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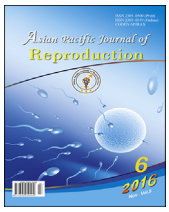

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Mitochondrion: Features, functions and comparative analysis of specific probes in detecting sperm cell damages

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ABSTRACT

In assisted reproduction, ejaculated semen is either washed immediately for in vitro fertilization or diluted and processed further for optimal low temperature liquid- or cryo-preservation. The extended handling of semen makes sperm vulnerable to organelle and/or membrane damages. In addition to several important functions, mitochondrion is chiefly responsible for production of detrimental free radicals in spermatozoa. Therefore, direct evaluation of mitochondrial activitymight be useful in semen evaluation protocol as an alternate, more objective and desirable parameter of spermatozoa quality. This is further aided by availability of wide spectral range of fluorescent probes that has advantage of simultaneous multi-parametric assay. Even with availability of several mitochondrial-specific probes, it is sometimes difficult for an investigator to select a most suited dye for experimentation. Therefore, in this review the literature pertaining to role of the mitochondria in the sperm cell, focusing on morphology, maintenance of trans-membrane potential, mechanism involved in generation of reactive species, and comparative analysis of a number of probes used for evaluating mitochondrial function in sperm cells are discussed. Our intention is to present concise information on the technical aspects of various probing methods, and this might be useful for investigators to design experimental approach by proper selection of the dye and for accurate interpretation of the results.

1. Introduction

The mammalian fertilization is a very complex process requiring a well-orchestrated series of events played by oocyte and sperm cell. For successful fertilization, one of the most essential attributes of spermatozoa is motility, required for transport through female genital tract, as well as in the approach to the waiting oocyte, and finally as an aid in penetration of zona pellucida [1]. Sperm motility is the result of flagellar movement of the sperm tail, achieved by ATP-derived energy, produced in mid-piece located mitochondrion. Evaluation of motility in assisted reproduction and in semen freezing laboratories is considered as an important attribute of sperm viability and in-direct measure of metabolism [2,3]. Therefore, it flows naturally to surmise that measurements of mitochondrial function might be useful as an alternate, more objective measure of sperm quality. It is crucial to understand the complexity of the mitochondrial compartments related to its functionality and to evaluate specific fluorescent probes used in assessing the morpho-functional features of mitochondria in different living- or fixed-cell types. Given the pivotal role mitochondria play in cellular life, this review covers mitochondrial features and functions vital in interpreting results and at the same time, elucidating finer points of various fluorescent probes employed to evaluate sperm mitochondrion.

2. Brief background of mitochondrion science

The earliest records on intracellular structures that probably represent mitochondria go back to the 1840s [4]. However, Altmann [5] was the first to recognize the ubiquitous occurrence of these structures, calling them “bioblasts” and concluding that they were “elementary organisms” living inside cells and carrying out vital functions. Benda introduced the name ‘mitochondrion’ in 1898 [6]. The word originates from the Greek “mitos” (thread) and “chondros” (granule), referring to the appearance of these structures during spermatogenesis. A brief history of literature related to development of knowledge source related to mitochondrion has been provided in Table 1.

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Table 1
Chronology of advances in mitochondrion science.

Time	Advance	Authors
2002	Mito SOX	Batandier *et al.* [7]
1996	MitoTracker®	Poot *et al.* [8]
1991	JC-1/JC-9	Smiley *et al.* [9]
1988	TMRM/TMRE	Ehrenberg *et al.* [10]
1981	Carbocyanines	Johnson *et al.* [11]
1980	Rhodamine 123	Johnson *et al.* [12]
1975	Evidence for electron transport-linked proton pumps	Papa *et al.* [13]
1967	Separation and characterization of inner and outer membrane	Chappell *et al.* [14]
1963	Mitochondria contains DNA	Nass *et al.* [15]
1963	Energy-linked uptake of Ca^{2+}	Chappell *et al.* [16]
1961	Chemiosmotic hypothesis	Mitchell [17]
1958	Protein synthesis by mitochondria	McLean *et al.* [18]
1957	Termed 'Powerhouse of the cell'	Siekevitz [19]
1952	Electron micrograph of mitochondria	Palade [20]
1948	Isolation of intact mitochondria	Hogeboom *et al.* [21]
1934	Isolation of mitochondria by cell fractionation	Bensley *et al.* [22]
1925	Association of CytC with cellular structures	Keilin [23]
1900	Janus Green B	Michaelis [24]
1898	Term 'mitochondrion' was coined	Benda [6]
1890	Mitochondria as 'Bioblast'	Altmann [5]

TMRE, tetramethylrhodamine ethyl ester; TMRM, tetramethylrhodamine methyl ester.

3. The sperm mitochondria

The mitochondria of mammalian spermatozoa are restricted to the mid-piece of the flagellum. They wrap helically around the outer dense fiber axoneme complex in a species-specific manner during spermiogenesis to form a cylinder-shaped mitochondrial sheath [25]. Within the sheath, adjacent mitochondria associate both end to end and along their lateral surfaces. This positioning of a concentrated array of mitochondria adjacent to the flagellum is believed to be an efficient way to provide at least some of the energy required for motility [26]. The typical mammalian sperm mid-piece contains approximately 50–75 mitochondria with one copy of mitochondrial DNA in each [27]. The fluorescent probes stain mid-piece of the spermatozoa containing mitochondria for functionality evaluation.

3.1. Features

The mitochondrion (0.75–3 μm in dia, [28]) is a double membrane-bound organelle found in all eukaryotic organisms (except red blood cells) where they make up as much as 10% of the cell volume [29]. They are pleomorphic organelles with structural variations depending on cell type, cell cycle stage and intracellular metabolic state [30–32].

Morphologically, mitochondrion has two distinct membranes, the outer mitochondrial membrane (OMM) with smooth boundaries and the inner mitochondrial membrane (IMM) forming many invaginations and tubes covering almost whole of the lumen, called cristae. The inner membrane and the lamellar structures are connected by narrow tube-like connections, called the cristae junctions [33]. It is remarkable to note that the inner and the outer membrane have completely different protein content and are functionally distinct [30,34]. Porin (30–35 kDa) is the most abundant protein of the OMM. Through the pores of the trans-membrane channel located in porins, passage of ions and small molecules is facilitated [35,36]. The maximal molecular weight of uncharged molecules, which can pass through the porins, is about 5 kDa [30,37]. For transport of proteins <5 kDa, binding of signaling sequence at their N-terminus with a large multi-subunit protein called 'translocase' of the OMM is required which then actively moves them across the membrane [38]. Mitochondrial pro-proteins are imported through specialized translocation complexes. Any damage to outer membrane permits proteins in the inter-membrane space to leak into the cytosol, leading to eventual cell death [39]. Outer membrane also contains many enzymes, which are involved in such diverse activities as degradation of tryptophan and oxidation of epinephrine.

The inner membrane contains 76% protein, which is more than any other cellular membrane [34]. It is a tight permeability barrier, with functional consequences. The IMM encloses chemiosmotic apparatus for energy production [40,41]. In the process of oxidation (ATP production) of glucose and free fatty acids by enzymes in the mitochondrial respiratory chain, protons (H^+) are pumped into the cell cytosol. The resulting proton gradient results in development of electrostatic potential across the inner membrane [42]. Cation fluorescent probes in differentiating between functional or apoptotic mitochondrion exploit this property of the inner membrane (Figure 1). Mitochondrial membrane potential (MMP, $\Delta\Psi m$) is an important index of the bio-energetics state of the spermatozoa. Due to folding of the inner membrane (cristae), it has much larger surface area in comparison to outer membrane. The adenosine-5'- triphosphate (ATP) synthesis complexes are located in the cristae. The number and size of cristae depends upon energy demand of that particular cell [34]. In addition to being home to 151 types of proteins, inner membrane is rich in an unusual phospholipid, 'cardiolipin' which makes it impermeable [30,38,43]. Binding property of fluorescent probes such as 10-*N*-nonyl acridine orange and Mito-ID Red with cardiolipin is exploited to measure functional status of sperm mitochondria (Table 2).

The matrix is the space enclosed by the IMM containing about 2/3rd of the total proteins in a mitochondrion, hundreds of enzymes, special ribosomes, tRNA and several copies of mitochondrial DNA [30,44]. The mitochondria-associated ER membrane is another structural element that is increasingly recognized for its critical role in cellular physiology and homeostasis [45,46].

3.2. Functions

Mitochondria are cellular organelles that play a key role in maintaining the cellular bio-energetic- and ion-homeostasis and are producers of free radicals [47] in spermatozoa. At the same time, it also plays a central role in regulation of apoptosis (programmed cell death). Similar to 'Janus', the mitochondrion presents two faces looking both forward and backward. On the one hand it is involved in the maintenance of viability and vitality, and on the other side plays a central role in the regulation of programmed cell death [48,49]. Thus, the mitochondrion may be considered the guardian of the gate between life and death [47]. Figure 2 depicts vital functions performed by mitochondrion.

3.3. ATP production

The principle role of a mitochondrion is production of ATP for energy homeostasis. The ATP production is achieved by