Sperm Mitochondrial Function

Subjects: Andrology

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Sperm motility is a fundamental requirement to ensure male fertility. Studies and interest for sperm motility started in 1919 when Lillie Frank Rattray, an American zoologist, author of the book “Problems of fertilization”, for the first time, talked on the energetic metabolism of spermatozoa. He said: “Spermatozoa are probably incapable of receiving nourishment outside of the gonad after they are fully differentiated; certainly in the case of external insemination there is no opportunity for the restitution of substance …”. Since those years, several studies focused on the “power plant” of the cell, the mitochondrion, demonstrating the key role of this organelle on cellular homeostasis and sperm motility. Mitochondrial sperm dysfunction is also implicated in the pathogenesis of seminal oxidative stress, a key element responsible of many cases of “apparently” idiopathic male infertility.

sperm motility asthenozoospermia mitochondrial function antioxidants

1. Sperm Mitochondria: Anatomy

Mammalian spermatozoa typically have between 50 and 75 mitochondria [1]. Mitochondria of spermatozoa show peculiar characteristics. They are exclusively confined in the mid-piece, tightly wrapped around the axoneme. During spermiogenesis, the mitochondria line up end-to-end and wrap helically around the flagellum to form the thick mitochondrial sheath, just under the outer plasma membrane of the cell [2][3]. The mitochondrial capsule is thus shaped by multiple disulphide bridges formed between cysteine and proline-rich selenoproteins [4][5][6]. This intricate attachment to the fibrous sheath makes sperm mitochondria particularly difficult to isolate by conventional separation method [7]. Moreover, many proteins and enzymes such as subunit VIb of the cytochrome oxidase [8], E1-pyruvate decarboxylase and creatine kinase (CK) [9][10] are isoforms present only in sperm mitochondria.

2. Sperm Mitochondrial Metabolism

2.1. A Long Debate: Glycolysis or Oxidative Phosphorylation?

Many studies on sperm mitochondrial bioenergetics have concentrated on the following question: what is the main biochemical pathway that provides the energy for sperm motility, glycolysis or oxidative phosphorylation (OXPHOS)? Studies carried out in several species, including humans, have often provided different and/or conflicting results. Many authors have reached the conclusion that glycolysis has a primary role in energy production in human sperm motility [11][12][13][14]. Other authors have underlined the importance of mitochondrial OXPHOS for sperm motility [15][16][17][18][19][20][21][22]. Moreover, the experimental conditions varied significantly from

one study to another and this complicated the interpretation of the results. Therefore, despite numerous studies, a clear conclusion cannot be drawn [6]. A reasonable concept that emerged from many studies is that these processes are not mutually exclusive and that spermatozoa exhibit a great versatility in their metabolism using glycolysis exclusively, mitochondrial OXPHOS exclusively or a combination of both pathways for energy production according to the substrates available in the female genital tracts [23][24]. Specifically, Zhu et al. had recently shown that mitochondrial oxidative phosphorylation is activated to produce ATP under low glucose condition. They incubated boar spermatozoa with different doses of glucose and they found that sperm progressive motility and straight-line velocity were significantly increased with decreasing glucose level in the incubation medium. They also showed that, in presence of the mitochondrial translation inhibitor d-chloramphenicol, mitochondrial protein synthesis, mitochondrial activity and ATP level were suppressed, and consequently, the linear motility speed decreased. Interestingly, despite the reduction of linear motility speed, total motility did not change [25]. These results suggest that sperm motility patterns depend on the substrates available and as a result on the biochemical pathway that is activated. While glycolysis is important for hyperactivated motility [26], the high-speed linear motility is induced via activating the mitochondrial activity in low glucose condition.

2.2. Reactive Oxygen Species and Sperm Mitochondria

Since mitochondria have a main role in sperm metabolism and energy production, they are the major reactive oxygen species (ROS) generator, as they convert approximately 1–2% of consumed oxygen into superoxide anions [27]. In spermatozoa, mitochondrial Complex I and Complex III are the major sites for ROS production [27]. An imbalance between impaired ROS production and antioxidants mechanisms may be extremely harmful for spermatozoa [28]. These cells are particularly susceptible to oxidative stress because they cannot restore damage caused by oxidative stress due to deficiency of cytoplasmic repair enzymes [29]. In contrast, at physiological concentrations, ROS are trigger for several reproductive mechanisms such as sperm capacitation, hyperactivation, acrosome reaction and oocyte fusion [30][31][32]. More recent research is changing the long-accepted dogma that ROS is ultimately a negative indicator of sperm function, thus indicating that ROS production can also reflect intense mitochondrial activity leading to increased sperm function [33].

3. Techniques to Study Sperm Mitochondria Function

Since sperm mitochondria have been related to sperm motility and fertilization, numerous tools have been developed to evaluate their function. Several parameters can be used to study sperm mitochondria. This include mitochondrial activity, MMP levels and mitochondrial calcium levels [34]. The use of fluorescent probes to detect changes in the MMP is the most popular tool used to evaluate mitochondrial function. These probes spread freely through the plasma membrane to the cell cytosol and accumulate electrophoretically in the mitochondrial matrix, due to the motive force of the proton and acting according to the ability of the mitochondria to pump the protons from the matrix to the intermembrane area [35][36][37]. Therefore, according to their properties, high concentrations of these fluorochromes accumulate in hyperpolarized mitochondria (high MMP), while lower concentrations are found in depolarized mitochondria (low MMP) and the intensity of the fluorescence correlates with MMP [38].

Many commercial fluorescent dyes are widely used, such as JC-1 (5,5,6,6-tetrachloro-1,1,3,3-tetraethylbenzimidazolyl carbocyanine iodide [36], Mito Tracker Green FM [39] and rhodamine 123 [40]. The JC-1 probe is one of the preferred fluorescent dyes for analyzing MMP and has been widely used for the analysis of spermatozoa in several species including humans [22][41], cattle [42], horse [43], ram [44], dog [45] and alpaca [46]. In 2004, Marchetti et al. compared the specificity of four fluorochromes in the evaluation of sperm MMP. They found that JC-1 is found only within the mitochondria and therefore provides the most accurate measurement of MMP [20]. Lugli et al. confirmed this finding, indicating that JC-1 is more reliable and specific for this type of evaluation than other probes [47]. Amaral and Ramalho-Santos found JC-1 more dynamic and suitable than MitoTracker Green and MitoTracker Red, as it is able to detect minimal changes in MMP [5][48]. Recently, Uribe et al. evaluated the usefulness of another fluorescent dye, tetramethyl rhodamine methyl ester perchlorate (TMRM) for measuring sperm MMP. They found that TMRM is able to accurately detect MMP variations comparable to the method widely used, JC-1 staining. In addition, TMRM was able to measure sperm MMP in the experimental conditions in which JC-1 had previously presented difficulties [49].

Finally, mitochondrial oxygen consumption is considered the central parameter of mitochondrial function. Studies on experimental animals have shown that mitochondrial oxygen consumption is positively correlated with traditional measures of sperm function including motility and vitality [50]. Therefore, measurement of oxygen consumption is another important biological endpoint for the study of sperm mitochondrial function and should be further investigated in future studies.

4. Asthenozoospermia and Sperm Mitochondrial Dysfunction

Almost forty years have passed since the first study relating mitochondrial function to sperm motility. Everson et al. reported a good correlation between sperm motility and MMP, comparing ejaculate from fertile men with those from patients whose spermatozoa showed reduced sperm motility [51]. Ruiz-Pesini et al. compared mitochondrial respiratory complex activities of 86 asthenozoospermic patients with those of 33 controls. Their results showed that semen samples of controls had substantially higher activities of complexes I, II and IV compared with those of patients with asthenozoospermia. Moreover, a direct and positive correlation was found in the whole population studied between spermatozoa motility and all the mitochondrial respiratory complex activities assayed (I, II, I+III, II+III, and IV). They suggested that more specific mitochondrial dysfunctions could be the underlying cause of idiopathic asthenozoospermia [18]. In a following study, these authors found that mitochondrial enzyme activities not only correlate with sperm motility but also with vitality and cell concentration [19]. As mitochondria are the major source of pro-oxidative agents, it is suggested that dysfunction of this organelle would have a fundamental role in the oxidative imbalance affecting sperm function [27]. Wang et al. [52] identified low MMP and high ROS production in spermatozoa from infertile patients, probably as a consequence of such mitochondrial injury. Other researchers have observed changes in mitochondrial function in sperm derived from infertile patients [53]. However, spermatozoa with high MMP have been identified in fertile men [20][21][54][55]. Paoli et al. [41], correlated MMP with increasing motility to establish MMP values corresponding to a precise sequence of gradually increasing motility. They evaluated 185 semen samples, divided into 13 motility classes (from 0 to 60%) with an increment of 5%

between classes and a second group of 28 semen samples showing nonlinear motility only, divided into five classes. A positive correlation was found between sperm motility and FL2 (percentage of sperm with high and low MMP) in all samples of both groups. In group A, the first 0 motility class, showed a mean FL2 of 11.5. This value increased gradually with increasing motility, reaching its highest mean value of 75.7% in the 60% motility class. They found both immotility and severe asthenozoospermia to be characterized by an extremely low MMP. These results diverge from those of Piasecka, whose study on 32 subjects with normal motility and 25 patients with asthenozoospermia identified two asthenozoospermic subpopulations, one with a low and the other with a high MMP, suggesting that the reduced motility might be caused not only by functional alteration of mitochondria but also by abnormal morphology of the axoneme, the dense fibers and the fibrous sheath [56]. Pelliccione et al. demonstrated the importance of structural defects in the mitochondrial membrane in asthenozoospermic semen samples, indicating a close correlation between forward motility and the percentage of the intermediate tract having a normal membrane [57].

Over the last years, proteomic studies have tried to identify dysfunctional proteins responsible for asthenozoospermia [58][59]. Recently, Nowicka-Bauer et al. compared proteomic profiles in spermatozoa of normozoospermic men and in patients with isolated asthenozoospermia. The results of this study were further supported by two additional mitochondrial tests (JC-1 and MitoSox Red) to establish a possible direct connection between the identified proteins and functional status of mitochondria [59]. In accordance with a previous study by Amaral et al. [58], they found that most of the identified dysfunctional proteins in low motility spermatozoa were of mitochondrial origin and a great proportion of them were engaged in cell metabolism and energy production being involved in tricarboxylic acid cycle (TCA), mitochondrial OXPHOS and the metabolism of butanoates, propanoates and pyruvates. These results strongly support the emerging idea that several bioenergetics metabolic pathways contribute to sperm motility (de)regulation [58][59].

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