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Mitochondria and fertility: the mitochondria critical role on spermatozoa function

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Abstract

Mitochondria of spermatozoa significantly differ, structurally and functionally, from the corresponding organelles of somatic cells. Nevertheless, sperm mitochondria, as well as the somatic ones, are the location of the oxidative phosphorylation (OXPHOS) process, which is necessary for the production of metabolic energy in the form of adenosine triphosphate (ATP). In addition to their basic role in oxidative energy generation, mitochondria are also a source of reactive oxygen species (ROS), which, at low concentrations, play a physiological role in many sperm processes.

It is commonly accepted that a proper functionality of mitochondria is necessary for a high quality of sperm cells; this last parameter, in its turn, is a prerequisite for a high sperm fertilizing ability.

Evaluation of mitochondrial respiratory efficiency represents therefore a valuable test that could integrate routine semen analysis.

Keywords: mitochondria, spermatozoa, oxidative phosphorylation, ROS, sperm motility

1. Introduction

Mitochondria are cellular cytoplasmic organelles which take part in a variety of cellular metabolic functions. They are involved in several pathways, such as the Krebs cycle, the oxidative decarboxylation of α-ketoacids, the β-oxidation of fatty acids, many reactions of the amino-acid metabolism and of the pyrimidine synthesis. Furthermore, mitochondria are actively implicated in other processes, such as cell differentiation, ROS generation, apoptosis, calcium signalling, iron metabolism, etc. However, these organelles are generally known as the energy-generating powerhouses of the cell, because they play a fundamental role in the production of adenosine triphosphate (ATP) through the sophisticated mechanism of the oxidative phosphorylation (OXPHOS).

This last process, which is the topic of this manuscript, requires the coordinated operation of two main components: the respiratory chain and the ATP-synthase, both located in the inner mitochondrial membrane.

The mitochondrial respiratory chain is involved in the transport of reducing equivalents from some electron-donors to the molecule of oxygen with the final formation of water. The energy released from these oxidation/reduction reactions is used to drive the synthesis of ATP from ADP. A strict coupling is therefore required between respiration, which is the electron transfer through respiratory chain complexes, and phosphorylation, which is necessary to synthesize ATP.

In addition to their basic role in ATP synthesis, mitochondria are a major source of reactive oxygen species (ROS), which are key mediators of cellular physiology and pathology.

Mitochondria of spermatozoa are different from the corresponding organelles of somatic cells, in both their morphology and biochemistry (Piomboni et al., 2012; Ferramosca and Zara, 2015).

They are helically arranged around the midpiece of sperm and show a peculiar morphology and arrangement. The biochemical difference is essentially related to the existence of specific enzyme isoforms, which are characterized by peculiar kinetic and regulatory properties.

It has been demonstrated that a proper functionality of mitochondria is necessary for a high quality of sperm cells and, in particular, for sperm motility. According to this hypothesis, structural and functional alterations are usually found in mitochondria from asthenozoospermic subjects.

However, not only motility but also several essential sperm functions require ATP as an energy source. Therefore, a careful and detailed investigation of mitochondrial bioenergetics of spermatozoa could provide more insight on the role of these organelles in the overall quality of the gametes. We are confident that evaluation of mitochondrial respiratory efficiency could integrate routine semen analysis in clinical investigation of male infertility.

2. A reliable tool for the evaluation of sperm respiratory efficiency

In 2008 we reported a relatively simple and fast method for analysing oxygen consumption, and therefore mitochondrial functionality, in individual human ejaculates (Ferramosca et al. 2008).

Human spermatozoa were incubated in hypotonic buffer to selectively disrupt the plasma membrane and were subsequently used for studies of mitochondrial respiration. The rupture of the plasma membrane, followed by the washing steps, caused the loss of the various metabolites contained inside the cells, thus exposing sperm mitochondria to an environment with a well- defined composition. At the same time, mitochondria maintained their intactness and functionality.

Oxygen uptake by hypotonically-treated spermatozoa was therefore measured at 36°C by using a Clark-type oxygen probe, in the presence of respiratory substrates (10 mM pyruvate and 10 mM malate) and 0.76 μM adenosine diphosphate (ADP) (Fig. 1). The ratio between the rate of oxygen uptake in presence of respiratory substrates plus ADP (V_3) and the rate of oxygen uptake in presence of the substrates alone (V_4), allowed for the calculation of a respiratory control ratio (or RCR).

In normozoospermic samples RCR was about 2.5, hence indicating a good coupling between respiration and phosphorylation. Interestingly, whereas the rates of oxygen uptake, as expected, changed with different sperm concentrations, the RCR values remained constant, thus demonstrating a linear response of the assay. The limit of sensitivity of this experimental system was found to be 1.5 x 10^7 sperm cells.

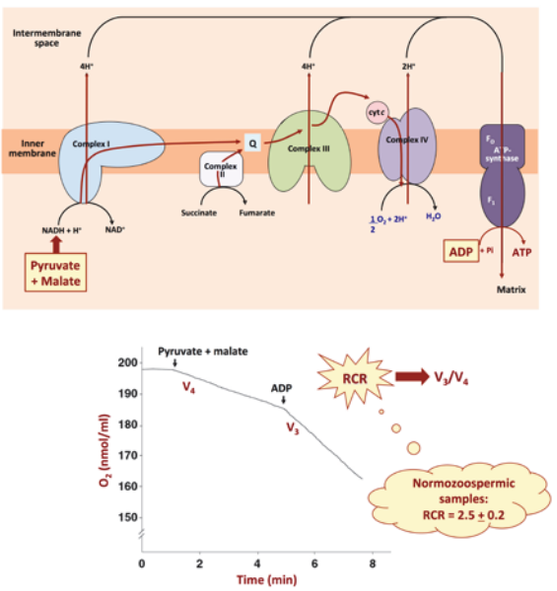

Figure 1: Assay of oxygen consumption in sperm cells

In order to test the possibility of a difference in mitochondrial function between progressively motile and less active sperm, we measured the respiration capacity of human sperm mitochondria from some asthenozoospermic subjects with a mean percentage of sperm motility of 36%. In these samples we found a significant reduction in the active state of respiration (V_3) when measured in the same experimental conditions used for normozoospermic samples. On the contrary, the V_4 value was almost unaffected, thus leading to a significantly lower value of RCR in asthenozoospermic subjects in comparison with the normozoospermic ones (Ferramosca et al. 2008).

The importance of mitochondrial functionality was also extended to hyperactivated motility observed during sperm capacitation. We meas-

ured the respiration capacity of human sperm mitochondria before and after swim up treatment (Stendardi et al., 2011). In sperm samples selected by swim up, we found a significant increase of about 10 fold in the V_3 and V_4 values. These results suggest that sperm motility strongly depends on mitochondrial respiratory function. Furthermore, high values of V_3 and V_4 obtained with sperm samples selected by swim up suggest that our experimental system responds also with a limited amount (less than 5 million) of sperm cells.

3. Sperm motility and mitochondrial respiratory efficiency

In a following study, we correlated sperm mitochondrial respiration, evaluated by the polarographic assay of oxygen consumption and RCR values, with variations in sperm motility (Ferramosca et al., 2012). Interestingly, we found a profile for RCR values with respect to a gradual increase or decrease in sperm motility (Fig. 2)

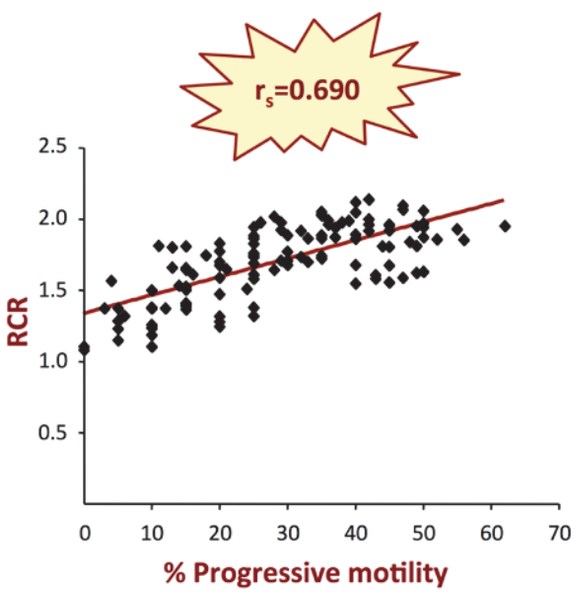

Figure 2: Sperm motility vs RCR values

In particular, V_3 (active state of mitochondrial respiration) positively correlated with progressive sperm motility, whereas V_4 (resting state of mitochondrial respiration) showed a lower positive correlation with sperm motility.
In the same study we also investigated a possible relationship between RCR values and the percentage of morphologic anomalies affecting the head, midpiece, or tail of spermatozoa. Interestingly, a strong negative correlation between mitochondrial respiration and defects in sperm midpiece, where mitochondria are exclusively localized, was found, suggesting that structural anomalies were accompanied by a parallel decrease in mitochondrial respiration. In agreement with these results, the anomalies in the sperm midpiece were also associated with decreased sperm motility.

4. Oxidative stress and sperm mitochondrial respiratory efficiency

For proper functionality of spermatozoa, an adequate level of ROS is required. Free radicals are involved in sperm hyperactivation and capacitation, acrosome reaction, spermatozoa-oocyte fusion, and other molecular events implicated in human fertility. However, high levels of ROS have negative influence on sperm quality and function.
What is the relationship between ROS and sperm mitochondria?
In this context we analyzed the dependence of mitochondrial respiration efficiency on seminal lipoperoxides (LPO) levels. LPO are a specific indicator of oxidative stress in seminal fluid. We found a strong negative correlation of RCR values with seminal LPO, suggesting that an increase of oxidative stress in seminal fluid (which is associated to an increase in the levels of LPO) was able to impair mitochondrial functionality.
Sperm DNA damage could be a marker of sperm quality and is often associated to oxidative stress. The analysis of dependence of sperm mitochondrial respiratory efficiency on sperm DNA fragmentation showed a strong negative correlation between RCR values and the percentage of DNA fragmentation.
When we analyzed V_3 and V_4 values, we found a moderate dependence of V_3 (which is the rate of oxygen consumption after the addition of ADP) on the levels of LPO and a strong correlation between V_4 and LPO. V_4 shows also a significant dependence on sperm DNA fragmentation, while V_3 seems to be independent.
We can therefore hypothesize that a condition of oxidative stress in the seminal fluid (which is

associated to an increase in the levels of LPO and in the percentage of DNA fragmentation) produces an increase in V_4, suggesting a stimulus of mitochondrial respiration, which is independent of ADP phosphorylation. These results suggest that oxidative stress, along with the concomitant phenomenon of sperm DNA fragmentation, negatively affects sperm mitochondrial respiration by an uncoupling between electron transport and ATP synthesis.

At least in principle, a double link exists between mitochondria and oxidative stress. In fact, on the one hand, mitochondria represent one of the ROS generators whereas, on the other hand, they might represent one of the ROS targets.

We eventually investigated the dependence of sperm mitochondrial respiratory efficiency on serum reactive oxygen species, a systemic indicator of oxidative stress (Fig. 3). Blood oxidative stress might also be a consequence of unhealthy lifestyles such as smoking, alcohol abuse, or exposition to chemical or electromagnetic pollution. Interestingly, we found a strong negative correlation between RCR values and a condition of oxidative stress. In fact, RCR values decreased at the increasing of serum reactive oxygen species. Also in this case, only V_4 showed a significantly correlation with radical species.

The analysis of blood oxidative status showed a parallel negative correlation between the levels of serum reactive oxygen species and the percentage of sperm progressive motility. Therefore, the analysis of blood oxidative status could be useful, together with the seminal profile, for the evaluation of sperm quality.

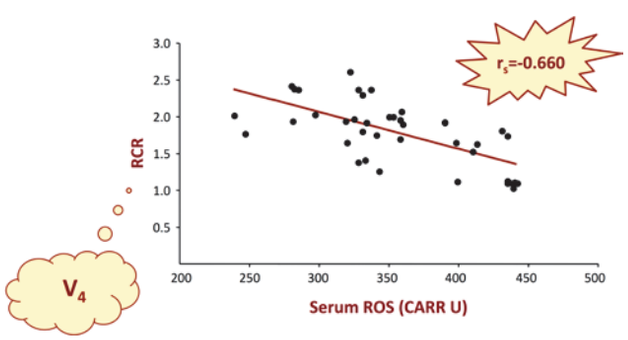

Figure 3: Serum ROMs vs RCR values

5. Varicocele and sperm mitochondrial respiratory efficiency

Varicocele is an enlargement of the veins that drain the testicle. This pathology is one of the main causes of male infertility and can impair sperm quality. Current evidence suggests that oxidative stress is a key element contributing to infertility in men with varicocele. Accordingly to these observations, we found a strong increase in the level of seminal LPO in varicocele samples and a higher incidence of spermatozoa with DNA damage in varicocele patients. Interestingly, in the same patients, we found for the first time an increase in the level of serum ROS, a systemic indicator of oxidative stress (Ferramosca et al., 2015).

The increased levels of ROS in serum and seminal fluid of varicocele patients negatively affected sperm mitochondrial respiration. In fact, when we analyzed the mitochondrial respiratory efficiency by polarography, we found that sperm mitochondria of varicocele patients showed a lower RCR values. In particular, we found a significant decrease in the V_3 values, indicating an impairment of active state of mitochondrial respiration (Ferramosca et al., 2015). We also observed a parallel increase in the percentage of structural defects in the sperm midpiece, corresponding to the sperm mitochondrial sheath.

The defective energy metabolism may play an important role in the impairment of sperm quality in varicocele patients, whose spermatozoa showed a decrease in motility and concentration when compared with control subjects.

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