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Energy metabolism in mammalian sperm motility

Alexandra Amaral

Department of Developmental Genetics, Max Planck Institute for Molecular Genetics, Berlin, Germany

Correspondence
Alexandra Amaral, Department of Developmental Genetics, Max Planck Institute for Molecular Genetics, Ihnestraße 63-73, 14195 Berlin, Germany.
Email: amaral@molgen.mpg.de

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Abstract

Mammalian sperm, the only cells that achieve their purpose outside their organism of origin, have to swim vigorously within the female reproductive tract to reach an oocyte. Flagellar dyneins drive sperm motility, which accounts for the consumption of high amounts of ATP. The two main ATP-producing metabolic pathways are compartmentalized in sperm: oxidative phosphorylation in the midpiece and glycolysis in the principal piece. The relative preponderance of these pathways has been discussed for decades (the so-called *sperm energy debate*). The debate has been muddled by species-specific variances and by technical constraints. But recent findings suggest that sperm from most mammalian species employ a versatile metabolic strategy to maintain motility according to the physiological environment. Different metabolic pathways likely coordinate by using exogenous and/or endogenous substrates in order to produce ATP efficiently. Defects in any of these pathways (glycolysis, mitochondrial oxidative phosphorylation, Krebs cycle, fatty acids oxidation, and ketone bodies oxidation, among others) may disturb sperm motility and be at the origin of male infertility. Understanding sperm bioenergetics is thus crucial for building new diagnostic tools, and for the development of treatments for patients presenting with low sperm motility. Some of these patients may benefit from personalized metabolic supplementations and dietary interventions.

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KEYWORDS

male infertility, mammalian sperm, sperm energy debate, sperm metabolism, sperm motility

1 | INTRODUCTION

Mammalian sperm, whose purpose is to eventually fertilize an oocyte, are the products of spermatogenesis, a differentiation process occurring in the seminiferous tubules within the testes (Clermont, 1972; Nishimura & L'Hernault, 2017). Spermatogenesis entails several genomic, epigenomic, metabolic, and morphological transformations. In the end, the male gamete is a compartmentalized, haploid cell, devoid of most cytoplasm and constituted by two main compartments: head and tail. The head comprises a highly compacted genome (which renders the cell almost silent in terms of

transcription and translation; Amaral & Ramalho-Santos, 2013) and the acrosome, a cap-like secretory vesicle that assists sperm penetration through the oocyte's vestments (Buffone et al., 2014). The tail or flagellum, with a 9 + 2 microtubules arrangement, is responsible for sperm motility (Lindemann & Lesich, 2016), a prerequisite for in vivo fertilization. The axoneme is surrounded by outer dense fibers, which are wrapped by the mitochondrial sheath in the midpiece, and the fibrous sheath in the principal piece. The mitochondrial sheath contains a number of peculiar mitochondria tethered to each other through intermitochondrial linkers and anchored to the cytoskeleton through ordered protein arrays on the outer mitochondrial membrane (Leung et al., 2021).

Upon spermiation (i.e., the release of sperm from the seminiferous epithelium) sperm are unable to fertilize an oocyte. During transit in the epididymis, proteins, lipids, and sugars derived from the epididymal lumen and from epididymal extracellular vesicles (epididymosomes) are incorporated in sperm, while posttranslational modifications of endogenous proteins occur (Barrachina et al., 2022; Breton et al., 2019). These molecular alterations induce a series of biochemical and physiological changes, including the activation of specific signaling pathways (Gervasi & Visconti, 2017) that prime the ability of sperm to move progressively in the female reproductive tract or in physiological media. Motility is propelled by the coordinated sliding of microtubule doublets and relies on the activity of dynein ATPases along the flagellum (Lin & NiCastro, 2018). Complex upstream regulatory events, involving calcium (Ca^{2+}) and cyclic adenosine monophosphate-dependent protein kinase signaling orchestrate sperm motility (Vyklicka & Lishko, 2020). Besides the alterations induced by the epididymis, sperm fertilization competence requires a second maturation step occurring in the female reproductive tract (but that can also be in vitro induced), dubbed capacitation (Gervasi & Visconti, 2016). This involves, among other physiological modifications, tyrosine phosphorylation of numerous proteins, and motility hyperactivation, that is, the instigation of an extremely vigorous motility pattern with high-amplitude asymmetrical flagellar beating (Suarez, 2008). Capacitated sperm are able to undergo the acrosome reaction, a Ca^{2+}-regulated exocytotic event that enables sperm to penetrate the zona pellucida and fuse with the oocyte plasma membrane (Hirohashi & Yanagimachi, 2018). Sperm movement, capacitation, and acrosome reaction are high energy-consuming cellular events. In order to carry out these (and other) physiological functions, sperm require remarkably more adenosine triphosphate (ATP) than other cells (Garrett et al., 2008; Miki, 2007). Energy metabolism is thus a key factor in sperm function.

This review will mainly focus on the catabolic pathways supporting mammalian sperm motility, an ability that accounts for the consumption of massive amounts of ATP. Sperm ATP content correlates with motility and swimming velocity, as exemplified by studies comparing rodent species with different levels of sperm competition (Tourmente et al., 2013). Species with higher sperm competition seem to be able to sustain larger amounts of intracellular ATP and to use ATP more efficiently, which translates in faster sperm speeds (Tourmente et al., 2019; Tourmente, Villar-Moya, Varea-Sanchez, et al., 2015). A clear understanding of the sperm metabolic pathways generating ATP is thus paramount to comprehend motility regulation. Because sperm motility defects are at the origin of several male infertility cases, sperm bioenergetics research is anticipated to prompt translational applications.

2 | SPERM: A CELL WITH PECULIAR BIOENERGETICS FEATURES

To meet their energetic demands after ejaculation, sperm can import exogenous substrates and/or use endogenous sources. Two main cellular compartmentalized metabolic pathways produce most of the sperm ATP: glycolysis in the principal piece, and oxidative phosphorylation (OXPHOS) in the midpiece (Visconti, 2012; Figure 1a). The former is faster and produces ATP in the subcellular compartment where it is most needed to fuel motility, but is less efficient in terms of net number of ATP molecules formed. The latter is more efficient, but depends on oxygen (O_2) availability, and generates ATP that has to be exported from the midpiece to the whole tail.

The available O_2 and exogenous substrates are not constant as sperm travel throughout the female reproductive tract, where semen and female fluids mix. Seminal fluid has high amounts of fructose and citrate, together with glucose, lactate, and free amino acids (Owen & Katz, 2005). Vaginal fluid contains glucose, lactate, and glycerol (Owen & Katz, 1999) and uterine and oviductal fluids contain glucose, pyruvate, and lactate in variable amounts, which depend on the stage of the female reproductive cycle (Table 1). Oxygen tensions also fluctuate throughout the female reproductive tract and during the cycle, both in species with menstrual cycles and in species with estrous cycles (Table 1 and references therein).

Beyond being polarized cells, with very high-energy demands, which are exposed to variable extracellular environments and have to compete with each other to achieve their aim, sperm cells use a singular proteome. This includes a number of isozymes and enzymes with unique properties (Figure 1b,c and references therein) that are expressed in the late (haploid) stages of spermatogenesis, presumably to improve the bioenergetics performance of the male gamete. All these peculiar bioenergetics features have to be considered in any sperm bioenergetics study.

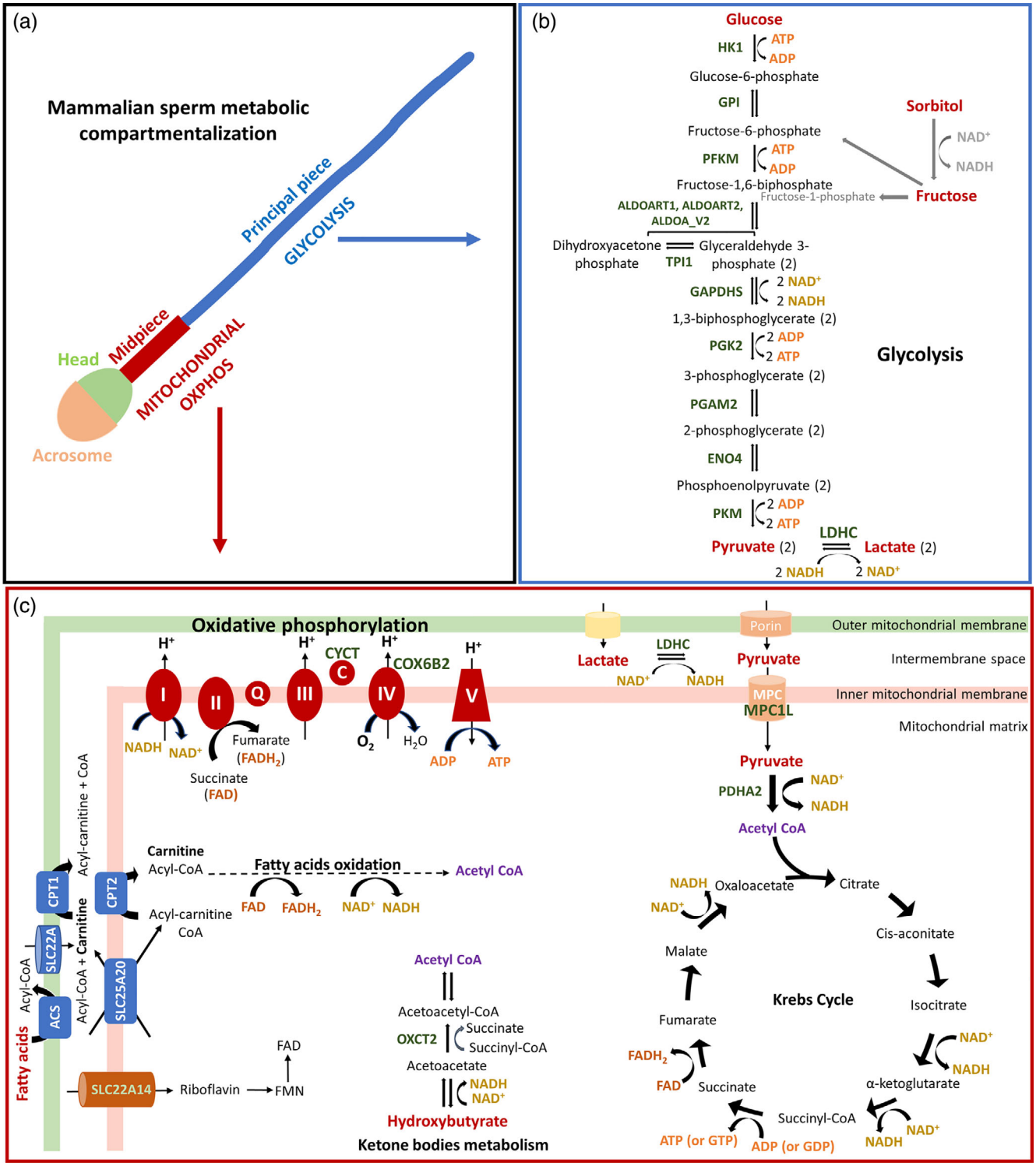

FIGURE 1 Legend on next page.

3 | SPERM ENERGY-PRODUCING METABOLIC PATHWAYS

Ejaculated sperm produce ATP mainly by catabolizing exogenous hexoses and monocarboxylates, but other exogenous (and endogenous) organic compounds, like fatty acids and ketone bodies, may also be used. Putative endogenous substrates are those produced or acquired during sperm biogenesis and epididymal storage and maturation. Exogenous nutrient molecules, available in seminal plasma and in the female reproductive tract, have to cross the sperm plasma membrane, and some also the mitochondrial membranes, and thus depend on membrane proteins to facilitate their transport. As shown by several reports, sperm are equipped with a range of specific membrane transporters (Table 2). These include glucose and fructose carriers (soluble carrier family 2; SLC2As; previously known as GLUTs) and monocarboxylate transporters (that facilitate the transport of lactate, pyruvate and ketone bodies over the plasma and mitochondrial membranes).

3.1 | Production of ATP in the principal piece: Glycolysis

Glycolysis is the conversion of glucose into pyruvate by a series of reactions involving the production of the coenzyme nicotinamide adenine dinucleotide (NADH) and resulting in the net gain of two ATP molecules per glucose consumed (Figure 1b). The fate of pyruvate is generally believed to depend on cellular O_2 levels. Under aerobic conditions, pyruvate is converted into acetyl CoA that enters the Krebs cycle and is ultimately oxidized to CO_2 in the mitochondrial electron transfer chain (ETC), where the regeneration of NAD^+ also occurs (Figure 1c). On the other hand, under anaerobic conditions, pyruvate is converted into lactate (fermentation), with the concomitant recycling of NADH to NAD^+, which can reintegrate the glycolytic pathway and sustain additional ATP production. However, some cells, such

FIGURE 1 Overview of the energy metabolic pathways responsible for ATP generation in mammalian sperm. (a) Schematic representation of mammalian sperm cell, with the main subcellular parts and bioenergetics compartmentalization indicated: oxidative phosphorylation (OXPHOS) in the midpiece and glycolysis in the principal piece. (b,c) Detailed metabolic pathways active in sperm. These rely on the function of several testis-specific proteins and proteins derived from alternative transcripts, uniquely expressed in post-meiotic germ cells (green): spermatogenic cell-specific hexokinase 1 isozyme (HK1; Mori et al., 1998; Mori et al., 1996); glucose-6-phosphate isomerase (GPI; Buehr & McLaren, 1981); spermatogenic cell-specific ATP-dependent 6-phosphofructokinase (PFKM; Nakamura et al., 2010); fructose-biphosphate aldolases A (ALDOART1, ALDOART2, ALDOA_V2; Gillis & Tamblyn, 1984; Vemuganti et al., 2007); triosephosphate isomerase (TPI1; Ijiri et al., 2013; Russell & Kim, 1996); glyceraldehyde 3-phosphate dehydrogenase, testis specific (GAPDHS; Welch et al., 2000; Welch et al., 1992); phosphoglycerate kinase 2 (PGK2; Boer et al., 1987; McCarrey & Thomas, 1987); phosphoglycerate mutase 2 (PGAM2; Broceno et al., 1995; Fundele et al., 1987); enolase 4 (ENO4; Edwards & Grootegoed, 1983; Nakamura et al., 2013), pyruvate kinase PKM (PKM; Feiden et al., 2007); L-lactate dehydrogenase C chain (LDHC; Blanco & Zinkham, 1963; Millan et al., 1987); cytochrome c, testis-specific (CYCT; Goldberg et al., 1977; Hennig, 1975); cytochrome c oxidase subunit 6B2 (COX6B2; Cheng et al., 2020; Huttemann et al., 2003); mitochondrial pyruvate carrier 1-like protein (MPC1L; Vanderperre et al., 2016); pyruvate dehydrogenase E1 component subunit alpha, testis specific form, mitochondrial (PDHA2; Dahl et al., 1990; Korotchkina et al., 2006); succinyl-CoA:3-ketoacid coenzyme A transferase 2, mitochondrial (OXCT2; Koga et al., 2000; Tanaka et al., 2002); solute carrier family 22 member 14 (SLC22A14). (b) *Sperm glycolytic pathway*. Glucose, fructose and sorbitol are oxidized to pyruvate, with the production of ATP and NADH. NAD+ can be regenerated by the conversion of pyruvate to lactate. (c) Energy metabolic pathways occurring in sperm mitochondria. Relevant carriers and shuttles are also depicted. *Oxidative phosphorylation*. A series of complexes forming the electron transfer chain (I–IV), together with ubiquinone (Q) and cytochrome C (C) transport electrons from reducing equivalents (NADH and succinate) to oxygen (O_2), with the production of water (H_2O). During this process, protons (H^+) are pumped to the intermembrane space and a quimio-osmotic proton gradient is generated across the inner mitochondrial membrane. The energy stored in this gradient is used by ATP synthase (V) to produce ATP. *Pyruvate metabolism and Krebs cycle*: Pyruvate and lactate can be imported to the mitochondria and generate acetyl CoA. Acetyl CoA enters the Krebs cycle, originating ATP or GTP, and NADH and succinate that are used in oxidative phosphorylation. Note that although most of the reactions in the Krebs cycle are reversible, the cycle is depicted as unidirectional. *Fatty acids oxidation*: Fatty acids are transferred to mitochondria as acyl-CoA by the carnitine shuttle. The process involves acyl-CoA synthases (ACS), mitochondrial carnitine/acylcarnitine carrier protein (SLC25A20) and carnitine *O*-palmitoyltransferases 1 and 2, mitochondrial (CPT1, CPT2). Acyl-CoA is oxidized to acetyl CoA,which enters the Krebs cycle. NADH (that can be used in OXPHOS) is also produced. *Ketone bodies metabolism*: Ketone bodies like hydroxybutyrate and acetoacetate are also oxidized to acetyl CoA in the mitochondrial matrix. *Riboflavins transport*: SLC22A14-mediated riboflavin transport is important for sperm energy metabolism, as riboflavin is the precursor of the flavine nucleotides FMN and FAD (coenzymes of many mitochondrial metabolic enzymes).

TABLE 1 Oxygen tensions and substrates (glucose, pyruvate and lactate) levels detected in the female reproductive tract (uterus and oviduct) of different mammalian species. Individual values vary throughout the female reproductive cycle

Molecule	Species	Uterus	Oviduct	References
Oxygen (%)	Human	1.8	ND	Yedwab et al. (1976)
		2.5	ND	Ottosen et al. (2006)
	Rhesus monkey	ND	<1.0–9.8	Maas et al. (1976)
		1.7	4.6–7.9	Fischer and Bavister (1993)
	Rat	3.3–6.2	ND	Mitchell and Yochim (1968)
		2.9–5.2	ND	Yochim and Mitchell (1968)
		1.0–3.2	ND	Yedwab et al. (1976)
		3.3–6.5	ND	Kaufman and Mitchell (1990)
	Hamster	0.65–6.5	ND	Kaufman and Mitchell (1990)
		4.5–7.8	3.9–6.9	Fischer and Bavister (1993)
		4.6	ND	Kaufman and Mitchell (1994)
	Guinea pig	2.7–5.7	ND	Garris and Mitchell (1979)
	Rabbit	ND	7.8	Mastroianni Jr. and Jones (1965)
		3.1–7.8	5.5–8.3	Fischer and Bavister (1993)
	Pig	7.6–10.1	7.6–10.1	Garcia-Martinez et al. (2018)
Glucose (mM)	Human	3.15	0.50–3.11	Dickens et al. (1995), Gardner et al. (1996), Tay et al. (1997)
	Mouse	0.61	1.09–5.19	Gardner and Leese (1990), Harris et al. (2005)
	Bovine	3.78–4.54	1.87–2.74	Hugentobler et al. (2008)
Pyruvate (mM)	Human	0.10	0.17–0.24	Dickens et al. (1995), Gardner et al. (1996), Tay et al. (1997)
	Mouse	0.25	0.14–0.37	Gardner and Leese (1990), Harris et al. (2005)
	Bovine	0.08–0.11	0.09–0.12	Hugentobler et al. (2008)
Lactate (mM)	Human	5.87	4.87–10.50	Dickens et al. (1995), Gardner et al. (1996), Tay et al. (1997)
	Mouse	9.41	4.79–11.68	Gardner and Leese (1990), Harris et al. (2005)
	Bovine	0.95–1.09	5.35–6.66	Hugentobler et al. (2008)

Abbreviation: ND, not determined.

as cancer cells and sperm, may use fermentation even in aerobic conditions (the so-called Warburg effect; Asgari et al., 2015).

Mammalian sperm fibrous sheath, a cytoskeletal structure located in the principal piece, serves as a scaffold that anchors all glycolytic enzymes, thus constituting a localized source of ATP (Figure 1a; Y. H. Kim et al., 2007; Krisfalusi et al., 2006). Mounting evidence has shown that ATP production via glycolysis is required for mammalian sperm function. Notable, every step of sperm glycolysis is catalyzed by male germline-specific isozymes (Figure 1b), arising either from novel genes, or from variant transcripts. Some of these enzymes (GAPDHS, PGK2, ENO4, and LDHC) were deleted by gene targeting, and all mouse knockout (KO) models generated presented with male infertility or subfertility, along with sperm with motility defects and reduced ATP levels (Danshina et al., 2010; Miki et al., 2004; Nakamura et al., 2013; Odet et al., 2008). Likewise, incubation of sperm with glycolysis inhibitors is deleteriously to motility (Takei et al., 2014; Williams & Ford, 2001).

In vitro studies on sperm glycolysis usually use glucose as substrate, in concentrations similar to the ones found in the female reproductive tract. In addition to glucose, sperm are able to metabolize other substrates through the glycolytic pathway, such as fructose (abundant in the seminal plasma) and sorbitol (a constituent of seminal plasma and uterine fluid), and both can sustain motility (Cao et al., 2009; Goodson et al., 2012). Fructose can be phosphorylated by hexokinase or by ketohexokinase, resulting in the formation of either fructose-6-phosphate or fructose-1-phosphate (Figure 1b). Sorbitol is converted to fructose by the enzyme sorbitol dehydrogenase, which is expressed along the entire length of rodent sperm flagellum (Cao et al., 2009; T. Kobayashi et al., 2002).

TABLE 2 Membrane transporters specific for hexoses, monocarboxylates, citrate, and carnitine detected in mammalian sperm. Members of the SLC2A family facilitate the transport of glucose (SLC2A1, 2, 3, 8, and 9) and other hexoses, including fructose (SLC2A2 and 5), across the sperm plasma membrane. Monocarboxylates, such as pyruvate, lactate, and ketone bodies, cross sperm membranes via SLC16A1 and 7. The mitochondrial pyruvate carrier (MPC), a complex composed of MPC/SLC54 family members, is involved in the import of pyruvate into mitochondria. Citrate transport is mediated by sodium-coupled citrate transporter (SLC13A5) and mitochondrial citrate transport protein (SLC25A1). Organic cation/carnitine transporter (OCTNs) SLC22 subfamily members A5 and A21 are solute carriers with high affinity for carnitine

Transporter	Species	Sperm subcellular localization	References
Hexoses			
SLC2A1 (GLUT1)	Human, rat, bull	Acrosome, principal piece	Angulo et al. (1998)
	Mouse	ND	S. T. Kim and Moley (2008)
	Donkey	Acrosome	Bucci, Spinaci, et al. (2010)
	Boar, stallion, dog	Acrosome, tail	Bucci, Isani, et al. (2010)
SLC2A2 (GLUT2)	Human, rat, bull	Midpiece (human and bull), acrosome (rat)	Angulo et al. (1998)
	Donkey	Acrosome, principal piece	Bucci, Spinaci, et al. (2010)
	Boar, stallion, dog	Acrosome, tail (stallion and dog)	Bucci, Isani, et al. (2010)
SLC2A3 (GLUT3)	Human, rat, bull	Midpiece, head (human and bull)	Angulo et al. (1998)
	Boar	Head, midpiece	Medrano et al. (2006)
	Mouse	ND	S. T. Kim and Moley (2008)
	Donkey	Principal piece	Bucci, Spinaci, et al. (2010)
	Boar, stallion, dog	Acrosome (boar), tail (stallion), principal piece (dog)	Bucci, Isani, et al. (2010)
SLC2A5 (GLUT5)	Human	Head, midpiece, tail	Burant et al. (1992)
	Human, rat, bull	Head, midpiece, principal piece	Angulo et al. (1998)
	Mouse	ND	S. T. Kim and Moley (2008)
	Donkey	Principal piece	Bucci, Spinaci, et al. (2010)
	Boar, stallion, dog	Acrosome, tail	Bucci, Isani, et al. (2010)
SLC2A8 (GLUT8)	Human, mouse	Acrosome	Schurmann et al. (2002)
	Mouse	Acrosome, midpiece, principal piece	Gomez et al. (2006), S. T. Kim and Moley (2007, 2008)
SLC2A9a (GLUT9a) and SLC2A9b (GLUT9b)	Mouse	Acrosome, midpiece, principal piece	S. T. Kim and Moley (2007, 2008)
Monocarboxylates			
SLC16A1 (MCT1)	Hamster	Head	Garcia et al. (1994)
	Mouse	Midpiece	C. Chen et al. (2016), Kishimoto et al. (2015), Mannowetz et al. (2012)
SLC16A7 (MCT2)	Hamster	Tail	Garcia et al. (1995)
	Mouse	Midpiece, principal piece	C. Chen et al. (2016), Kishimoto et al. (2015), Mannowetz et al. (2012)
MPC1 (SLC54A1), MPC2 (SLC54A2), and MPC1-like (SLC54A3)	Mouse	Midpiece	Vanderperre et al. (2016)

TABLE 2 (Continued)

Transporter	Species	Sperm subcellular localization	References
Citrate			
SLC13A5	Human	Midpiece (plasma membrane)	Bhutia et al. (2017)
SLC25A1	Human	Midpiece (inner mitochondrial membrane)	Cappello et al. (2012)
Carnitine			
SLC22A5 (OCTN2)	Mouse	Principal piece	D. Kobayashi et al. (2007)
SLC22A21 (OCTN3)	Mouse	Midpiece	

Abbreviation: ND, not determined.

Furthermore, sperm may be able to synthesize and degrade glycogen, which would be converted to glucose-6-phosphate and enter glycolysis. But to date such ability was only shown in dogs (Albarracin et al., 2004; Ballester et al., 2000; Palomo et al., 2003).

3.2 | Production of ATP in the midpiece: Oxidative phosphorylation and beyond

3.2.1 | Mitochondrial oxidative phosphorylation

Mitochondrial OXPHOS, the most efficient ATP producing pathway, is the final stage of cellular respiration, where electron donors generated in other pathways are used, together with O_2, to produce ATP. Located in the inner mitochondrial membrane, four multi-subunit complexes (I–IV) and two electron-transfer molecules (ubiquinone and cytochrome C) make up the ETC. These successively transport electrons from NADH and succinate, to O_2, the final acceptor, with the formation of H_2O (Figure 1c). Concomitant to this electron flow is the generation of an electrochemical gradient of protons across the inner mitochondrial membrane. ATP synthase (complex V) drives protons back to the mitochondrial matrix and uses the proton-motive force to synthesize ATP.

As previously mentioned, the production of ATP by OXPHOS is restricted to the sperm midpiece, where mitochondria are localized. At least two sperm-specific proteins of the ETC were identified: cytochrome c, testis-specific (CYCT; Goldberg et al., 1977; Hess et al., 1993) and cytochrome c oxidase subunit 6B2 (COX6B2; Huttemann et al., 2003). Although capable of generating progeny, *Cyct* KO mice showed reduced male fertility and their sperm had low ATP levels and decreased motility (Narisawa et al., 2002). Interestingly, tumor cells expressing COX6B2 (and whose expression is enhanced by hypoxia) show enhanced complex IV activity and increased OXPHOS, and display a proliferative advantage in low oxygen (Cheng et al., 2020). Thus, similarly to glycolysis, OXPHOS seems to have adapted to the sperm-specific energy demands. Different experimental evidences illustrate the relevance of OXPHOS for sperm swimming. Human sperm motility correlates with oxygen consumption and respiratory efficiency (Ferramosca et al., 2012) and with the levels of ETC proteins (Amaral et al., 2007). Moreover, incubation of sperm with ETC inhibitors results in reduced ATP levels and decreased motility (St John et al., 2005).

3.2.2 | Krebs cycle and pyruvate and lactate metabolism

Krebs cycle (citric acid cycle or tricarboxylic acid cycle), which also operates in the mitochondrial matrix (Figure 1c), is one of the primary sources of the reducing equivalents that fuel the ETC. It consists on a series of reactions carried out by eight enzymes that oxidize acetyl coenzyme A (CoA) and produce NADH, flavin adenine dinucleotide, guanosine triphosphate (GTP) or ATP and CO_2.

Pyruvate and lactate are the main sperm mitochondrial substrates. These can be taken from exogenous sources, facilitated by monocarboxylate carriers (Table 2), or derive from glycolysis. An increasing expression of two subunits of mitochondrial pyruvate carriers, MPC2 and MPC1L (mitochondrial pyruvate carrier 1-like protein; specific to post meiotic male germ cells) occurs during spermiogenesis, suggesting that the import of pyruvate into mitochondria might be important for sperm function (Vanderperre et al., 2016). Once inside the mitochondrial matrix, pyruvate is oxidized to

acetyl CoA by the pyruvate dehydrogenase complex (PDHc). This consists of different enzymes, of which pyruvate dehydrogenase E1 (PDH, the enzyme catalyzing the first step) has a testis-specific isozyme (PDHA2), with particular regulatory properties (Dahl et al., 1990; Gerez de Burgos et al., 1994; Korotchkina et al., 2006). Both PDHA2 and dihydrolipoamide dehydrogenase, another component of the PDHc, undergo capacitation-dependent tyrosine phosphorylation in hamster sperm, and seem to be required for hyperactivation (Kumar et al., 2006; Mitra et al., 2005; Mitra & Shivaji, 2004).

Owing to the expression of the testis-specific lactate dehydrogenase also in mitochondria (Burgos et al., 1995; Burkhart et al., 1982), sperm might use lactate as an aerobic energy source (Storey & Kayne, 1977). LDHC could operate in a branched-chain-2-hydroxy acid/2-oxo acid shuttle (Burgos et al., 1982; Coronel et al., 1986) or in a lactate/pyruvate shuttle (Calvin & Tubbs, 1978; Gallina et al., 1994), possibly together with a malate/aspartate shuttle (Brooks, 1978; Burgos et al., 1982; Calvin & Tubbs, 1978; Kane, 2014). These shuttles, whose operation in sperm was not confirmed in vivo, would allow the transfer of reducing equivalents to the sperm mitochondria.

3.2.3 | Fatty acids oxidation, glycerol, and ketone bodies catabolism

Acetyl CoA may originate from other sources, such as fatty acids beta-oxidation and ketone bodies catabolism, both of which also contribute to sperm ATP production. Indeed, ketone bodies, such as hydroxybutyrate (detected in uterine and oviductal fluids at least in ewes; Tripathi et al., 2016), are able to support mouse sperm motility (Tanaka et al., 2004). These can be converted into acetyl-CoA by the activity of the haploid germ cell-specific protein succinyl-CoA:3-ketoacid coenzyme A transferase 2, mitochondrial (OXCT2; Figure 1c; Koga et al., 2000), a sperm mitochondrial protein with high enzymatic activity (Tanaka et al., 2003).

Beta oxidation involves a repeated sequence of reactions that result in the conversion of fatty acids to acetyl-CoA (Figure 1c). Fatty acids may be taken up from exogenous sources by specific transporters or result from the cleavage of cellular phospholipids by phospholipase A2. In any case, fatty acids are converted to acyl CoA that is then transferred to the mitochondrial matrix (where beta-oxidation undergoes) by the carnitine shuttle. Sperm from some species may also to be able to internalize exogenous acyl CoA by a process not involving carnitine, but whose molecular mechanisms have not been defined (Carey et al., 1981; Ferramosca et al., 2008). At any rate, carnitine, which can be imported to sperm with the help of solute carrier family 22 members 5 and 21 (SLC22A5 and SLC22A21; Table 2; D. Kobayashi et al., 2007), seems to be critical for sperm function. Indeed, the levels of carnitine and acetyl-carnitine in sperm are extremely high, and free carnitine was suggested to have an important role in the sperm mitochondrial matrix, by buffering the excess of acetyl CoA derived from different catabolic processes (Jeulin & Lewin, 1996).

The idea that sperm could use membrane phospholipids and triglycerides for energy metabolism is not new (Jones & Bubb, 2000; Storey, 1980) and it would explain the observation that sperm sustain motility even in the absence of exogenous substrates (Amaral et al., 2011). Proteomic studies of human and horse sperm suggest that beta-oxidation contribute to sperm ATP generation and motility, which was corroborated by the decline in motility observed upon incubation with etomoxir, a carnitine palmitoyltransferase I (CPT1) inhibitor (Amaral et al., 2013; Swegen et al., 2015). Dolphin and pig sperm movement are also sensitive to etomoxir, with more pronounced effects on the former (Alves et al., 2021; Lombo et al., 2021). Interestingly, dolphin sperm motility seems to depend almost entirely on endogenous fatty acids oxidation, most probably as an adaptation to the carbohydrate-depleted marine environment. Stronger evidence for the importance of fatty acids as energy source for sperm motility was given by *Slc22a14* KO mice, whose males are infertile (Kuang et al., 2021). SLC22A14 is a testis-specific riboflavin (precursor of flavin nucleotides) transporter localized in the sperm inner mitochondrial membrane (Figure 1c). Its disruption resulted in sperm with defective fatty acids oxidation, Krebs cycle, and OXPHOS, along with decreased ATP levels and motility defects. As prove of evidence, the outcomes were phenocopied by wild-type mice fed a riboflavin-deficient diet.

In addition to fatty acids, phospholipid hydrolysis yields glycerol, which can enter the glycolytic pathway by the sequential conversion to glycerol-3-phosphate (by glycerol kinase [GK]) and dihydroxyacetone phosphate (by glycerol-3-phosphate dehydrogenase [GPD]), an endogenous metabolic pathway that seems to be active in pig sperm (Jones & Gillan, 1996; Jones & Milmlow, 1997). It has also been proposed that glycerol could be transported by aquaglyceroporins, namely AQP7, whose expression in human and bull sperm tail was correlated with motility (Fujii et al., 2018; Yeung et al., 2010). However, the association between sperm AQP7 and glycerol metabolism remains to be established. Moreover, the contribution of the enzymes GK and GPD to sperm ATP production is unclear. The GKs GYKL1 and GK2 are testis-specific proteins specifically localized to the mitochondria of haploid germ cells (Sargent

et al., 1994). Male mice deficient in either *Gykl1* or *Gk2* are infertile, showing defects in mitochondrial sheath formation during spermatogenesis (Chen et al., 2017; Shimada et al., 2019). The expression of testicular GPD2 in the testis is also restricted to haploid germ cells (Rajkovic et al., 2004; Weitzel et al., 2003), and this protein may be involved in mouse sperm capacitation and hyperactivation (Kota et al., 2010).

3.3 | Contribution of each pathway: The sperm energy debate

The contribution of glycolysis and OXPHOS as sources of the ATP that fuel mammalian sperm motility has been the subject of a debate that has last for decades (for a historic overview see Storey, 2008). The question of whether diffusion of ATP from the mitochondria to the distal end of the flagellum could be fast enough to support microtubules sliding led to the suggestion that local glycolysis would have a preponderant role. In addition, it was suggested that the oxygen levels sperm encounter in the female reproductive tract would be too low to support mitochondrial respiration, again putting the emphasis on glycolysis (Mastroianni Jr. & Jones, 1965; Yedwab et al., 1976). The current premise is that sperm of a given species are adapted to the substrate and oxygen contents of the female reproductive tract of that species, with for instance, horses relying more on OXPHOS, and mouse and human on glycolysis. Apart from species-specific differences, the debate has also been baffled by methodological discrepancies between the dozens of studies performed.

Here, it will be argued that this debate is somehow artificial. To begin with, the arguments once raised to suggest that OXPHOS-derived ATP could not fuel sperm motility were contradicted. ATP diffusion from the mitochondria may not be a problem, at least in species with smaller tails; and in species with longer sperm tails, like rodents, the problem could be overcome by the action of ATP transfer shuttles (Ford, 2006; Takei et al., 2014). On the other hand, the O_2 levels in mammalian female reproductive tract are relatively low (Table 1), but not much lower than the levels found in other organs (Ast & Mootha, 2019; Ortiz-Prado et al., 2019) and may be enough to support sperm respiration in most species. Actually, the O_2 tensions that sperm are subjected to during their travel in the female reproductive tract are not constant, since periodic fluctuations and changes according to the stage of the female cycle were described (Keeley & Mann, 2019). The levels of the available exogenous substrates also oscillate during sperm travel (Table 1 and references therein), and thus sperm should be bioenergetically adaptable. Lastly, since all metabolic pathways are interconnected, it is challenging to study each one separately, as inhibiting or stimulating one particular pathway may have effects on many others. To this extent, glycolisable substrates like glucose, activate glycolysis, but can also activate OXPHOS. Likewise, pyruvate and lactate, usually used as mitochondrial substrates, are involved in the last glycolytic step.

Genetic approaches, glycolysis and ETC inhibitors, and OXPHOS uncouplers have been widely used to study the preponderance of glycolysis versus OXPHOS in mouse sperm motility. Pioneering work from Mukai and Okuno (2004) suggested that glycolysis supports mouse sperm motility, even in aerobic conditions. Sperm beat frequency could be equally maintained by glucose, fructose, pyruvate, and lactate. OXPHOS inhibition resulted in decreased sperm motility and ATP levels, but was rescued by glucose. On the other hand, motility and ATP content could not be maintained in the presence of respiratory substrates when glycolysis was suppressed (Mukai & Okuno, 2004). These outcomes were corroborated using human sperm and a combination of laser tweezers, fluorescence imaging and robotics (Nascimento et al., 2008). As previously mentioned, KO mice for different sperm-specific glycolytic enzymes (GAPDHS, PGK2, and LDHC) have impaired male fertility, with sperm motility defects and low ATP levels (Danshina et al., 2010; Miki et al., 2004; Odet et al., 2008). Since mitochondrial oxygen consumption was normal in *Gapdhs* KO mice, the outcomes were interpreted as a proof of concept for the preponderance of glycolysis. However, the observation that adverse effects only occur if sperm are incubated with glucose, and glycolytic intermediates accumulate, resulted in an alternative interpretation (Ford, 2006). In such conditions, glucose acts as a metabolic poison, by allowing the ATP consuming steps of glycolysis to operate. Similarly, 2-deoxy-glucose, the glycolysis inhibitor used by Mukai and Okuno (2004), is metabolized to 2-deoxyglucose-6-phosphate (that cannot be further metabolized), and thus entails ATP consumption. On the other hand, the defects observed by the targeted disruption of *Pgk2* were less severe, most probably due to the action of alternative pathways that bypass the PGK2 step (Danshina et al., 2010). The phenotypic differences between the two KO models are likely triggered by alterations in signaling and metabolic pathways (Huang et al., 2017). Sperm from mice lacking LDHC, which do not hyperactivate (Odet et al., 2008), have normal pyruvate levels and NAD/NADH ratio (most probably due to the activity of LDHA), but low glucose consumption (Odet et al., 2011). The authors hypothesized that LDHC is part of a macromolecular complex involved in ATP homeostasis. To further complicate the subject, the phenotype of *Ldhc*-null sperm depends on the mouse strain (Odet et al., 2013).

At any rate, studies using *Ldhc* KOs add to other studies showing that both glycolysis and OXPHOS can support mouse sperm motility, but that the glycolytic pathway is required for hyperactivation (Goodson et al., 2012). Variations on the relative usage of glycolysis and OXPHOS relate to differences in sperm performed in closed related mouse species, with species with higher sperm respiration/glycolysis ratio showing higher ATP content and motility, and faster and more progressive sperm (Tourmente, Villar-Moya, Rial, et al., 2015). Sperm from *Slc22a14* KO mouse (previously mentioned), which have defects in fatty acids oxidation, Krebs cycle, and OXPHOS, showed increased glycolysis, a (failed) attempt to compensate the energy demands (Kuang et al., 2021). These observations illustrate the ability of sperm to reprogram their energetic metabolism and validate the theory that several metabolic pathways are essential for normal sperm function.

Studies in nonrodent species point to the same idea. Glucose supports human sperm motility for extended periods (Amaral et al., 2011) and glycolysis seems to be required for hyperactivation (Williams & Ford, 2001). Although metabolic tracing analyses suggested that human sperm rely primarily on glycolysis (Calvert et al., 2019; Hereng et al., 2011), proteomic and metabolomic analyses, data mining, and in silico modeling, supported the notion that mitochondrial metabolic pathways (triggered by either exogenous or endogenous substrates) also contribute to motility (Amaral et al., 2013; Amaral, Castillo, et al., 2014; Amaral, Paiva, et al., 2014; Asghari et al., 2017; Paiva et al., 2015; Reynolds, Ismail, et al., 2017). Starved human sperm seem to use mitochondrial pathways to fuel swimming for several hours (although with less vigor than when incubated with nutrients), but these do not support hyperactivation. Upon the addition of substrates, motility recovers to normal levels and sperm can hyperactivate (Marin-Briggiler et al., 2021). Altogether, these findings highlight the energetic flexibility of sperm in a species usually believed to rely mainly on glycolysis-derived ATP. Metabolic flexibility was also reported in sperm from other species, including the ones on the other side of the spectrum, that is, supposed to rely on OXPHOS, such as equine (Darr et al., 2016; Davila et al., 2016; Losano et al., 2017).

Most of the studies reported were performed in noncapacitating, and thus nonphysiological, conditions, which sperm do not encounter in vivo. This is special important since independent groups have recently shown that capacitation involves changes in mouse sperm bioenergetics, including increases in ATP consumption (Sansegundo et al., 2022) and in mitochondrial activity, which may be required for hyperactivation and fertilization ability (Ferreira et al., 2021; Giaccagli et al., 2021). In addition, capacitation seems to trigger an increase in glucose uptake and consumption, and to stimulate both glycolysis and OXPHOS (Balbach et al., 2020; Hidalgo et al., 2020). Because the increase in OXPHOS was shown to be dependent on the glycolytic pathway, the two pathways might be linked.

The sperm energy debate has been studied using different species and miscellaneous technical conditions: (a) different sperm origins (ejaculated vs. epididymal); (b) different sperm status (capacitated vs. noncapacitated); (c) use of substrates and inhibitors at diverse concentrations and times of incubations; (d) use of dissimilar culture media formulations; and (e) diverse end-points (e.g., percentage of motile sperm, motility parameters, or sperm beat frequency to report motility). Also worth noting, sperm culture media usually have serum albumin that, with the exception of a few studies, contains fatty acids. And this is never taken in consideration for data interpretation. Moreover, in vitro conditions are inevitably different from in vivo ones, notably concerning O_2 tensions that are much higher in vitro, potentially hindering a true understanding of sperm bioenergetics. Having the available literature and these limitations in consideration, and although species-specific differences certainly exist, the studies performed so far suggest that sperm from most mammalian species have a wide range of active metabolic tools. The environment conditions, which change along the female reproductive tract, may elicit the differential use of specific metabolic pathways, to allow the most efficient ATP production.

4 | SPERM METABOLISM AND MALE (IN)FERTILITY

Asthenozoospermia (i.e., low sperm motility) is a very common etiology in patients with fertility problems, which call on assisted reproductive technologies (ART) to conceive. Although intracytoplasmic sperm injection allows even immotile sperm to fertilize an oocyte, the development of methods to enhance/rescue sperm motility is warranted. If successful, these could end up in pregnancy without medical intervention, or at least with the use of less invasive techniques. Given the role of metabolic pathways on sperm motility regulation, in vitro metabolic supplementation and in vivo metabolic adjustments are appealing. Both may require the identification of markers to diagnose specific energetic deficiencies. Although some work has been done, further studies aimed to identify metabolic markers and efficient supplementation strategies are warranted.

Mounting evidence suggests that metabolic faults are on the origin of sperm motility defects. To this extent, several studies reported deregulation of energy metabolism-related proteins in sperm samples/subpopulations with motility dysfunction (Amaral, Paiva, et al., 2014; Guo et al., 2019; Moscatelli et al., 2019; Saraswat et al., 2017; Yang et al., 2022). Alterations at the metabolome level were also described (Reynolds, Calvert, et al., 2017; Zhao et al., 2018) and the amounts of sperm metabolites seem to correlate with motility, while those of seminal plasma relate to sperm concentration and morphology (Engel et al., 2019).

Typifying the prospect of sperm in vitro metabolic adjustments, the supplementation of sperm with succinate (that enters the ETC at Complex II) rescued the motility of sperm from a patient with a defect on ETC Complex I (Folgero et al., 1993). Also interesting, although more difficult to understand, is the observation that transient sperm starvation may increase motility and fertilization ability and improve ART outcomes in murine and bovine models (Navarrete et al., 2019). On the other hand, dietary interventions may modulate rodent sperm metabolism in vivo. For instance, and although inconsistent results have been published (Gomez-Elias et al., 2019), a high-fat diet may induce alterations in sperm metabolism and decreased motility (Ferramosca et al., 2016). Such defects seem to be counteracted by a short-term intervention with metformin (a drug commonly used to treat Type II diabetes; McPherson and Lane, 2020) or by the inclusion of specific fatty acids in the diet (Ferramosca et al., 2017). Nutrition adjustments are thus likely to be effective as therapeutics for some cases of male infertility (Ferramosca & Zara, 2022).

Putative translational applications stimulated by the increased knowledge on sperm bioenergetics have other targets, such as male contraception and oncology. Interestingly, cancer cells express sperm-specific proteins, including metabolic enzymes, the expression of which may be responsible for energetic reprograming in tumors (Cheng et al., 2020; Ding et al., 2021; Sevostyanova et al., 2012). Although the attempts made so far were unsuccessful, enzymes such as GAPDHS and LDHC, are potential targets for the development of male contraceptives and cancer therapeutics (Ford & Waites, 1980; Frayne et al., 2009; Goldberg, 2021; Gupta, 2012; Muronetz et al., 2019).

5 | CONCLUSION

Mammalian sperm require large amounts of ATP to fuel a number of cellular activities, including motility. Since the capacity to move forward is essential for sperm fertilization ability (at least in vivo), a full understanding of the cellular pathways responsible for ATP production is paramount in andrology. It is well established that energy-producing metabolic pathways are compartmentalized in the male gamete, with glycolysis in the principal piece and OXPHOS in the midpiece. For decades we have been discussing which of these two pathways is more preponderant in sperm. It has been concluded that the answer may depend on the species, particularly on the O_2 and substrates available in the female reproductive tract. However, spatial and temporal fluctuations occur even in a same species, and the reported values differ between studies. Thus, with the data obtained to date, no real parallelisms can be drawn, and the *adaptation to the female tract conditions hypothesis* remains elusive.

As discussed, the debate has been bemused by several technical constraints, including the impossibility of studying each metabolic pathway in isolation. Anyhow, and although species-specific differences certainly exist, the outcomes obtained so far suggest that sperm from most mammalian species have a wide range of metabolic tools and can produce ATP (using both exogenous and endogenous substrates) through glycolysis, Krebs cycle, and fatty acids and ketone bodies oxidation, all of which converge in OXPHOS. Other pathways, such as amino acids oxidation, may also be relevant, but convincing supporting outcomes are lacking. The axoneme spreads along the entire flagellum and thus dynein ATPases hydrolyze ATP in the midpiece and in the principal piece. Different metabolic pathways might be active in the flagellum at the same time, producing ATP locally. Whether the ATP produced in mitochondria can reach the tip of the flagellum is probably irrelevant, as ATP can be produced nearby. The fate of glycolysis-derived pyruvate (and lactate) could be different on different principal piece locations, that is, those pyruvate (and lactate) molecules produced next to the midpiece would be directed to the mitochondria, while fermentation would have a more preponderant role in the lower part of the principal piece. The relative activity of each pathway may vary according to the (also variable) environment.

The conditions sperm are exposed to in the female reproductive tract need to be better established. And although this may be technically challenging, such environment should be, as far as possible, mimicked in in vitro studies on sperm bioenergetics. Standardization, for each species, of the conditions used in sperm metabolism research would allow a better comparison between studies and technical readouts. From a clinical point of view, it should be considered that defects in any energetic pathway can affect sperm motility and translate into asthenozoospermia. Metabolic

markers for the most common metabolic failures in the male gamete ought to be defined. Translational approaches based on nutritional interventions and on adjustments of the culture medium used to process semen before ARTs must be considered.

AUTHOR CONTRIBUTIONS

Alexandra Amaral: Conceptualization (equal); formal analysis (equal); writing—original draft (equal); writing—review and editing (equal).

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Data sharing is not applicable to this article as no new data were created or analyzed in this study.

ORCID

Alexandra Amaral https://orcid.org/0000-0003-2354-8258

RELATED WIREs ARTICLE

An interdisciplinary systems approach to study sperm physiology and evolution

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