Cell and Tissue Research (2019) 378:333–339
https://doi.org/10.1007/s00441-019-03028-4

REGULAR ARTICLE

Knockout of MCT1 results in total absence of spermatozoa, sex hormones dysregulation, and morphological alterations in the testicular tissue

Raquel L. Bernardino[1] · Warren N. D'Souza[2] · Luis Rato[3] · Jay L. Rothstein[2] · Tânia R. Dias[1,3,4] · Daniel Chui[2] · Sharon Wannberg[2] · Marco G. Alves[1] · Pedro F. Oliveira[1,5,6]

Received: 6 December 2018 / Accepted: 4 April 2019 / Published online: 9 May 2019

Abstract

Lactate is a key metabolite for the normal occurrence of spermatogenesis. In the testis, lactate is produced by the Sertoli cells and transported to germline cells. Monocarboxylate transporters (MCTs) are key players in that process. Among the family of MCTs, MCT1 is at least partly responsible for lactate uptake by the germ cells. We aimed to perform a first assessment of the role of MCT1 in male reproductive potential. Mct1 conditional knockout (cKO) mice were used for morphometric evaluation, testicular morphology, and sperm parameter assessment. Serum steroid hormones levels were also measured. cKO animals showed a decrease in gonadosomatic index, testis weight, and seminiferous tubular diameters. Deletion of MCT1 also causes morphological changes in the organization of the seminiferous tubules and on Sertoli cell morphology. These changes resulted in failure of spermatogenesis with depletion of germ cells and total absence of spermatozoa. MCT1 cKO animals presented also hormonal dysregulation, with a decrease in serum 17β-estradiol levels. In conclusion, MCT1 is pivotal for male reproductive potential. Absence of MCT1 results in maintenance of undifferentiated spermatogonia pool and compromised sperm production.

Keywords Lactate · Male fertility · MCT1 · Seminiferous tubules · Spermatozoa

Introduction

The prevalence of infertility has been increasing worldwide. This global health issue is reflected by the decline in total fertility rates in developed countries, even with the growing recurrence to assisted reproductive technologies (Agarwal et al. 2015). Although infertility has a multifactorial and complex etiology, the male factor is still highly unexplored and poorly understood. It was estimated that 2 to 12% of men worldwide are infertile (Martin et al. 2007; Agarwal et al. 2015; Kumar and Singh 2015). Recent data highlighted that the increase in male factor infertility may be related to a 50–60% decline in sperm counts over the last four decades (Levine et al. 2017). In addition, this is affecting more young men within reproductive ages with approximately 1 in 20 young men presenting decreased sperm counts (Barratt et al. 2018). Among other factors, current lifestyle factors have been associated with a negative impact on spermatogenesis and sperm quality (Sharpe 2010; Rato et al. 2013).

The metabolic cooperation established between Sertoli and germ cells is pivotal for spermatogenesis, as the former provide the nutritional support required by the latter during their development. That process is mainly mediated through glucose metabolism that is essential for the normal development of spermatogenesis (Dias et al. 2013). Sertoli cells, the somatic cells of seminiferous tubules, take up glucose from the extracellular compartment by the glucose transporters (GLUTs),

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00441-019-03028-4) contains supplementary material, which is available to authorized users.

✉ Pedro F. Oliveira
pfobox@gmail.com

[1] Department of Microscopy, Laboratory of Cell Biology, Institute of Biomedical Sciences Abel Salazar (ICBAS) and Multidisciplinary Unit for Biomedical Research (UMIB), University of Porto, Rua de Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal

[2] Amgen Inc., Thousand Oaks, CA, USA

[3] University of Beira Interior, Covilhã, Portugal

[4] LAQV/REQUIMTE - Laboratory of Bromatology and Hydrology, Faculty of Pharmacy, University of Porto, Porto, Portugal

[5] Department of Genetics, Faculty of Medicine, University of Porto, Porto, Portugal

[6] i3S-Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal

and metabolize it to pyruvate in a process known as glycolysis. The majority of pyruvate is then converted by lactate dehydrogenase (LDH) into lactate (Oliveira et al. 2015). This lactate produced by Sertoli cells will be then used as metabolic substrate for developing germ cells. However, different developmental stages of germ cells have different metabolic needs. While spermatogonia and spermatozoa present glycolytic capacity using glucose as main energy substrate (Grootegoed and Den Boer 1990; Boussouar and Benahmed 2004), the post-meiotic germ cells (spermatocytes and spermatids) are dependent on lactate as they do not possess the machinery to metabolize glucose.

The uptake of lactate by germ cells is controlled by facilitated-diffusion through monocarboxylate transporters (MCTs). There are several isoforms of MCTs expressed by testicular cells. MCT1 is express in spermatozoa tail, spermatids, and Sertoli cells (Brauchi et al. 2005; Galardo et al. 2007; Oliveira et al. 2011; Mannowetz et al. 2012). MCT1 is also present in Leydig cells, which are responsible for the production of androgens in the testis (Nakai et al. 2006). These studies highlight the ubiquitous expression of MCT1 among testicular cells, which may indicate an important role in male fertility potential. However, its role in male reproductive function is not fully elucidated. Herein, we propose to study the relevance of MCT1 on testicular environment, spermatogenesis, and sperm parameters.

Material and methods

Animals

Twelve male mice (C57Bl/6) were housed in our accredited animal facilities and maintained in type III-H cages (Tecniplast, Italy) with food and water ad libitum, at a constant room temperature (20 ± 2 °C), on a 12-h cycle of artificial lighting and noise level (< 55 dB). All procedures involving animals were performed according to the "Guide for the Care and Use of Laboratory Animals" published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and the European directives for the care and handling of laboratory animals (Directive 2010/63/EU). In accordance with the Portuguese law (ordinance no. 1005/92 of 23 October), the research team requested a permission to perform this animal experimentation study to the Portuguese Veterinarian and Food Department (ordinance no. 1005/92 of 23 October). The animals were euthanized at 5 months of age.

Mct1 conditional knockout mice generation

Briefly, Mct1 conditional knockout (cKO) mice were created by targeting C57Bl/6 embryonic stem (ES) cells (The Jackson Laboratory) with a targeting vector containing genomic Mct1 exon 4 flanked by loxP sites inserted within upstream and downstream intronic sequences, followed by upstream and downstream arms of homology and a Neomycin selectable cassette flanked by FRT sites. Correctly targeted ES cells were confirmed by PCR and genomic southern and microinjected into albino C57Bl/6 (Taconic) blastocyst to generate chimeric mice. Germ line transmission of the conditionally targeted Mct1 allele as well as removal of any selectable markers flanked by FRT sites was accomplished through breeding with systemic FLP expressing transgenic mice (The Jackson Labs). Homozygous Mct1 cKO mice were created by the breeding of heterozygous Mct1 cKO mice that lacked the FLP transgene and selectable markers. Further breeding to tissue-specific Cre driver mice will generate conditionally knock out Mct1 tissue, with the use of tamoxifen, because of Cre recombinase its tamoxifen-dependent activity. Cre fused to mutated hormone-binding domains of the estrogen receptor. The Cre is inactive but can be activated by the synthetic estrogen receptor ligand 4-hydroxytamoxifen, therefore allowing for external temporal control of Cre activity.

Tamoxifen preparation and administration

Sunflower oil (T-8267, Sigma, Germany) was sterilized and used as vehicle. Tamoxifen (Tm, T5648, Sigma, Germany) was dissolved in ethanol (200 mg/mL) and supplemented with sunflower oil at a concentration of 20 mg/mL. The solution was placed on a tube rotator for 4 h at 4 °C to form an emulsion. The rats were weighted and an intraperitoneal injection (IP) of Tm was given in a final concentration of 100 mg/kg. Mice were daily injected with 100 mg/kg of Tm for 5 consecutive days after completing 80 days of age.

Epididymal sperm parameters

After the animals' sacrifice, testes and one epididymis of each animal were immediately collected. The assessment of epididymal sperm parameters was performed as previously described by Rato and collaborators (Rato et al. 2013). Briefly, epididymis was placed in pre-warmed (37 °C) HBSS (pH 7.4) solution, minced with a scalpel blade, and incubated for 5 min (37 °C) to allow spermatozoa dispersion. Sperm motility was evaluated by spreading a drop of sperm suspension in a pre-warmed slide (37 °C). Motile sperm percentage was assessed in 10 random fields, using an optical microscope (×100 magnification), and the average value was used as the total sperm motility. Epididymal sperm concentration was determined using a Neubauer counting chamber. Sperm cells were counted under an optical microscope (×100 magnification).

Sperm viability was assessed by eosin-nigrosin staining technique. A total of 333 spermatozoa were counted in random fields under a bright-field microscope. Dead spermatozoa stained pink, as the loss of membrane integrity allows the cells to take up eosin, whereas live cells appear white. Nigrosin stains the background in a dark violet color for a better visualization of the cells.

Sperm morphology was evaluated in Diff-Quik (Baxter Dale Diagnostics AG, Dubinger, Switzerland) stained smears according to manufacturers' instructions. A total of 333 spermatozoa were evaluated, in random fields. To be classified as normal, a sperm cell must have a hook-shaped head and no defects of head, neck, or tail. Otherwise, spermatozoa were considered abnormal.

Histology parameters

The right testis was placed in Davidson's modified fixative for 48 h. Then, the tissue processing was as following: 70% ethanol for 5 min, 90% ethanol for 30 min, 95% ethanol for 30 min, three changes of 100% ethanol for 30 min each, one change of xylene for 30 min and another for 5 min, two changes of 60 °C paraffin for 1 h each, and finally placed in a paraffin block. The tissue paraffin blocks were sectioned at 4 μm, and the resultant sections were stained with hematoxylin and eosin, and mounted with Coverquick 2000 on microscope slides (Cardiff et al. 2014). The staining cross sections with hematoxylin and eosin were used to measure the average diameter of the seminiferous tubules and morphometric analysis.

Determination of testosterone and 17β-estradiol levels in serum

Serum was collected right after animals' sacrifice. Testosterone and 17β-estradiol (E_2) levels were determined using commercial kits, namely, the Mouse Testosterone ELISA Kit (Crystal Chem, Elk Grove Village, MI, USA) and the Estradiol ELISA Kit (Cayman Chemical Company, Ann Arbor, MI, USA) according to manufacturer's instructions. The ELISA kits used had detection limits of approximately 25 ng/mL (for testosterone) and 4 ng/mL (for estradiol).

Statistical analysis

Experimental data are shown as mean ± SEM ($n = 6$ for each condition). Statistical analysis was performed by one-way ANOVA using GraphPad Prism 6 (GraphPad Software, San Diego, USA). Results with $p < 0.05$ were considered significant.

Results

Mct1 conditional knockout mice present abnormal testicular histological architecture with disrupted spermatogenesis

Body weight was not significantly different between wild-type (WT) (30.9 ± 1.43 g) and cKO mice (28.8 ± 0.14 g). However, there was a decrease in the testicular weight of cKO mice which resulted in a decrease in the gonadosomatic index in cKO animals (0.46 ± 0.05) relative to WT group (0.76 ± 0.05) (Table 1).

Testicular tissue of Mct1 cKO mice presented altered histological architecture when compared with WT mice. Figure 1a shows a cross section of WT mice testis, in which it is possible to observe a regular morphology and the presence of spermatozoa in the lumen of the seminiferous tubules. On the other hand, the cKO mice showed abnormal morphological features including Sertoli cell vacuolization and germ cell degeneration and disorganization. In addition, the amount of spermatozoa in the lumen of these mice seminiferous tubules is very low or absent, which denotes a compromised spermatogenesis (Fig. 1b).

The diameter of the seminiferous tubules was measured by microscopic analysis of the histological sections. The average diameter of the seminiferous tubules of WT mice was 199 ± 4 μm. cKO mice showed a significant decrease of about 30% in the diameter of the seminiferous tubules (140.9 ± 2.1 μm) (Table 1).

The reproductive parameters of Mct1 conditional knockout mice were severely compromised

To assess the sperm quality of cKO mice, we tried to evaluate the following parameters: concentration, motility, viability, and morphology (Table 2). Nevertheless, it was not possible to evaluate any of these parameters in the cKO mice, since the number of spermatozoa was either insufficient to be analyzed or completely absent.

17β-estradiol serum levels were decreased in Mct1 conditional knockout mice

Mct1 cKO mice presented a significant lower concentration of 17β-estradiol in the serum (21.95 ± 9.68 pmol/L) when compared with the levels detected in the serum of WT mice (49.14 ± 5.10 pmol/L). However, the levels of testosterone detected in the serum of WT and cKO mice did not present significant alterations, being 1358 ± 37.2 pmol/L and 1845 ± 351 pmol/L, respectively (Fig. 2).

Table 1 Average values of the body weight, testicular weight, gonadosomatic index, and seminiferous tubules diameter in wild-type (WT) and Mct1 conditional knockout (cKO) mice

Morphometric parameters	WT mice	cKO mice	*p* value
Body weight (g)	30.9 ± 1.43	28.8 ± 0.14	0.91
Right testis weight (g)	0.11 ± 0.005*	0.07 ± 0.006	0.0061
Left testis weight (g)	0.11 ± 0.01*	0.06 ± 0.01	0.011
Gonadosomatic index	0.76 ± 0.05*	0.46 ± 0.05	0.021
Seminiferous tubular diameter (μm)	199.4 ± 4.4*	140.9 ± 2.1	0.0079

*Significant difference relative to cKO mice

Discussion

The production of lactate by Sertoli cells is crucial to the development and differentiation of germ cells into fully competent spermatozoa (Erkkilä et al. 2002). Thus, it is essential to unveil the mechanisms by which this metabolite is transported to germ cells controlling spermatogenesis. Lactate is exported by Sertoli cells to the extracellular compartment where it will be taken up by germ cells through MCTs. These transporters are relevant to the establishment of a metabolic cooperation between different mammalian cell types (Poole and Halestrap 1993). Among the different isoforms of MCTs, MCT1, MCT2, and MCT4 seem to have an important role in the transport of lactate within testis (Brauchi et al. 2005; Oliveira et al. 2011; Mannowetz et al. 2012). Although MCT4 has a low affinity for lactate, its role in lactate export by mammalian Sertoli cells was highlighted in several studies (Bonen 2001; Brauchi et al. 2005; Galardo et al. 2007; Oliveira et al. 2012; Rato et al. 2012). MCT2 is known to have a high affinity for lactate, but its expression was only described in round spermatids (Brauchi et al. 2005), while MCT1 is a medium-affinity isoform that is expressed in Sertoli, Leydig, and germ cells. Thus, there is evidence that MCT1 is the most relevant regulator of lactate efflux/influx in testicular cells (Draoui and Feron 2011; Halestrap and Price 1999; Draoui and Feron 2011). In this study, it was observed that MCT1 is of extreme relevance for the sustainability of male fertility. The absence of MCT1 in cKO animals resulted in spermatogenesis failure, as observed by the almost inexistent spermatozoa presence. The failure in sperm production can be explained by defects on lactate transport and uptake through MCT1 in testicular cells. Although this metabolite can still be produced and excreted into the intratubular fluid by MCT4, it has a lower affinity for lactate than MCT1, thus compromising lactate availability for germ cells. In addition, MCT1 absence will lead to a lower uptake of lactate by lactate-dependent germ cells. In fact, pachytene spermatids only express MCT1 and MCT4, while round spermatids also express MCT2. Without MCT1, lactate uptake is inefficient, resulting in spermatogenesis arrest. Lactate is also an anti-apoptotic agent for germ cells, especially spermatocytes and spermatids (Erkkilä et al. 2002), which may also explain why only very few cells were able to differentiate into spermatozoa. cKO mice

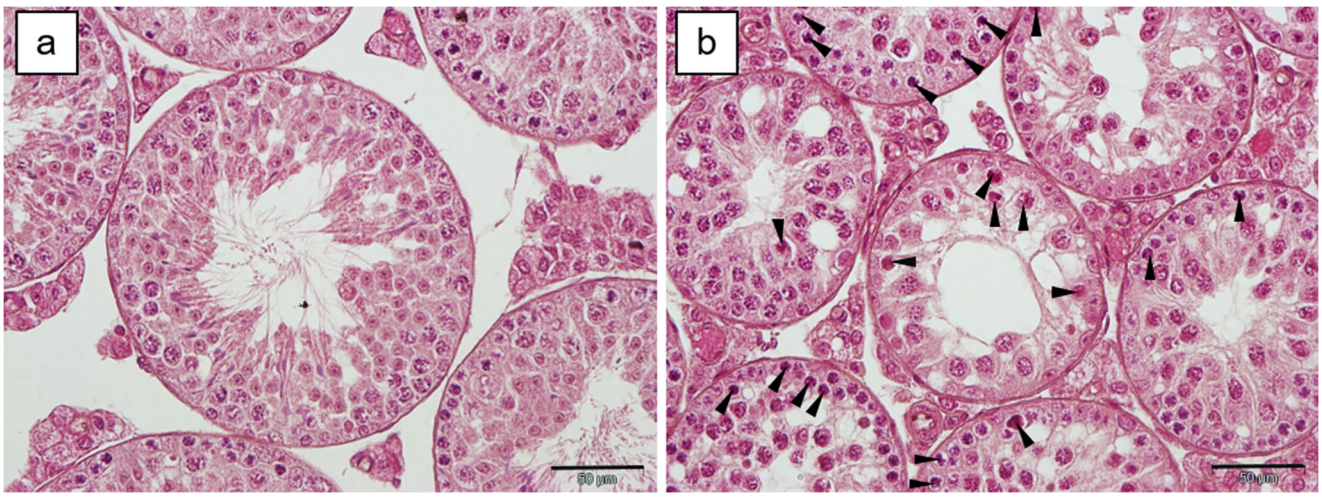

Fig. 1 Histological structure of mouse testis as assessed in paraffin sections. Hematoxylin and eosin staining. **a** Wild-type mice: seminiferous tubules have normal shape and arrangement of Sertoli cells. Visible spermatozoa in lumen of seminiferous tubule. **b** Mct1 conditional knockout mice: Sertoli cells have irregular morphology, with the presence of vacuoles. Germ-line cell shortages, with absence of spermatozoa in the lumen of semiferous tubules. Arrows show cells with apoptotic morphology. Bar scale: 50 μm

Table 2 Sperm concentration, motility, viability, and morphology of wild-type (WT) and Mct1 conditional knockout (cKO) mice

Reproductive parameters	WT mice	cKO mice
Sperm concentration (cell ml^{-1})	$3.5 \times 10^7 \pm 2.3 \times 10^6$*	0[a]
Sperm motility (%)	78.3 ± 2.7*	0[a]
Sperm viability (%)	30.6 ± 3.0*	0[a]
Sperm morphology (% abnormal sperm)	18.89 ± 0.9*	0[a]

*Significant difference relative to cKO mice

[a] Insufficient/absent spermatozoa to conduct the analysis

presented atypical morphological features including marked Sertoli cell cytoplasmic vacuolization and germ cell degeneration. The nature and the mechanisms of Sertoli cell vacuolization are not clearly understood. Some authors have described that vacuoles created in Sertoli cell cytoplasm as the spaces remaining after germ cell degeneration (Russell et al. 1993), which may also be happening with these animals, since we observed degeneration of germ cells. Eid and collaborators (Eid et al. 2012) showed that the vacuoles formed in the cytoplasm of the Sertoli cells may be of autophagic nature, sometimes in response to some toxic agent or some change in the equilibrium state.

Spermatogenesis is highly regulated by hormones, mostly testosterone and estradiol. In the testis, Leydig cells are the main producers of testosterone. Although these cells also express MCT1, cKO mice presented a normal testosterone production illustrating that MCT1 may have a secondary role in Leydig cells as MCT1 absence did not have a major impact in the normal function of these cells. Testosterone can be irreversibly converted to E2 by the enzyme cytochrome P450 aromatase (Lambard et al. 2005). It was described that spermatogonia, spermatocytes, spermatids, and spermatozoa are an important source of estrogens due to the expression of cytochrome P450 aromatase (Nitta et al. 1993). We were able to detect a decrease in the serum concentration of E2 in MCT1 cKO animals. However, germ cell aromatase may not be enough to change E2 concentration in serum. In adult testis, germ and Sertoli cells present lower expression of aromatase, unlike the Leydig cells, which present greater expression of this enzyme (Lardone et al. 2017). cKO mice may have altered aromatase expression in Leydig cells, causing lowered E2 concentration in serum. Also, factors secreted by Sertoli cells affect aromatase activity of Leydig cells (Carreau et al. 1988). Since we observed an adverse effect of MCT1 absence in Sertoli cells, it is quite likely that Leydig cell aromatase activity is affected due to loss of paracrine control and thus affects the production of E2. Estrogens are essential for male reproduction through the modulation of several important regulators (Bernardino et al. 2015; Bernardino et al. 2016). In addition, estrogens are also a survival factor for germ cells (Ebling et al. 2000; Correia et al. 2014, 2015).

In conclusion, MCT1 is crucial for the normal occurrence of spermatogenesis and for testicular integrity of mice. Animals with the absence of MCT1 presented spermatogenesis arrest and compromised fertility. This study highlights the possible essential role for MCT1 in mammalian spermatogenesis in a first attempt to report some of the molecular mechanisms that may mediate those effects. Nevertheless, further studies are needed to unveil the relevance of MCT1 in male reproduction.

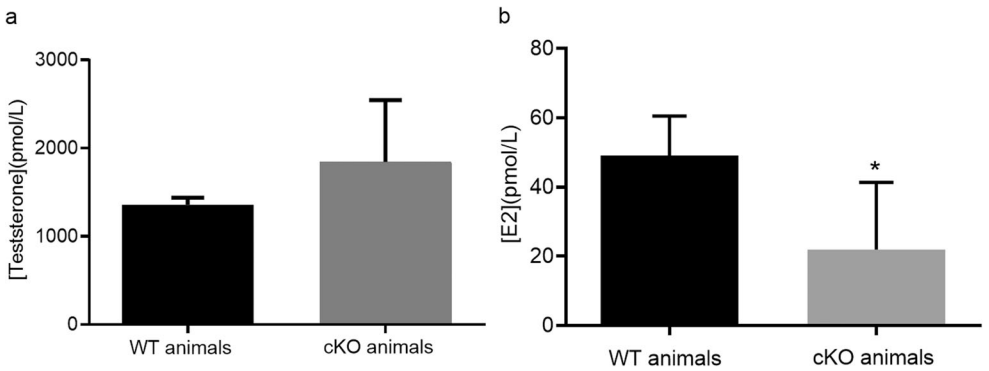

Fig. 2 Hormonal measurements in serum. **a** Testosterone levels in serum of wild-type (WT) and Mct1 conditional knockout (cKO) mice. **b** The 17β-estradiol (E_2) levels in serum of WT and cKO mice. Results are expressed as mean ± SEM ($n = 6$ for each condition). *Significantly different relative to WT mice ($p < 0.05$)

Acknowledgments The authors are thankful to Ângela Alves for the technical support.

Funding information This work was supported by "Fundação para a Ciência e a Tecnologia" (FCT) to Raquel L. Bernardino (SFRH/BD/103105/2014) and Tânia R. Dias (SFRH/BD/109284/2015). The work was co-funded by FEDER through the COMPETE/QREN, FSE/POPH to Marco G. Alves (PTDC/BIM-MET/4712/2014 and PTDC/MEC-AND/28691/2017); Pedro F. Oliveira (PTDC/BBB-BQB/1368/2014); UMIB (PEst-OE/SAU/UI0215/2014); co-funded by the EU Framework Programme for Research and Innovation H2020 (POCI/COMPETE2020).

Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. This article does not contain any studies with human participants performed by any of the authors.

Informed consent Not applicable.

References

Agarwal A, Mulgund A, Hamada A, Chyatte MR (2015) A unique view on male infertility around the globe. Reprod Biol Endocrinol 13:37

Barratt CL, De Jonge CJ, Sharpe RM (2018) Man up': the importance and strategy for placing male reproductive health centre stage in the political and research agenda. Hum Reprod 33:541–545

Bernardino RL, Martins AD, Jesus TT, Sá R, Sousa M, Alves MG, Oliveira PF (2015) Estrogenic regulation of bicarbonate transporters from SLC4 family in rat Sertoli cells. Mol Cell Biochem 408:47–54

Bernardino RL, Costa AR, Martins AD, Silva J, Barros A, Sousa M, Sá R, Alves MG, Oliveira PF (2016) Estradiol modulates Na^+-dependent HCO_3^- transporters altering intracellular pH and ion transport in human Sertoli cells: a role on male fertility? Biol Cell 108:179–188

Bonen A (2001) The expression of lactate transporters (MCT1 and MCT4) in heart and muscle. Eur J Appl Physiol 86:6–11

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

Brauchi S, Rauch MC, Alfaro IE, Cea C, Concha II, Benos DJ, Reyes JG (2005) Kinetics, molecular basis, and differentiation of L-lactate transport in spermatogenic cells. Am J Physiol Cell Physiol 288: C523–C534

Cardiff RD, Miller CH, Munn RJ (2014) Manual hematoxylin and eosin staining of mouse tissue sections. Cold Spring Harb Protoc 2014: 655–658

Carreau S, Papadopoulos V, Drosdowsky M (1988) Stimulation of adult rat Leydig cell aromatase activity by a Sertoli cell factor. Endocrinology 122:1103–1109

Correia S, Alves MR, Martins AD, Cavaco JE, Oliveira PF, Socorro S (2014) Effect of 17 [beta]-estradiol on germ cell apoptosis by modulating the expression of SCF/c-kit, FasL/FasR and regucalcin. Endocr Abstr 35:P702

Correia S, Cardoso HJ, Cavaco JE, Socorro S (2015) Oestrogens as apoptosis regulators in mammalian testis: angels or devils? Expert Rev Mol Med 17:e2

Dias TR, Martins AD, Reis VP, Socorro S, Silva BM, Alves MG, Oliveira PF (2013) Glucose transport and metabolism in Sertoli cell: relevance for male fertility. Curr Chem Biol 7:282–293

Draoui N, Feron O (2011) Lactate shuttles at a glance: from physiological paradigms to anti-cancer treatments. Dis Model Mech 4:727–732

Ebling FJ, Brooks AN, Cronin AS, Ford H, Kerr JB (2000) Estrogenic induction of spermatogenesis in the hypogonadal mouse. Endocrinology 141:2861–2869

Eid N, Ito Y, Otsuki Y (2012) Enhanced mitophagy in Sertoli cells of ethanol-treated rats: morphological evidence and clinical relevance. J Mol Histol 43:71–80

Erkkilä K, Aito H, Aalto K, Pentikäinen V, Dunkel L (2002) Lactate inhibits germ cell apoptosis in the human testis. Mol Hum Reprod 8:109–117

Galardo MN, Riera MF, Pellizzari EH, Cigorraga SB, Meroni SB (2007) The AMP-activated protein kinase activator, 5-aminoimidazole-4-carboxamide-1-bD-ribonucleoside, regulates lactate production in rat Sertoli cells. J Mol Endocrinol 39:279–288

Grootegoed J, Den Boer P (1990) Energy metabolism of spermatids: a review. In: Hamilton DW, Waites GMH (eds) WHO Special Programme of Research, Development and Research Training in Human Reproduction & Family Health International. Cellular and molecular events in spermiogenesis: proceedings of a symposium held at Oaxtepec, Mexico, 11–13 March 1987 / edited by D. W. Hamilton, G. M. H. Waites. University Press, Cambridge

Halestrap AP, Price NT (1999) The proton-linked monocarboxylate transporter (MCT) family: structure, function and regulation. Biochem J 343:281

Kumar N, Singh AK (2015) Trends of male factor infertility, an important cause of infertility: a review of literature. J Hum Reprod Sci 8:191–196

Lambard S, Silandre D, Delalande C, Denis-Galeraud I, Bourguiba S, Carreau S (2005) Aromatase in testis: expression and role in male reproduction. J Steroid Biochem Mol Biol 95:63–69

Lardone M, Argandona F, Florez M, Parada-Bustamante A, Ebensperger M, Palma C, Piottante A, Castro A (2017) Overexpression of CYP19A1 aromatase in L eydig cells is associated with steroidogenic dysfunction in subjects with Sertoli cell-only syndrome. Andrology 5:41–48

Levine H, Jørgensen N, Martino-Andrade A, Mendiola J, Weksler-Derri D, Mindlis I, Pinotti R, Swan SH (2017) Temporal trends in sperm count: a systematic review and meta-regression analysis. Hum Reprod Update 23:646–659

Mannowetz N, Wandernoth P, Wennemuth G (2012) Basigin interacts with both MCT1 and MCT2 in murine spermatozoa. J Cell Physiol 227:2154–2162

Martin JA, Hamilton BE, Sutton PD, Ventura SJ, Menacker F, Kirmeyer S, Munson ML (2007) Births: final data for 2005. Natl Vital Stat Rep 56:1–103

Nakai M, Chen L, Nowak RA (2006) Tissue distribution of basigin and monocarboxylate transporter 1 in the adult male mouse: a study using the wild-type and basigin gene knockout mice. Anat Rec A Discov Mol Cell Evol Biol 288:527–535

Nitta H, Bunick D, Hess R, Janulis L, Newton SC, Millette CF, Osawa Y, Shizuta Y, Toda K, Bahr JM (1993) Germ cells of the mouse testis express P450 aromatase. Endocrinology 132:1396–1401

Oliveira P, Alves M, Rato L, Silva J, Sa R, Barros A, Sousa M, Carvalho R, Cavaco J, Socorro S (2011) Influence of 5α-dihydrotestosterone and 17β-estradiol on human Sertoli cells metabolism. Int J Androl 34:612–620

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

Oliveira PF, Martins AD, Moreira AC, Cheng CY, Alves MG (2015) The Warburg effect revisited—lesson from the Sertoli cell. Med Res Rev 35:126–151

Poole RC, Halestrap AP (1993) Transport of lactate and other monocarboxylates across mammalian plasma membranes. Am J Phys Cell Phys 264:C761–C782

Rato L, Alves MG, Socorro S, Carvalho RA, Cavaco JE, Oliveira PF (2012) Metabolic modulation induced by oestradiol and DHT in immature rat Sertoli cells cultured in vitro. Biosci Rep 32:61–69

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

Russell LD, Ettlin RA, Hikim APS, Clegg ED (1993) Histological and histopathological evaluation of the testis. Int J Androl 16:83–83

Sharpe RM (2010) Environmental/lifestyle effects on spermatogenesis. Philos Trans R Soc Lond Ser B Biol Sci 365:1697–1712