Correlation of sperm motility with changes of mitochondrial membrane potential difference

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Introduction

Metabolic processes in biological cells are complex. However, there are some functions that are performed only in specific types of cells. Spermatozoa are the example of cells of functions of very well defined, single task: effective fertilization. The success of attaining this task is closely related to the ability of converting intracellular energy into the kinetic energy of the cell progressive movement, which can be quantized by measuring the sperm motility. The reduction in sperm cell functions and ability of functioning outside an organism, causes that sperm cells seems to be extremely attractive for studying the elementary intercellular energetic conversion processes or some chosen part of such a processes.

Mitochondria play significant role in many metabolic cell processes. In mitochondria the energy from food molecules (e.g. glucose) is converted into energy available for cell metabolic processes, and for that reason they are sometimes named "cellular power plants". During the citric acid cycle (Krebs cycle) NADH and $FADH_2$ molecules are produced. The energy released during successive oxidation-reduction reactions of NADH and $FADH_2$ allows pumping protons (H+ ions) from the mitochondrial matrix to the intermembrane space. As a result, an electrical potential difference $\Delta\Psi_m$ at the mitochondrial membrane develops causing polarization of the membrane. $\Delta\Psi_m$ is an important parameter of mitochondrial functionality and an indirect evidence of energy status of the cell. In particular, due to $\Delta\Psi_m$, the molecular motor enzyme ATP synthase undergoes the necessary conformational changes allowing for ATP (adenosine triphosphate) molecule synthesis. The energy stored in ATP is the main energy source for majority of cellular functions.

The main task of this study is to find a relationship between the changes in membrane potential difference $\Delta\Psi_m$ and the corresponding changes in bovine sperm motility. The underlying expectation is that the internal, microscopic measure for intermembrane potential energy (proportional to $\Delta\Psi_m$) , may by quantified by the outward, macroscopic measure such as sperm motility, which we can determine with our method based on a numerical processing of the optical contrast of the sperm images [1].

To indicate changes in $\Delta\Psi_m$ the organelle-specific lipophilic cationic dyes JC-1 and JC-9 were used. For low membrane potential, these dyes exist in a monomeric form of green (510-520 nm) fluorescence. High $\Delta\Psi m$ causes formation of J-aggregates of red-orange (590 nm) fluorescence. The combination of dye staining with flow cythometry technique and fluorescence microscopy imaging allowed us to study some aspects of mitochondrial activity of thousands of individual spermatozoa in each semen sample.

1. A. Sozańska, K. Kolwas, J. Galas, N. Błocki, A. Czyżewski, *Simple optical method of qualitative assessment of sperm motility: preliminary results*. Proceedings of SPIE, vol. 5959, 176, (2005)

Microscopic examination of the mitochondrial membrane potential difference and sperm viability

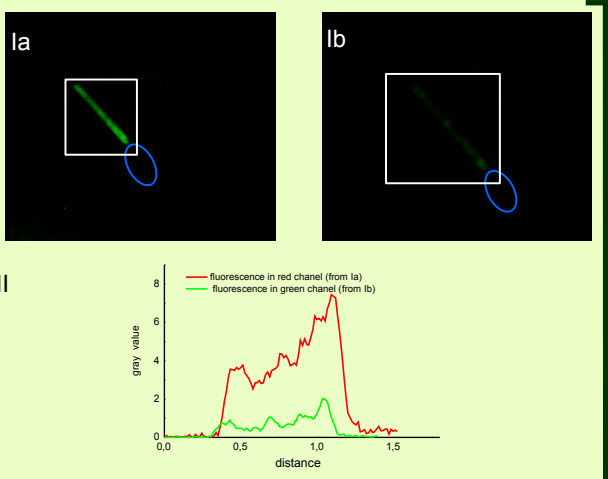

Fig.1. Fluorescence microscopy evaluation of fluorescently stained spermatozoa with JC-1: from the red (Ia) and from the green channel (Ib) of the same spermatozoa, Fig.(II) shows the fluorescence intensities for both channels

We used cryopreserved bull semen collected by Łowicz Semen Storage Centre which was warmed to the temperature of 37°C in the water bath and then centrifuged (x208 g). The best semen was collected by the swim-up technique and diluted. A sperm motility was accessed using our method [1] based on a numerical processing of the optical contrast of the sperm images of a movie which illustrates the dynamics of sperm cells movement. An appropriate analysis of a grey scale level of the superimposed images allowed as to quantise sperm motility .

A semen from three bulls was used to check if correlations between the mitochondrilal membrane potential difference $\Delta\Psi_m$ and the sperm motility exists. Each sample was separate to 5 and for each of them different times of incubation were chosen to differentiate the motility in successive trials. Each sample was than separated into 3 parts (spermatozoa plus JC-1 dye for the flow cythometry measurements; spermatozoa plus JC-1 dye for microscopic measurements and spermatozoa plus JC-1 plus CCCP as a control probe). The motility has been accessed at the beginning of each fluorometric measurements.

Results

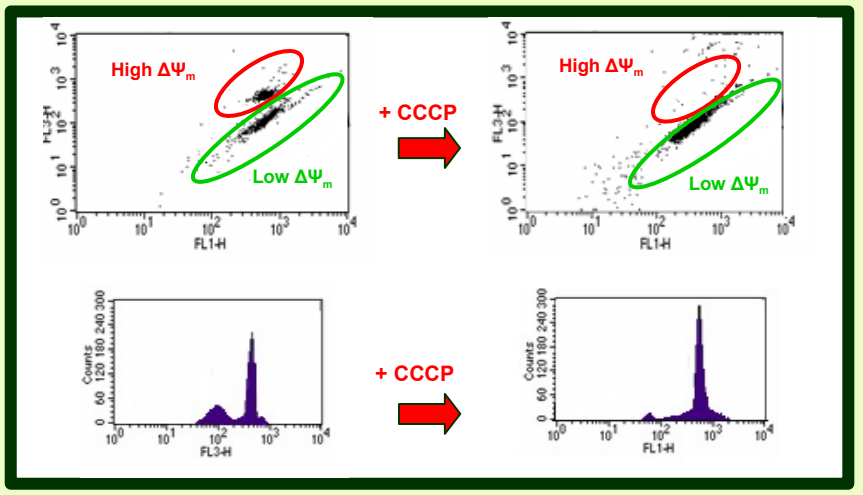

Fig. 2. Influence of the depolarizing agent CCCP on mitochondrial membrane potential ($\Delta\Psi_m$) of spermatozoa stained with JC-1, of initial motility **M = 45% ± 1.5**

The metabolitically active spermatozoa, stained with JC-1and JC-9 forms two distinct populations. A bright red-orange fluorescence (area inside red ellipse in Fig.2) is emitted by J-aggregates and marks the population with high membrane polarization $\Delta\Psi_m$. The population with low membrane potential is contained within area of green ellipse in Fig. 2).

For each sample which was analyzed with flow cythometric techniques, a negative control was performed, to checked that the staining was working properly. Samples were treated with CCCP which cancels the transmembrane potential difference $\Delta\Psi_m$ due to its protonophoric activity.

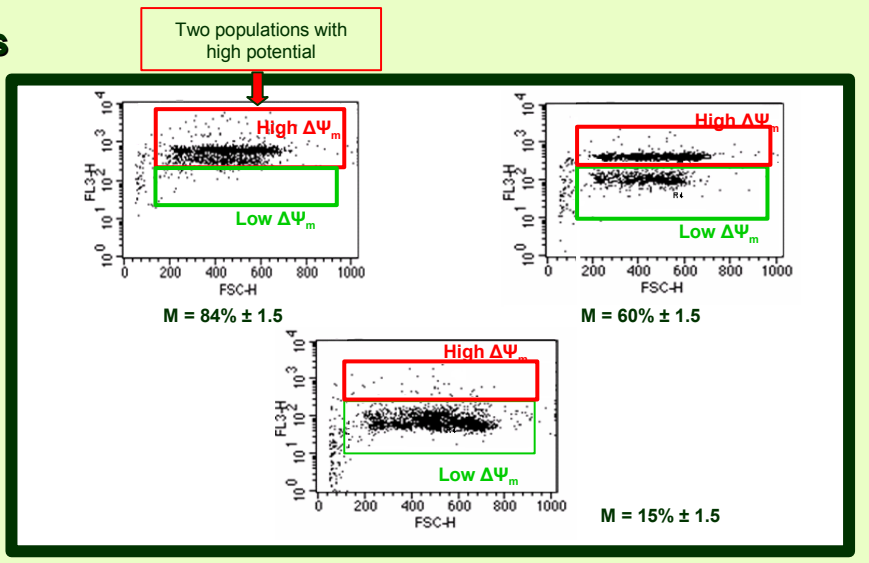

Fig. 3. Dot plots resulting from flow cythometric analysis of spermatozoa stained with JC-1, for different sperm motilities.

Figure 3 shows some populations characterized by high and low intermemrane polarization $\Delta\Psi_m$ for semen of different motilities from the bull nr I. We have got some similar plots for other bull samples. We can notice that the population of spermatozoa with high potential decrease when the sperm motility falls down.

It is interesting that for some motile spermatozoa samples, we can observed two (as shown in Fig. 3) or more intermediate populations with high $\Delta\Psi_m$.

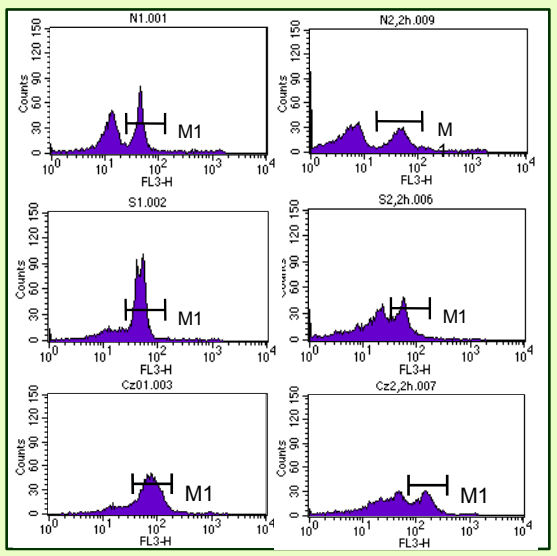

Fig. 4. The decrease in fluorescence intensity with time (2 hours of incubation) for mitochondria stained with JC-9

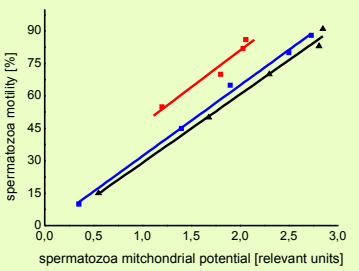

Fig. 5. Relation of the membrane potential difference $\Delta\Psi_m$ to the sperm motility for samples from three bulls.

Bull breed number	Correlation coefficient
I	0,995
II	0,997
III	0,996

Table I. Correlations coeficients for between microscopic and fluorometric assessment of spermatozoa mitochondrial activity and motility for 3 bulls.

The measure for the mitochondrial membrane potential difference $\Delta\Psi_m$ was set from cythometric measurements as the ratio of green to red fluorescence of sperm stained with lipophilic dyes JC-1 and JC-9. This ratio is dependent only on membrane potential, and not a mitochondrial size, shape or density.

As it is shown in Fig. 5 and Table I, there exist a strong correlation between changes in sperm motility and the mitochondrial membrane potential difference $\Delta\Psi_m$.

We can notice that mitochondrial activity of spermatozoa coming from bull from various breed can be different.

Conclusions

The goal of this study was to check if the correlation exists between sperm motility and the intermembrane mitochondrial potential difference $\Delta\Psi_m$. The preliminary results which we presented here are confirmative, but a larger statistics is still needed for quantitative analysis of the effect.

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