the proliferation of male germ cells and SCs remains debatable, the following evidence supports that insulin plays a key role in spermatogenesis: (1) testes provide a constitutive source of insulin for spermatogenesis, while peripheral insulin levels fluctuate in response to diet and the circadian rhythm and (2) SCs secrete insulin to provide adequate levels of this hormone to germ cells that do not have direct access to plasma insulin due to the BTB (Schoeller et al., 2012).

Reproduction and metabolism are two events linked by metabolic signals (for review see (Comninos et al., 2013)), particularly leptin, which appears to influence male reproduction by modulating SC metabolism. Leptin

is a hormone mainly, but not exclusively, secreted by adipose tissue. It was only discovered twenty years ago and many of its functions remain unknown (Halaas et al., 1995). Leptin was first recognized for its role as a satiety factor. It was later found that leptin had a crucial role in glucose homeostasis, after observing that leptin deficient mice were diabetic and obese (Ingalls et al., 1950; Hummel et al., 1966). Moreover, a key feature of this animal model is its infertility, illustrating a crucial role for leptin in reproductive function (Mounzih et al., 1997). Although the role of this hormone in female reproductive function is well established, the available information concerning its role in male reproductive function remains elusive. We have recently shown that human SCs express the leptin receptor and that leptin modulates human SC metabolism (Martins et al., 2015). It was shown that GLUT2 protein levels increased after exposure to the concentrations of leptin known to be present in lean individuals. Besides, the concentrations of leptin found in lean and obese patients increased LDH activity. Importantly, leptin decreased the production of acetate, illustrating that this hormone may be pivotal for acetate metabolism in these cells (Martins et al., 2015). This was a first assessment of the molecular mechanisms by which leptin controls human SC metabolism and illustrates novel insights on obesity-induced male infertility.

Another peripheral hormone that has been recently considered relevant for the metabolic control of SCs is melatonin. This hormone is synthetized *via* serotonin (a neurotransmitter), which has tryptophan as the precursor. This essential amino acid is obtained from the human diet, mainly from meat and turkey, cheese, walnut, sweet cherry, avocado, banana, tomato, soy protein, and bread. Previous reports have highlighted its beneficial effects on male fertility due to its antioxidant properties (Cruz et al., 2014) and the capacity to interact with reproductive axis (for review see (Barrett and Bolborea, 2012)). Furthermore, a role for melatonin on the maintenance of glucose metabolism homeostasis was described in various systems (for review see (Cipolla-Neto et al., 2014)) and the presence of its specific receptors on testicular tissue, particularly in SCs (Rocha et al., 2014) suggested the hormone of "darkness" as regulator of SCs metabolism. We have recently demonstrated that SCs express both melatonin receptors 1A and 1B and that melatonin-exposed SCs express higher GLUT1 protein levels, which were followed by higher glucose consumption (Rocha et al., 2014). Notably, in these conditions, melatonin-exposed SCs did not produce higher amounts of lactate, probably due to a significant decrease in LDH protein expression and activity. Moreover, SCs exposed to melatonin produced and accumulated less acetate than insulin-exposed cells. These findings illustrate that melatonin regulates SC metabolism, and thus may affect spermatogenesis. Interestingly, this pineal hormone cooperates with insulin in the regulation of glucose homeostasis. In fact, SCs exposed to insulin plus melatonin produced more lactate and maintained the protein levels of glycolysis-related enzymes and transporters when compared with SCs cultured in insulin-only or melatonin-only conditions (Rocha et al., 2014). These data suggest that insulin and melatonin, together, may improve spermatogenesis by enhancing the nutritional support of spermatogenesis by SCs. In fact, the antioxidant properties of melatonin have been regularly described to improve male reproductive dysfunctions related to pathological conditions and with the exposure to toxicants (Rocha et al., 2015). However, further studies are needed to explore the role of the synergistic action between these hormones on SC metabolism.

Within seminiferous tubules, SCs are heavily targeted by sex hormones (androgens and estrogens), which are known modulators of SC metabolism. Androgens are particularly important for the initiation and maintenance of spermatogenesis. LCs synthesize the "male hormones", especially T, within the testes through a steroidogenic process where cholesterol acts as precursor. Several steps are involved in the synthesis of T and some of the intermediary androgenic substrates, as is the case of dehydroepiandrosterone (DHEA) and its metabolite 7-oxo-dehydroepiandrosterone (7-oxo-DHEA), are also reported to affect SC metabolism (Dias et al., 2015a). A recent study from our group has shown that DHEA stimulates oxidative metabolism in human SCs without altering its oxidative profile. DHEA increased the glycolytic efficiency of human SCs since it led to an increase in lactate production without altering glucose consumption (Dias et al., 2015a). On the other hand, 7-oxo-DHEA has no relevant androgenic activity by itself, is not convertible to androgens and thus is considered a more potent therapeutic agent than DHEA (Hampl et al., 2003). Although 7-oxo-DHEA has not altered the oxidative profile of human SCs, it was reported as a potent metabolic modulator of these cells. Exposure of human SCs to 7-oxo-DHEA led to an increased glycolytic flux, stimulating not only lactate production, the metabolic fuel of germ cells, but also glucose and pyruvate consumption. Moreover, 7-oxo-DHEA also stimulated the production of acetate by human SCs, which may then be used by germ cells for membrane remodelling (Oliveira et al., 2012; Dias et al., 2015a).

T and its metabolite 5α-dihydrotestosterone (5α-DHT) arise as major modulators of these "nurse cells" metabolism. The metabolic control of SCs by these androgens is mediated, in part, through modulation of glycolysis-related transporters and enzymes, since SCs exposed to 5α-DHT shifted from a Warburg-like metabolism to an oxidative metabolism. In those conditions, SCs presented an increased glucose uptake, which was not followed by an increased production of lactate (Oliveira et al., 2011; Rato et al., 2012b). The increased levels of GLUT3 may explain the high glucose consumption, since according to our previous results (Martins et al., 2013), the expression of GLUT3 protein is several times higher than GLUT1, illustrating a

possible compensatory mechanism through modulation of these transporters. The metabolic reprogramming of SCs may result from the impairment of key mechanisms involved in lactate production, such as decreased lactate synthesis by LDH enzyme and/or decreased lactate transport *via* MCT4, observed in 5α-DHT-exposed SCs. The decrease in lactate production promoted by 5α-DHT-exposed SCs can also be a consequence of a lower cellular conversion of pyruvate to lactate catalysed by LDHA, as the mRNA levels of LDHA were found to be decreased (Oliveira et al., 2011; Rato et al., 2012b; Martins et al., 2013). Of note, results obtained *in vitro* do not exactly mirror physiologic conditions. Nevertheless, these findings reveal that SCs possess a high metabolic flexibility, which is particularly important during conditions that change the metabolic profile of the testes.

Analogously to androgens, it was described that in the lizard *Hemidactylus flaviviridis*, E_2 markedly suppressed lactate production by SCs in a dose- and time-dependent manner (Khan and Rai, 2004). The mechanisms by which estrogens modulate SC metabolism are not fully understood, but it is clear that they compromise the viability of germ cells by suppressing the production of lactate. The role of E_2 in SC metabolism was investigated and it was reported that it promoted the production of high amounts of alanine (Oliveira et al., 2011; Rato et al., 2012b). This is very relevant as the appearance of high alanine content is associated with a reduced redox cytosolic state (Oliveira et al., 2011; Rato et al., 2012b). These *in vitro* observations do not exactly represent an *in vivo* condition, but the isolated effect of E_2 on SC metabolism clearly illustrates that non-physiologic conditions, such as those that induce profound alterations in E_2 concentration, may compromise the normal function of spermatogenesis by targeting SC metabolism.

Altogether, the studies discussed provide clear evidence that SC carbohydrate metabolism, particularly glucose metabolism, is tightly controlled by several hormones, probably due to the place that SCs physically occupy in the seminiferous tubules and the functions they perform. It is pivotal to clarify to what extent hormones affect one of the main functions of SCs: the nutritional support of spermatogenesis.

Paracrine and autocrine regulation of Sertoli cell metabolism

Apart from the endocrine effects exerted by hormones, SCs are also targeted by a myriad of products and sub-products that control their metabolism *via* paracrine and/or autocrine regulation (Table 1). However, few works have explored these mechanisms in detail. The relationship between SCs and germ cells is bidirectional. On one hand, SCs control the fate of germ cell line through their physical and metabolic support. On the other hand, germ cells also influence the function of the somatic testicular cells. The basic fibroblast growth factor (bFGF), which belongs to a large family of peptide growth factors secreted by germ cells (Han et al., 1993), is involved in the functioning and maintenance of spermatogenesis by exerting its effects through specific receptors present in SCs (Han et al., 1993). bFGF controls several SC functions, including regulation of transferrin secretion and stimulation of glucose metabolism through up-regulation of GLUT1 and LDHA transcript levels, along with LDH activity (Riera et al., 2002a). The mechanisms by which bFGF controls glucose transport and lactate production differ. As discussed, the first depends on the PI3K/PKB pathway, while lactate transport is mediated by mitogen-activated protein kinase (MAPK) (Riera et al., 2003). MAPK enhances lactate production by stimulating LDH activity (Riera et al., 2003). However, this pathway may also participate in bFGF-stimulated lactate production resulting from the inhibition of PDC, since SCs exposed to bFGF increased the mRNA levels of all four PDK isoforms present in SCs and phosphorylated levels of PDC without modifying PDP mRNA levels (Regueira et al., 2015).

Epidermal growth factor (EGF) is a cytokine that promotes cell proliferation and controls several cellular functions. Within the seminiferous epithelium, EGF is not only able to stimulate lactate production by SCs through modulation of LDH levels, but also inhibits the aromatization of T (Mallea et al., 1986), though the mechanisms remain to be fully understood. Besides EGF, other cytokines can also function as metabolic modulators, including the multifunctional interleukin-1 (IL-1), which exists in two functional isoforms within testes: IL-1α and IL-1β. IL-1α is produced either by SCs and germ cells. IL-1α favours the expression of LDH and lactate production in cultured SCs (Nehar et al., 1998) although the information regarding the control of glucose uptake is scarce. Conversely, IL-1β stimulates glucose uptake in SCs by increasing GLUT1 transcript levels. This may be a crucial control point of glucose transport, since SCs express GLUT1 and GLUT3, but only GLUT1 was proved to be regulated by IL-1β (Galardo et al., 2008). In addition, IL-1β also enhances lactate production by increasing LDH activity (Riera et al., 2001). The capacity that cytokines show as metabolic regulators is particularly relevant within the seminiferous tubules, since these factors are associated and required in inflammatory and immune responses. Therefore, it is of major importance to understand how these endogenous factors impact spermatogenesis by metabolically modulating SCs in conditions of inflammatory responses or when the immune privilege is lost.

L-carnitine (3-hydroxy-4-N-trimethylaminobutyrate) is present throughout the male reproductive tract (Jeulin and Lewin, 1996) and is also reported as a positive metabolic modulator of SCs. This water soluble quaternary amine is synthesized only in small amounts by the liver, kidney, and brain, having the essential amino acids methionine and lysine as precursors. Thus, most L-carnitine is derived from the human diet,

especially from red meat, fish, and dairy products. L-carnitine functions as an antioxidant and is particularly important in the transport of long-chain fatty acids, such as acyl-carnitines, into the mitochondria. Notably, *in vitro* supplementation with L-carnitine enhanced the production of lactate and pyruvate, the activity of LDH and hexose transport in cultured SCs (Palmero et al., 2000). Arachidonic acid is an essential polyunsaturated omega-6 fatty acid (20:4) abundant in red meat, chicken and eggs. This essential fatty acid was also suggested to regulate lactate production, stimulate glucose uptake and LDH activity, as well as increase LDHA mRNA levels in SCs (Meroni et al., 2003). The paracrine factor peritubular modifying substance (PModS) derived from peritubular cells also plays a relevant role in SC physiology. Two isoforms of PModS have been described, PModS-A and PModS-B, which are structurally and functionally related (Skinner et al., 1988). PModS stimulates SC differentiation, probably by a tyrosine phosphorylation dependent event (Norton et al., 1994) and it also stimulates lactate production at various stages of pubertal development (Mullaney et al., 1994).

The multifaceted roles played by SCs is vital for the success of spermatogenesis. This process is continuum and has high-energy demands, illustrating that the presence of metabolic sensors is essential to make SCs sensitive to energy fluctuations. When energy levels are low, SCs "turn on" energy-producing pathways such as glycolysis and fatty acid oxidation through simple stimulation by a decrease in adenosine triphosphate (ATP). Under energy deficiency, testicular ATP levels significantly decrease and may be used by SCs to form adenosine (Rato et al., 2014b). This sub-product results from ATP degradation and activates the energy sensor 5' adenosine monophosphate-activated protein kinase (AMPK), thereby stimulating glycolysis *via* AMPK. Galardo and collaborators (2010) suggested that activation of AMPK increases glucose transport, probably due to an increase in GLUT1, since AMPK activation down-regulates GLUT3 levels. AMPK also favours lactate production by increasing MCT4 expression while decreasing MCT1 expression. Since MCT4 levels presented much lower affinity for lactate than MCT1 it has been proposed to mainly serve as lactate exporter (Galardo et al., 2007).

Recently, Regueira and collaborators (2014) hypothesized that peroxisome proliferator-activated receptors (PPAR) – α, β/δ and γ might control lactate production in SCs. PPARα, PPARβ/δ and PPARγ are members of the nuclear-hormone receptor superfamily expressed by SCs. PPARα and PPARβ/δ act as catabolic regulators, whereas PPARγ mostly regulates anabolic metabolism. Activation of PPARβ/δ in SCs increased lactate production without being involved in the regulation of glucose transport or lactate production and export. PPARβ/δ leads to a phosphorylation of PDC, which is favoured, by increased levels of several PDK. As a result, the activity of PDC declines, lowering the conversion of acetyl-coA into pyruvate and increasing the availability of pyruvate for lactate conversion (Regueira et al., 2014).

Lipid metabolism in Sertoli cells is under regulation of hormones and endogenous factors

Lipids are important for spermatogenesis since they act as "fuel" for SCs and are required for developing germ cell membrane remodelling. FSH modulates SCs' lipid metabolism by increasing acetate incorporation into SC lipids (Guma et al., 1997). These effects were also mirrored by insulin action and not only increased acetate incorporation, but also ATP citrate lyase activity, highlighting the relevance of insulin on fatty acid synthesis. Insulin signalling is essential for SC metabolism, since its absence shifts SC metabolism from glycolysis to Krebs cycle, which compromises germ cell development (Oliveira et al., 2012). Moreover, as discussed, we reported that human SCs produce high amounts of acetate (Alves et al., 2012b) which is crucial in the synthesis of lipids and phospholipid precursors.

Sex steroids also modulate lipid metabolism of SCs. The effects of 5α-DHT in SC metabolism were similar to those of insulin, though not so pronounced (Oliveira et al., 2011). Conversely, E_2 stimulated acetate production by increasing the expression of acetyl-coA hydrolase transcript levels, thus contributing to the formation of sub-products essential for the maintenance of lipid synthesis. Mature SCs efficiently convert 18 carbon polyunsaturated fatty acids (PUFAs) into 22- and 24-carbon PUFAs and express the enzymes necessary for this metabolic process in high levels, including Δ5 and Δ6 desaturases and fatty acid elongases (Saether et al., 2003). However, PUFA synthesis may be disrupted by hormonal deregulation since cultured SCs exposed to T decreased the activities of both Δ5 and Δ6 desaturases, depressing the steps in which Δ5 and Δ6 desaturases are involved (Carosa et al., 2005). This is of great relevance since decreased activity of Δ5 and Δ6 desaturases potentially limits the incorporation of long chain PUFAs into sperm membranes, which ultimately compromises sperm membrane fluidity and flexibility. PUFAs contain more than one double bond in their backbone and the introduction of more lipids with double bonds enhances the flexibility and fluidity of sperm membrane (Israelachvili et al., 1980). The process of lipid oxidation is also under strict control of PPARα, PPARβ/δ and PPARγ. These transcription factors function as sensors of fatty acids and fatty acid derivatives and thus control the metabolic pathways involved in lipid and energy metabolism. Interestingly, the activation of PPARα and PPARβ/δ increased the expression of fatty acid transporter CD36 in SCs, favouring fatty acid uptake (Regueira et al., 2014). Furthermore, PPAR activation increased phosphorylation of acetyl-coA carboxylase (ACC) and as a result of increased ACC phosphorylation, the enzyme activity declined prompting to fatty acyl-CoA uptake into the mitochondria for subsequent

oxidation, where mRNA levels of L-carnitine palmitoyltransferase 1, long chain and medium chain dehydrogenases enzymes were also up-regulated (Regueira et al., 2014). In summary, PPARα and PPARβ/δ are essential for lipid oxidation in SCs, but the upstream regulation of the PPAR system remains elusive.

Diabetes mellitus and Sertoli cell metabolism: a clear example

Diabetes mellitus (DM) is one of the most prominent public health threats in modern societies and its prevalence is rapidly increasing (American Diabetes Association, 2015). According to the most recent numbers, the global prevalence of DM in men within reproductive age is growing and the number of diabetic patients has reached 3.82 billion worldwide (Scully, 2012). Statistics estimated that in the next 20 years, the world will have nearly 6 billion people suffering from DM (Scully, 2012). The growing epidemics of DM and its associated complications has presented challenges and opportunities, particularly in the field of reproductive biology. Compelling evidence suggests that DM adversely affects male reproductive function at multiple levels (Zitzmann, 2009; Corona et al., 2011; Bernardino et al., 2013; Rato et al., 2013, 2015a). Although a number of human and animal studies have shown an association between diabetic stages and the subsequent reproductive outcomes (Rato et al., 2013, 2014b, 2015a; Dias et al., 2015b), the real impact of DM on male reproductive health is still a matter of debate. Metabolic diseases, such as DM, induce large fluctuations of T and other hormone levels, as well as metabolic factors, directly affecting the function of mature SCs as observed in a decreased expression of mRNA levels of inhibin B and AR in SCs from progressive stages of DM (Rato et al., 2015b). Thus, SC dysfunction is caused by their state of maturation and that may be on the basis of the reproductive problems associated with metabolic diseases. Moreover, it is also expected that the metabolic performance of SCs will be affected.

SCs metabolically respond under T deficiency induced by progressive stages of DM (prediabetes and type 2 diabetes mellitus (T2DM)). Rato and collaborators (2015b) cultured SCs in different concentrations of T, mimicking what happens in progressive states of DM, and observed significant metabolic alterations with the more pronounced effects being concurrent with the lower levels of T. T deficiency associated to different diabetic stages disrupts the metabolism of SCs by compromising rate-limiting steps of glycolysis. However, under severe conditions of DM these somatic cells are still able to adapt their metabolism in order to enhance glucose uptake. Indeed, when exposed to T levels detected in the prodromal stage of DM, SCs were not able to uptake glucose as efficiently as those cells exposed to T2DM-like conditions, although no alterations were observed on GLUT1 and GLUT3 protein levels. SCs exposed to prediabetic conditions preferentially consumed pyruvate, whereas SCs exposed to T2DM-like conditions largely consumed glucose, in a mechanism where GLUT3 plays a key role. Still, SCs cultured under T deficiency levels associated to prediabetic and T2DM-like conditions did not produce higher amounts of lactate, since part of the pyruvate produced/consumed was used to produce alanine. Also, T deficiency induced by the different development stages of DM did not favour the glycolytic flux of SCs (Dias et al., 2015b; Rato et al., 2015a,b). The glucose taken up by SCs was not efficiently converted into lactate, being partly redirected to alternative metabolic pathways, such as glycogen synthesis, in order to ensure endogenous reserves of glucose that allow them to face the energy requirements of germ cells during unfavourable conditions. As discussed, SCs are able to use glycogen since they express enzymes involved in the glycogen metabolism (Leiderman and Mancini, 1969; Slaughter and Means, 1983). The testes of diabetic animals present an increased accumulation of glycogen precursors, such as uridine diphosphate glucose (Spiro, 1984), thus supporting the hypothesis that glycogenesis may be of high relevance within the testicular *milieu*, particularly under diabetic conditions. These data point towards a crucial effect for androgen deficiency (in particular T levels) induced by DM stages in SC glucose metabolism. Although prediabetic and diabetic conditions promote multiple alterations in testicular homeostasis that go far beyond T deficiency, the works discussed show that metabolic alterations on SCs may contribute to the decreased male fertility associated with DM progression. However, the most used drug for the treatment of DM, metformin, may help to protect male reproductive health against the deleterious effects of DM (Ferreira et al., 2015; Meneses et al., 2015a,b). Recent studies highlight that metformin improves male reproductive function and the spermatogenic index of diabetic males (Morgante et al., 2011). Moreover, we have also shown that metformin may be a suitable antidiabetic drug for DM patients at reproductive age, since exposure of rat SCs to this drug led to an increased production of lactate (Alves et al., 2014b), which provides nutritional support and has an anti-apoptotic effect in developing germ cells. The impact of DM in male reproductive potential is still a matter of debate but it is evident that diabetic males face severe fertility problems that may be surpassed or attenuated by antidiabetic drugs, such as metformin. Nevertheless, in the last few years, there is growing evidence that natural products may also be an interesting source of phytochemicals with potential to modulate SC metabolism.

The impact of natural products on Sertoli cell metabolism

There are several natural compounds, often present

in plant-based foods and beverages, which are daily consumed and have been identified for their ability to influence male reproductive function (Nantia et al., 2009; Dias et al., 2014a). Despite the advances in this field in the last years, the understanding of how natural products control male fertility, especially SC metabolism, is still a matter of great interest and needs further research. Many natural compounds have been studied due to their antifertility properties (Akbarsha and Murugaian, 2000; Aladakatti and Ahamed, 2005) since some of them are able to cross BTB causing disruption of SCs or of Sertoli cell–germ cell junctional complexes. However, other natural products show promising properties to improve male fertility (Dias et al., 2014b, 2015b; Martins et al., 2014) and in this context, they have been studied with the purpose of improving SC metabolism. For instance, caffeine is one of the most widely consumed psychoactive substances and is present in several supplements and beverages. The concentrations of caffeine corresponding to low and moderate consumption of caffeine-rich beverages (5 and 50 μM) increased lactate production, but only cells exposed to 50 μM showed increased expression of glucose transporters (Dias et al., 2015b). Therefore, moderate consumption of caffeine seems to be harmless or even beneficial to male reproductive health, since it stimulates lactate production by SCs, which can promote germ cell survival (Dias et al., 2015b). Another example is white tea (*Camellia sinensis L.*) which is ascribed to be rich in phenolic compounds and was reported as a potent antioxidant agent with ameliorating properties relative to DM (Oliveira et al., 2015b) and male fertility (Dias et al., 2014b, 2015b). In fact, rat SCs exposed to a white tea extract, containing high levels of caffeine and catechins, showed decreased glucose consumption due to a decrease in GLUT1 levels. No significant alterations were observed in SCs GLUT3 levels thus evidencing that white tea action on GLUTs is selective and not compensated by GLUT3. Although glucose uptake is significantly decreased, SCs exposed to white tea extract increased lactate production. This was not followed by changes in LDH or MCT4 levels, but a significant increase in LDH activity. Thus, the supplementation with a white tea extract may be helpful to improve male reproductive health (Martins et al., 2014). Daily white tea consumption by prediabetic rats also improved glucose tolerance, insulin sensitivity and prevented testicular oxidative stress, leading to improvement in sperm parameters, namely sperm concentration, motility and viability (Oliveira et al., 2015b). It was also shown that the high antioxidant properties of white tea extracts are determinant for their use as media additive for the preservation of spermatozoa at room temperature (Dias et al., 2014b). These studies indicate that some natural products and phytochemicals usually consumed in western societies may be very valuable to counteract male reproductive tract dysfunctions. The understanding of the molecular mechanisms involved in male fertility dysfunction induced by certain diseases is a good approach to find new possible targets for natural product action as modulators of spermatogenesis.

Conclusion

In recent years, cell metabolism has emerged as a hot topic for research in areas such as cancer and metabolic diseases. However, concerning male fertility, this subject has been overlooked. A successful spermatogenesis is dependent on the metabolic cooperation between SCs and germ cell line. Thus, given the relevance of this process to determining male reproductive potential, it is crucial to disclose and clarify the underlying mechanisms controlling SC metabolism and their interaction with germ cells. Although the effects of several factors and hormones in SCs metabolism are known for many years, new and exciting data are arising. In contrast to what happened before, researchers working in reproductive biology give more detailed attention to the metabolic cooperation established in the testis between SCs and germ cells. Moreover, several metabolic diseases, especially DM, have become a major health problem and have increased to pandemic proportions. Most of these diseases are reported to impair glucose metabolism in SCs and lead to modifications in sperm quality, highlighting the relevance of glucose homeostasis and metabolism in testicular cells. In addition, environmental toxicants have also been shown to disrupt the metabolic cooperation between SCs and germ cells. Deregulation of SC functions impairs male fertility. Thus, it has been highlighted that the *in vitro* study of toxicant impact on SC functions can be a suitable model to evaluate male reproductive toxicology of compounds. Notably, dietary habits may also modulate SC metabolism. Indeed, consumption of some plant-based products has been highlighted due to the beneficial effects on SC glycolytic profile. However, further efforts will be needed to fully understand SCs energy metabolism, its metabolic products and how these cells metabolically adapt to different conditions. Alterations in the mechanisms and signalling events that control SC metabolism will compromise the fate of germ cells and hence spermatogenesis.

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References

Akbarsha M.A. and Murugaian P. (2000). Aspects of the male reproductive toxicity/male antifertility property of andrographolide in

albino rats: Effect on the testis and the cauda epididymidal spermatozoa. Phytother. Res. 14, 432-435.

Aladakatti R.H. and Ahamed R.N. (2005). Changes in Sertoli cells of albino rats induced by azadirachta indica a. Juss leaves. J. Basic Clin. Physiol. Pharmacol. 16, 67-80.

Alves M.G. and Oliveira P.F. (2013). Diabetes mellitus and male reproductive function: Where we stand? Int. J. Diabetol. Vasc. Dis. Res. 1, 1-3.

Alves M.G., Oliveira P.F., Socorro S. and Moreira P.I. (2012a). Impact of diabetes in blood-testis and blood-brain barriers: Resemblances and differences. Curr. Diabetes Rev. 8, 401-412.

Alves M.G., Dias T.R., Silva B.M. and Oliveira P.F. (2014a). Metabolic cooperation in testis as a pharmacological target: From disease to contraception. Curr. Mol. Pharmacol. 7, 83-95.

Alves M.G., Martins A.D., Cavaco J.E., Socorro S. and Oliveira P.F. (2013a). Diabetes, insulin-mediated glucose metabolism and Sertoli/blood-testis barrier function. Tissue Barriers 1, e23992.

Alves M.G., Martins A.D., Rato L., Moreira P.I., Socorro S. and Oliveira P.F. (2013b). Molecular mechanisms beyond glucose transport in diabetes-related male infertility. Biochim. Biophys. Acta Mol. Basis Dis. 1832, 626-635.

Alves M.G., Socorro S., Silva J., Barros A., Sousa M., Cavaco J.E. and Oliveira P.F. (2012b). *In vitro* cultured human Sertoli cells secrete high amounts of acetate that is stimulated by 17beta-estradiol and suppressed by insulin deprivation. Biochim. Biophys. Acta Mol. Cell Res. 1823, 1389-1394.

Alves M.G., Martins A.D., Vaz C.V., Correia S., Moreira P.I., Oliveira P.F. and Socorro S. (2014b). Metformin and male reproduction: Effects on Sertoli cell metabolism. Br. J. Pharmacol. 171, 1033-1042.

American Diabetes Association. (2015). Classification and diagnosis of diabetes. Diabetes Care 38 Suppl, S8-S16.

Arambepola N.K., Bunick D. and Cooke P.S. (1998a). Thyroid hormone effects on androgen receptor messenger rna expression in rat Sertoli and peritubular cells. J. Endocrinol. 156, 43-50.

Arambepola N.K., Bunick D. and Cooke P.S. (1998b). Thyroid hormone and follicle-stimulating hormone regulate mullerian-inhibiting substance messenger ribonucleic acid expression in cultured neonatal rat Sertoli cells. Endocrinology 139, 4489-4495.

Barrett P. and Bolborea M. (2012). Molecular pathways involved in seasonal body weight and reproductive responses governed by melatonin. J. Pineal Res. 52, 376-388.

Bernardino R.L., Martins A.D., Socorro S., Alves M.G. and Oliveira P.F. (2013). Effect of prediabetes on membrane bicarbonate transporters in testis and epididymis. J. Membr. Biol. 246, 877-883.

Boussouar F. and Benahmed M. (2004). Lactate and energy metabolism in male germ cells. Trends Endocrinol. Metab. 15, 345-350.

Carosa E., Radico C., Giansante N., Rossi S., D'Adamo F., Di Stasi S.M., Lenzi A. and Jannini E.A. (2005). Ontogenetic profile and thyroid hormone regulation of type-1 and type-8 glucose transporters in rat Sertoli cells. Int. J. Androl. 28, 99-106.

Catalano S., Rizza P., Gu G., Barone I., Giordano C., Marsico S., Casaburi I., Middea E., Lanzino M., Pellegrino M. and Ando S. (2007). Fas ligand expression in tm4 Sertoli cells is enhanced by estradiol "in situ" production. J. Cell. Physiol. 211, 448-456.

Caviglia D., Scarabelli L. and Palmero S. (2004). Effects of carnitines on rat Sertoli cell protein metabolism. Horm. Metab. Res. 36, 221-225.

Cheng C.Y., Wong E.W., Yan H.H. and Mruk D.D. (2010). Regulation of spermatogenesis in the microenvironment of the seminiferous epithelium: New insights and advances. Mol. Cell. Endocrinol. 315, 49-56.

Chiba H., Osanai M., Murata M., Kojima T. and Sawada N. (2008). Transmembrane proteins of tight junctions. Biochim. Biophys. Acta Biomembranes 1778, 588-600.

Cipolla-Neto J., Amaral F.G., Afeche S.C., Tan D.X. and Reiter R.J. (2014). Melatonin, energy metabolism, and obesity: A review. J. Pineal Res. 56, 371-381.

Comninos A.N., Jayasena C.N. and Dhillo W.S. (2013). The relationship between gut and adipose hormones, and reproduction. Hum. Reprod. Update 20, 153-174.

Corona G., Monami M., Rastrelli G., Aversa A., Sforza A., Lenzi A., Forti G., Mannucci E. and Maggi M. (2011). Type 2 diabetes mellitus and testosterone: A meta-analysis study. Int. J. Androl. 34, 528-540.

Cruz M.H., Leal C.L., da Cruz J.F., Tan D.X. and Reiter R.J. (2014). Role of melatonin on production and preservation of gametes and embryos: A brief review. Anim. Reprod. Sci. 145, 150-160.

Dias T.R., Alves M.G., Oliveira P.F. and Silva B.M. (2014a). Natural products as modulators of spermatogenesis: The search for a male contraceptive. Curr. Mol. Pharmacol. 7, 154-166.

Dias T.R., Alves M.G., Tomas G.D., Socorro S., Silva B.M. and Oliveira P.F. (2014b). White tea as a promising antioxidant medium additive for sperm storage at room temperature: A comparative study with green tea. J. Agric. Food Chem. 62, 608-617.

Dias T.R., Alves M.G., Almeida S.P., Silva J., Barros A., Sousa M., Silva B.M., Silvestre S.M. and Oliveira P.F. (2015a). Dehydroepiandrosterone and 7-oxo-dehydroepiandrosterone in male reproductive health: Implications of differential regulation of human Sertoli cells metabolic profile. J. Steroid Biochem. Mol. Biol. 154, 1-11.

Dias T.R., Alves M.G., Bernardino R.L., Martins A.D., Moreira A.C., Silva J., Barros A., Sousa M., Silva B.M. and Oliveira P.F. (2015b). Dose-dependent effects of caffeine in human Sertoli cells metabolism and oxidative profile: Relevance for male fertility. Toxicology 328, 12-20.

Dym M. and Fawcett D. (1970). The blood-testis barrier in the rat and the physiological compartmentation of the seminiferous epithelium. Biol. Reprod. 3, 308-326.

Elkington J.S. and Fritz I.B. (1980). Regulation of sulfoprotein synthesis by rat Sertoli cells in culture. Endocrinology 107, 970-976.

Erkkila K., Aito H., Aalto K., Pentikainen V. and Dunkel L. (2002). Lactate inhibits germ cell apoptosis in the human testis. Mol. Hum. Reprod. 8, 109-117.

Ferreira C., Sousa M., Rabaca A., Oliveira P.F., Alves M.G. and Sa R. (2015). Impact of metformin on male reproduction. Curr. Pharm. Des. 21, 3621-3633.

Fritz I.B., Rommerts F.G., Louis B.G. and Dorrington J.H. (1976). Regulation by fsh and dibutyryl cyclic amp of the formation of androgen-binding protein in Sertoli cell-enriched cultures. J. Reprod. Fertil. 46, 17-24.

Galardo M.N., Riera M.F., Pellizzari E.H., Cigorraga S.B. and Meroni S.B. (2007). The amp-activated protein kinase activator, 5-aminoimidazole-4-carboxamide-1-b-d-ribonucleoside, regulates lactate production in rat Sertoli cells. J. Mol. Endocrinol. 39, 279-288.

Galardo M.N., Riera M.F., Pellizzari E.H., Chemes H.E., Venara M.C., Cigorraga S.B. and Meroni S.B. (2008). Regulation of expression of Sertoli cell glucose transporters 1 and 3 by fsh, il1 , and bfgf at two

different time-points in pubertal development. Cell Tissue Res. 334, 295-304.

Galardo M.N., Riera M.F., Pellizzari E.H., Sobarzo C., Scarcelli R., Denduchis B., Lustig L., Cigorraga S.B. and Meroni S.B. (2010). Adenosine regulates Sertoli cell function by activating ampk. Mol. Cell. Endocrinol. 330, 49-58.

Griswold M.D. (1998). The central role of Sertoli cells in spermatogenesis. Semin. Cell Dev. Biol. 9, 411-416.

Guma F.C., Wagner M., Martini L.H. and Bernard E.A. (1997). Effect of fsh and insulin on lipogenesis in cultures of Sertoli cells from immature rats. Braz. J. Med. Biol. Res. 30, 591-597.

Hadley M.A., Byers S.W., Suarez-Quian C.A., Kleinman H.K. and Dym M. (1985). Extracellular matrix regulates Sertoli cell differentiation, testicular cord formation, and germ cell development *in vitro*. J. Cell Biol. 101, 1511-1522.

Halaas J.L., Gajiwala K.S., Maffei M., Cohen S.L., Chait B.T., Rabinowitz D., Lallone R.L., Burley S.K. and Friedman J.M. (1995). Weight-reducing effects of the plasma protein encoded by the obese gene. Science 269, 543-546.

Hall P.F. and Mita M. (1984). Influence of follicle-stimulating hormone on glucose transport by cultured Sertoli cells. Biol. Reprod. 31, 863-869.

Hampl R., Pohanka M., Hill M. and Stárka L. (2003). The content of four immunomodulatory steroids and major androgens in human semen. J. Steroid Biochem. Mol. Biol. 84, 307-316.

Han I.S., Sylvester S.R., Kim K.H., Schelling M.E., Venkateswaran S., Blanckaert V.D., McGuinness M.P. and Griswold M.D. (1993). Basic fibroblast growth factor is a testicular germ cell product which may regulate Sertoli cell function. Mol. Endocrinol. 7, 889-897.

Holness M.J. and Sugden M.C. (2003). Regulation of pyruvate dehydrogenase complex activity by reversible phosphorylation. Biochem. Soc. Trans. 31, 1143-1151.

Hummel K.P., Dickie M.M. and Coleman D.L. (1966). Diabetes, a new mutation in the mouse. Science 153, 1127-1128.

Ingalls A.M., Dickie M.M. and Snell G.D. (1950). Obese, a new mutation in the house mouse. J. Hered. 41, 317-318.

Israelachvili J.N., Marcelja S. and Horn R.G. (1980). Physical principles of membrane organization. Q. Rev. Biophys. 13, 121-200.

Jeulin C. and Lewin L.M. (1996). Role of free l-carnitine and acetyl-l-carnitine in post-gonadal maturation of mammalian spermatozoa. Hum. Reprod. Update 2, 87-102.

Jutte N.H., Jansen R., Grootegoed J.A., Rommerts F.F. and van der Molen H.J. (1983). Fsh stimulation of the production of pyruvate and lactate by rat Sertoli cells may be involved in hormonal regulation of spermatogenesis. J. Reprod. Fertil. 68, 219-226.

Kaiser G.R.R.F., Monteiro S.C., Gelain D.P., Souza L.F., Perry M.L.S. and Bernard E.A. (2005). Metabolism of amino acids by cultured rat Sertoli cells. Metabolism. 54, 515-521.

Khan U.W. and Rai U. (2004). *In vitro* effect of fsh and testosterone on Sertoli cell nursing function in wall lizard *hemidactylus flaviviridis* (ruppell). Gen. Comp. Endocrinol. 136, 225-231.

Kokk K., Veräjänkorva E., Wu X.-K., Tapfer H., Põldoja E. and Pöllänen P. (2003). Immunohistochemical detection of glucose transporters class i subfamily in the mouse, rat and human testis. Med. Lith. 40, 156-160.

Leiderman B. and Mancini R.E. (1969). Glycogen content in the rat testis from postnatal to adult ages. Endocrinology 85, 607-609.

Li M.W., Cheng C.Y. and Mruk D.D. (2014). Sertolin mediates blood-testis barrier restructuring. Endocrinology 155, 1520-1531.

Lui W.Y., Mruk D., Lee W.M. and Cheng C.Y. (2003). Sertoli cell tight junction dynamics: Their regulation during spermatogenesis. Biol. Reprod. 68, 1087-1097.

Maclean J.A., Hu Z., Welborn J.P., Song H.W., Rao M.K., Wayne C.M. and Wilkinson M.F. (2013). The rhox homeodomain proteins regulate the expression of insulin and other metabolic regulators in the testis. J. Biol. Chem. 288, 34809-34825.

Mallea L.E., Machado A.J., Navaroli F. and Rommerts F.F. (1986). Epidermal growth factor stimulates lactate production and inhibits aromatization in cultured Sertoli cells from immature rats. Int. J. Androl. 9, 201-208.

Martins A.D., Alves M.G., Bernardino R.L., Dias T.R., Silva B.M. and Oliveira P.F. (2014). Effect of white tea (*camellia sinensis* (l.)) extract in the glycolytic profile of Sertoli cell. Eur. J. Nutr. 53, 1383-1391.

Martins A.D., Alves M.G., Simoes V.L., Dias T.R., Rato L., Moreira P.I., Socorro S., Cavaco J.E. and Oliveira P.F. (2013). Control of Sertoli cell metabolism by sex steroid hormones is mediated through modulation in glycolysis-related transporters and enzymes. Cell Tissue Res. 354, 861-868.

Martins A.D., Moreira A.C., Sá R., Monteiro M.P., Sousa M., Carvalho R.A., Silva B.M., Oliveira P.F. and Alves M.G. (2015). Leptin modulates human Sertoli cells acetate production and glycolytic profile: A novel mechanism of obesity-induced male infertility? Biochim. Biophys. Acta Mol. Basis Dis. 1852, 1824-1832.

Marzowski J., Sylvester S.R., Gilmont R.R. and Griswold M.D. (1985). Isolation and characterization of Sertoli cell plasma membranes and associated plasminogen activator activity. Biol. Reprod. 32, 1237-1245.

Meneses M.J., Sousa M., Alves M.G. and Oliveira P.F. (2015a). The antidiabetic drug metformin and male reproductive function: An overview. Int. J. Diabetol. Vasc. Dis. Res. 3, 1-2.

Meneses M.J., Silva B.M., Sousa M., Sa R., Oliveira P.F. and Alves M.G. (2015b). Antidiabetic drugs: Mechanisms of action and potential outcomes on cellular metabolism. Curr. Pharm. Des. 21, 3606-3620.

Meroni S.B., Riera M.F., Pellizzari E.H. and Cigorraga S.B. (2002). Regulation of rat Sertoli cell function by fsh: Possible role of phosphatidylinositol 3-kinase/protein kinase b pathway. J. Endocrinol. 174, 195-204.

Meroni S.B., Riera M.F., Pellizzari E.H., Schteingart H.F. and Cigorraga S.B. (2003). Possible role of arachidonic acid in the regulation of lactate production in rat Sertoli cells. Int. J. Androl. 26, 310-317.

Morgante G., Tosti C., Orvieto R., Musacchio M.C., Piomboni P. and De Leo V. (2011). Metformin improves semen characteristics of oligo-terato-asthenozoospermic men with metabolic syndrome. Fertil. Steril. 95, 2150-2152.

Mounzih K., Lu R. and Chehab F.F. (1997). Leptin treatment rescues the sterility of genetically obese ob/ob males. Endocrinology 138, 1190-1193.

Mruk D.D. and Cheng C.Y. (2004). Sertoli-Sertoli and Sertoli-germ cell interactions and their significance in germ cell movement in the seminiferous epithelium during spermatogenesis. Endocr. Rev. 25, 747-806.

Mullaney B.P., Rosselli M. and Skinner M.K. (1994). Developmental regulation of Sertoli cell lactate production by hormones and the testicular paracrine factor, pmods. Mol. Cell. Endocrinol. 104, 67-73.

Nantia E.A., Moundipa P.F., Monsees T.K. and Carreau S. (2009). Medicinal plants as potential male anti-infertility agents: A review. Andrologie 19, 148-158.

Nehar D., Mauduit C., Boussouar F. and Benahmed M. (1998). Interleukin 1alpha stimulates lactate dehydrogenase a expression and lactate production in cultured porcine Sertoli cells. Biol. Reprod. 59, 1425-1432.

Norton J.N., Vigne J.L. and Skinner M.K. (1994). Regulation of Sertoli cell differentiation by the testicular paracrine factor pmods: Analysis of common signal transduction pathways. Endocrinology 134, 149-157.

Novellasdemunt L., Obach M., Millan-Arino L., Manzano A., Ventura F., Rosa J.L., Jordan A., Navarro-Sabate A. and Bartrons R. (2012). Progestins activate 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3 (pfkfb3) in breast cancer cells. Biochem. J. 442, 345-356.

O'Brien D.A., Gabel C.A. and Eddy E.M. (1993). Mouse Sertoli cells secrete mannose 6-phosphate containing glycoproteins that are endocytosed by spermatogenic cells. Biol. Reprod. 49, 1055-1065.

Oliveira P.F., Sousa M., Barros A., Moura T. and Rebelo da Costa A. (2009). Membrane transporters and cytoplasmatic ph regulation on bovine Sertoli cells. J. Membr. Biol. 227, 49-55.

Oliveira P.F., Alves M.G., Rato L., Silva J., Sa R., Barros A., Sousa M., Carvalho R.A., Cavaco J.E. and Socorro S. (2011). Influence of 5alpha-dihydrotestosterone and 17beta-estradiol on human Sertoli cells metabolism. Int. J. Androl. 34, e612-620.

Oliveira P.F., Alves M.G., Rato L., Laurentino S., Silva J., Sa R., Barros A., Sousa M., Carvalho R.A., Cavaco J.E. and Socorro S. (2012). Effect of insulin deprivation on metabolism and metabolism-associated gene transcript levels of *in vitro* cultured human Sertoli cells. Biochim. Biophys. Acta Gen. Subjects 1820, 84-89.

Oliveira P.F., Martins A.D., Moreira A.C., Cheng C.Y. and Alves M.G. (2015a). The warburg effect revisited—lesson from the Sertoli cell. Med. Res. Rev. 35, 126-151.

Oliveira P.F., Tomás G.D., Dias T.R., Martins A.D., Rato L., Alves M.G. and Silva B.M. (2015b). White tea consumption restores sperm quality in prediabetic rats preventing testicular oxidative damage. Reprod. Biomed. Online 31, 544-556.

Oonk R.B. and Grootegoed J.A. (1987). Identification of insulin receptors on rat Sertoli cells. Mol. Cell. Endocrinol. 49, 51-62.

Oonk R.B., Jansen R. and Grootegoed J.A. (1989). 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. 139, 210-218.

Oresti G.M., Reyes J.G., Luquez J.M., Osses N., Furland N.E. and Aveldano M.I. (2010). Differentiation-related changes in lipid classes with long-chain and very long-chain polyenoic fatty acids in rat spermatogenic cells. J. Lipid Res. 51, 2909-2921.

Palmero S., Prati M., Bolla F. and Fugassa E. (1995). Tri-iodothyronine directly affects rat Sertoli cell proliferation and differentiation. J. Endocrinol. 145, 355-362.

Palmero S., Bottazzi C., Costa M., Leone M. and Fugassa E. (2000). Metabolic effects of l-carnitine on prepubertal rat Sertoli cells. Horm. Metab. Res. 32, 87-90.

Petersen C. and Soder O. (2006). The Sertoli cell-a hormonal target and'super'nurse for germ cells that determines testicular size. Horm. Res. 66, 153-161.

Rardin M.J., Wiley S.E., Naviaux R.K., Murphy A.N. and Dixon J.E. (2009). Monitoring phosphorylation of the pyruvate dehydrogenase complex. Anal. Biochem. 389, 157-164.

Rato L., Socorro S., Cavaco J.E. and Oliveira P.F. (2010). Tubular fluid secretion in the seminiferous epithelium: Ion transporters and aquaporins in Sertoli cells. J. Membr. Biol. 236, 215-224.

Rato L., Alves M.G., Socorro S., Cavaco J.E. and Oliveira P.F. (2011). Blood testis barrier: How does the seminiferous epithelium feed the developing germ cells. Endothelium and Epithelium: Composition, Functions and Pathology. Nova Biomedical. New York, NY. pp 137-155.

Rato L., Alves M.G., Socorro S., Duarte A.I., Cavaco J.E. and Oliveira P.F. (2012a). Metabolic regulation is important for spermatogenesis. Nat. Rev. Urol. 9, 330-338.

Rato L., Alves M.G., Socorro S., Carvalho R.A., Cavaco J.E. and Oliveira P.F. (2012b). Metabolic modulation induced by estradiol and dht in immature rat Sertoli cells cultured *in vitro*. Biosci. Rep. 32, 61-69.

Rato L., Alves M.G., Dias T.R., Lopes G., Cavaco J.E., Socorro S. and Oliveira P.F. (2013). High-energy diets may induce a pre-diabetic state altering testicular glycolytic metabolic profile and male reproductive parameters. Andrology 1, 495-504.

Rato L., Alves M.G., Cavaco J.E. and Oliveira P.F. (2014a). High-energy diets: A threat for male fertility? Obes. Rev. 15, 996-1007.

Rato L., Duarte A.I., Tomas G.D., Santos M.S., Moreira P.I., Socorro S., Cavaco J.E., Alves M.G. and Oliveira P.F. (2014b). Pre-diabetes alters testicular pgc-1alpha/sirt3 axis modulating mitochondrial bioenergetics and oxidative stress. Biochim. Biophys. Acta Bioenergetics 1837, 335-344.

Rato L., Alves M.G., Dias T.R., Cavaco J.E. and Oliveira P.F. (2015a). Testicular metabolic reprogramming in neonatal streptozotocin-induced type 2 diabetic rats impairs glycolytic flux and promotes glycogen synthesis. J. Diabetes Res. 2015, 13.

Rato L., Alves M.G., Duarte A.I., Santos M.S., Moreira P.I., Cavaco J.E. and Oliveira P.F. (2015b). Testosterone deficiency induced by progressive stages of diabetes mellitus impairs glucose metabolism and favors glycogenesis in mature rat Sertoli cells. Int. J. Biochem. Cell Biol. 66, 1-10.

Regueira M., Riera M.F., Galardo M.N., Pellizzari E.H., Cigorraga S.B. and Meroni S.B. (2014). Activation of ppar alpha and ppar beta/delta regulates Sertoli cell metabolism. Mol. Cell. Endocrinol. 382, 271-281.

Regueira M., Artagaveytia S.L., Galardo M.N., Pellizzari E.H., Cigorraga S.B., Meroni S.B. and Riera M.F. (2015). Novel molecular mechanisms involved in hormonal regulation of lactate production in Sertoli cells. Reproduction 150, 311-321.

Reis M.M.S., Moreira A.C., Sousa M., Mathur P.P., Oliveira P.F. and Alves M.G. (2015). Sertoli cell as a model in male reproductive toxicology: Advantages and disadvantages. J. Appl. Toxicol. 35, 870-883.

Riera M.F., Meroni S.B., Pellizzari E.H. and Cigorraga S.B. (2003). Assessment of the roles of mitogen-activated protein kinase and phosphatidyl inositol 3-kinase/protein kinase b pathways in the basic fibroblast growth factor regulation of Sertoli cell function. J. Mol. Endocrinol. 31, 279-289.

Riera M.F., Meroni S.B., Schteingart H.F., Pellizzari E.H. and Cigorraga S.B. (2002a). 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. 173, 335-343.

Riera M.F., Meroni S.B., Schteingart H.F., Pellizzari E.H. and Cigorraga S.B. (2002b). 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. 173, 335-343.

Riera M.F., Galardo M.N., Pellizzari E.H., Meroni S.B. and Cigorraga S.B. (2009). Molecular mechanisms involved in Sertoli cell adaptation to glucose deprivation. Am. J. Physiol. Endocrinol. Metab. 297, E907-914.

Riera M.F., Meroni S.B., Gomez G.E., Schteingart H.F., Pellizzari E.H. and Cigorraga S.B. (2001). Regulation of lactate production by fsh, il1beta, and tnfalpha in rat Sertoli cells. Gen. Comp. Endocrinol. 122, 88-97.

Robinson R. and Fritz I.B. (1979). Myoinositol biosynthesis by Sertoli cells, and levels of myoinositol biosynthetic enzymes in testis and epididymis. Can. J. Biochem. 57, 962-967.

Rocha C.S., Rato L., Martins A.D., Alves M.G. and Oliveira P.F. (2015). Melatonin and male reproductive health: Relevance of darkness and antioxidant properties. Curr. Mol. Med. 15, 299-311.

Rocha C.S., Martins A.D., Rato L., Silva B.M., Oliveira P.F. and Alves M.G. (2014). Melatonin alters the glycolytic profile of Sertoli cells: Implications for male fertility. Mol. Hum. Reprod. 15, 299-311.

Saether T., Tran T.N., Rootwelt H., Christophersen B.O. and Haugen T.B. (2003). Expression and regulation of delta5-desaturase, delta6-desaturase, stearoyl-coenzyme a (coa) desaturase 1, and stearoyl-coa desaturase 2 in rat testis. Biol. Reprod. 69, 117-124.

Samih N., Hovsepian S., Aouani A., Lombardo D. and Fayet G. (2000). Glut-1 translocation in frtl-5 thyroid cells: Role of phosphatidylinositol 3-kinase and n-glycosylation. Endocrinology 141, 4146-4155.

Schoeller E.L., Albanna G., Frolova A.I. and Moley K.H. (2012). Insulin rescues impaired spermatogenesis *via* the hypothalamic-pituitary-gonadal axis in akita diabetic mice and restores male fertility. Diabetes 61, 1869-1878.

Scully T. (2012). Diabetes in numbers. Nature 485, S2-3.

Setchell B.P. (1980). The functional significance of the blood-testis barrier. J. Androl. 1, 3-10.

Sharpe R.M. (1994). Regulation of spermatogenesis. In: The physiology of reproduction, Knobill E. N.J. (ed). Raven Press Ltd. New York. pp 1363-1434.

Sharpe R.M., McKinnell C., Kivlin C. and Fisher J.S. (2003). Proliferation and functional maturation of Sertoli cells, and their relevance to disorders of testis function in adulthood. Reproduction 125, 769-784.

Shubhada S., Glinz M. and Lamb D.J. (1993). Sertoli cell secreted growth factor. Cellular origin, paracrine and endocrine regulation of secretion. J. Androl. 14, 99-109.

Skinner M.K. and Griswold M.D. (1980). Sertoli cells synthesize and secrete transferrin-like protein. J. Biol. Chem. 255, 9523-9525.

Skinner M.K., Fetterolf P.M. and Anthony C.T. (1988). Purification of a paracrine factor, p-mod-s, produced by testicular peritubular cells that modulates Sertoli cell function. J. Biol. Chem. 263, 2884-2890.

Slaughter G.R. and Means A.R. (1983). Follicle-stimulating hormone activation of glycogen phosphorylase in the Sertoli cell-enriched rat testis. Endocrinology 113, 1476-1485.

Spiro M.J. (1984). Effect of diabetes on the sugar nucleotides in several tissues of the rat. Diabetologia 26, 70-75.

Villarroel-Espíndola F., Maldonado R., Mancilla H., vander Stelt K., Acuña A.I., Covarrubias A., López C., Angulo C., Castro M.A., Carlos Slebe J., Durán J., García-Rocha M., Guinovart J.J. and Concha I.I. (2013). Muscle glycogen synthase isoform is responsible for testicular glycogen synthesis: Glycogen overproduction induces apoptosis in male germ cells. J. Cell. Biochem. 114, 1653-1664.

Walker W.H. and Cheng J. (2005). FSH and testosterone signaling in Sertoli cells. Reproduction 130, 15-28.

Yamashita H., Itsuki A., Kimoto M., Hiemori M. and Tsuji H. (2006). Acetate generation in rat liver mitochondria; acetyl-coa hydrolase activity is demonstrated by 3-ketoacyl-coa thiolase. Biochim. Biophys. Acta 1761, 17-23.

Yoshimoto M., Waki A., Yonekura Y., Sadato N., Murata T., Omata N., Takahashi N., Welch M.J. and Fujibayashi Y. (2001). Characterization of acetate metabolism in tumor cells in relation to cell proliferation: Acetate metabolism in tumor cells. Nucl. Med. Biol. 28, 117-122.

Zitzmann M. (2009). Testosterone deficiency, insulin resistance and the metabolic syndrome. Nat. Rev. Endocrinol. 5, 673-681.

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