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CORRELATIONS BETWEEN ATP LEVELS OF HUMAN SPERMATOZOA AND CLASSICAL SEMEN PARAMETERS

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Abstract: The main focus of this study was to estimate whether there is a correlation between adenosine triphosphate (ATP) levels in human spermatozoa and classical semen parameters. Native sperm from 98 patients undergoing intracytoplasmic sperm injection (ICSI) procedure was used for ATP determination. The concentration and motility were automatically detected with CASA (Computer Assisted Sperm Analysis). Evaluation of morphology was performed according to Tygerberg-Kruger strict criteria. ATP concentration in sperm was measured by a bioluminescence assay. The statistical analyzes were done by using SPSS 21.0.

Our data showed a statistically significant correlation between ATP levels and concentration, motility, viscosity and morphology of the spermatozoa. ATP amount per sperm was found to be positively correlated with active spermatozoa motility (class A: R=0.456; class B: R=0.393), concentration (R=0.396) and morphology (R=0.245). A negative correlation between ATP levels and viscosity was observed (R= -0.321).

Patients with high ATP levels have better sperm morphology, higher concentration and motility and lower viscosity. Thus, it is concluded that the ATP concentration in human spermatozoa may serve as a potential physiological biomarker in combination with classical sperm parameters.

INTRODUCTION

Male infertility diagnosis is mainly based on the conventional semen analysis as specified in the manual of the World Health Organization (WHO), with a strong

emphasis on the evaluation of semen volume, sperm concentration, motility and morphology. About 75% of men with reduced fertility are oligozoospermic (very low sperm concentration) or asthenozoospermic (sperm with reduced motility). Both conditions are difficult to treat and the etiology is not always clear. Over the last decade scientists' attention shifted to another probable factor for male infertility – the bioenergetic status of spermatozoan mitochondria. One of the main mitochondrial function parameters in sperm determining fertile potential is the production of ATP. The profound study of that particular mitochondrial indicator is an important step in understanding the causes leading to male infertility.

Spermatogonia, mature sperm and Sertoli cells exhibit a high glycolytic activity, while spermatocytes and spermatids produce ATP, mainly by oxidative phosphorylation (Robinson and Fritz, 1981; Grootegoed et al, 1984; Nakamura et al, 1984; Bajpai et al, 1998; Meinhardt et al, 1999).

The issue whether the main source of ATP production for movement is the glycolytic pathway or the oxidative phosphorylation still remains, as it is unclear how synthesized ATP is transported from the mitochondria to the final sections of the sperm tail and dynein ATPases. The presence of ATP shuttles for ATP transportation in human sperm has not been proven yet. The experimental inhibition of the respiratory chain or genetic defects in the proteins lead to severely reduced sperm mobility. On the other hand, defects in glycolytic enzymes also have such negative effects (Ruiz-Pesini et al, 2000; St John et al, 2005).

ATP, synthesized by sperm mitochondria serves as a provider of the motor activity and the processes of capacitation, hyper activation and acrosome reaction. That is why ATP seems to be promising marker for establishment of sperm functionality.

MATERIALS AND METHODS

Semen analysis

The results obtained in this study were based on data from the standard semen analysis and ATP levels in 98 patients aged between 24 and 54 years suffering from infertility and undergoing ICSI procedure. Samples were collected and analyzed from November 2013 to September 2014 at Nadezhda, Women's Health Hospital. The semen samples were obtained by masturbation following 3-5 days a sexual abstinence. The analysis of the ejaculate was carried out with an automatic analyzer, strictly following the World Health Organization requirements. The ejaculate was collected in a non-cytotoxic, sterile plastic container. After the container was received, it was placed in an incubator with 5% CO_2 and temperature of 36°C for 10-30 minutes in order to liquefy the ejaculate. After the liquefaction, the sample was treated in a sterile laminar flow hood. The standard semen analysis involved volume measurement with a sterile syringe, viscosity and colour evaluation. The pH was determined by using litmus strips

(pH range 4.5-10). Fresh samples were analyzed according to WHO criteria (WHO laboratory manual for the examination and processing of human semen - 5th Edition, 2010). Automatic reading with counting chamber MAKLER was used for concentration and motility determination. 5 µl of seminal sample was taken, using an automatic pipette. The sample was put in MAKLER chamber and was examined under microscope with negative phase contrast, 20x magnification objective lens. The chamber depth was 0,01 µm. The cover glass centre had a square grid sized 1x1 mm, containing 100 small squares.

The counted sperm number in 10 squares showed the concentration in 1 ml (in millions). Sperm motility was assessed by counting motile and immotile sperm and calculated as a percentage of the total concentration.

CASA - Computer Assisted Sperm Analysis

The concentration and motility were measured using MedeaLAB-CASA (Fig. 1). Concentration above 15 million sperm/ml was considered as normal. Samples with higher concentration (above 150 million sperm/ml) were diluted with seminal plasma from the same patient. Samples with a concentration lower than 15 million sperm/ml were excluded from the analysis. In the study, an Olympus CX-41 microscope was used, equipped with a camera that was connected to a computer and the recorded data were analyzed via specialized software. Negative phase contrast objective lens with 20x magnification were used. A drop of 5 µl was applied to the MAKLER chamber and after that the system recorded and analyzed the received data. Two chambers were made using each patient sample and were recorded on two different fields from each chamber. Depending on the velocity and trajectory of sperm movement, spermatozoa were divided into 4 categories – class A, B, C and D. Class A and B spermatozoa were categorized as progressively motile; class C – non-progressively motile and class D – immotile.

The computer-assisted semen analysis is a fast and sensitive method, therefore it is preferred for such experiments.

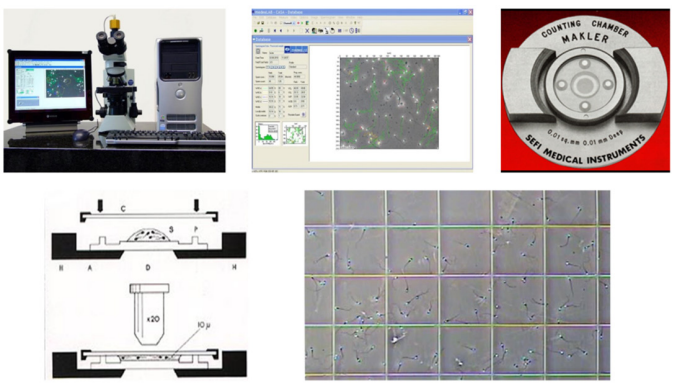


Figure 1. Automatic computer-assisted sperm analysis – CASA (MedeaLab)

Morphological evaluation strict criteria Tygerberg-Kruger

Sperm morphology assessment was performed in accordance with the strict criteria of Tygerberg-Kruger (Carell and Liu, 2001; Zini and Bielecki, 2001). For spermatozoa staining commercial Sperm Stain Kit (Microptic) was used.

Quantification of ATP in sperm

ATP Determination Kit A22066 from Life Technologies (Invitrogen) was used for ATP quantitation. ATP Determination Kit is a bioluminescent assay for the quantitative determination of ATP with a recombinant luciferase and its substrate D-luciferin (Fig. 2).

$$\text{Luciferin} + \text{ATP} + O_2 \xrightarrow[\text{Luciferase}]{Mg^{2+}} \text{Oxyluciferin} + \text{AMP} + \text{Pyrophosphate} + CO_2 + \text{light}$$

Figure 2. Quantitative ATP determination by using a recombinant firefly luciferase and its substrate D- luciferin. The assay is based on a simple luciferase assay, which requires ATP for the enzymatic reaction to produce luminescent light (emission maximum is approximately 560 nm at pH 7.8).

Statistical analysis

Statistical analyzes were carried out using SPSS software (version 21.0). Correlations between ATP levels of human spermatozoa and classical semen parameters (sperm concentration, motility, morphology and viscosity) were analyzed by Spearman's rank correlation ($P < 0.05$).

RESULTS AND DISCUSSION

Characteristics of the classical semen parameters in the studied patients

The median sperm concentration of the samples was 79.11 x 106/ml and the median morphology was 8%. The median percentage of classes A, B, C and D of the studied patients was 19.27%, 20.17%, 9.28% and 51.27%, respectively (Fig. 3).

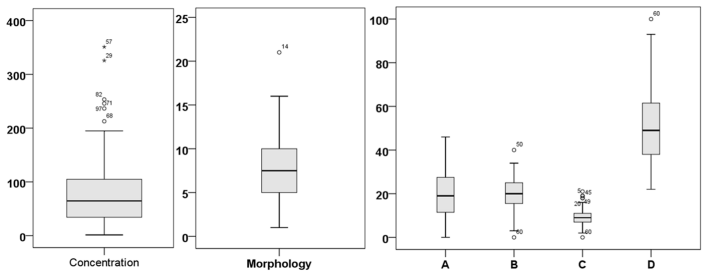
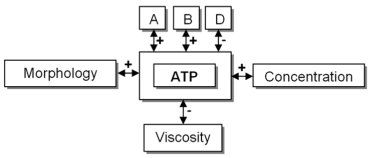


Figure 3. Box plots showing the variation of selected semen parameters in the studied population of patients – concentration (million spermatozoa/ml), morphology and motility (% of classes A, B, C and D). Box plots show 10th, 25th, 50th, 75th and 90th percentiles with horizontal lines.

Correlations between ATP levels and classical semen parameters

The analyzed results proved the presence of statistically reliable correlation between the standard sperm indicators and ATP levels in the spermatozoa with the following parameters: concentration, motility, morphology and viscosity. The correlation with progressive motile sperm (classes A and B), concentration and morphological assessment was positive, while the correlation with viscosity was negative (Fig. 4).



	Correlation coefficient (P value)	
ATP	A	0,456** (0,001)
	B	0,393** (0,004)
	C	0,243 (0,075)
	D	-0,432** (0,009)
	Concentration	0,396** (0,033)
	Morphology	0,245* (0,048)
	Viscosity	-,321**

Figure 4. Correlations between ATP levels of human spermatozoa and classical semen parameters

In 1987, Megory and colleagues examined the relationship of this energy parameter (ATP) with some of the basic indicators of semen analysis (concentration, motility, morphology). Using multiple regression analysis, the authors found a significant association of ATP only with sperm concentration, but found no relationship to motility and morphology. According to the authors, sperm ATP levels were not relevant to the IVF outcome - the presence or absence of implantation. Ultimately, the authors concluded that ATP is not relevant enough to be included in the human ejaculate routine analysis (Megory et al, 1987). The results obtained from our study also showed a significant relationship between ATP levels and the sperm concentration (R = 0.396), but unlike these authors, a statistically significant relationship was established with motility (class A: R=0.456, P=0.001; class B: R=0.393, P=0.004) and morphology (R=0.245, P=0.048).

Charles Vigee and colleagues (1992) examined ATP in human sperm; the levels they measured were comparable to the levels presented in our study - between 0.005 μM and 0.02 μM in 105 sperm count. The authors found a difference in ATP concentration during sample analysis in normozoospermic and oligozoospermic patients (ATP 123.1 ± 21.6 to 90.0 ± 24.5 pmol/106 sperm, respectively), but unlike Megory et al (1987) and our results the correlation was not statistically significant. Moreover, they found that sperm with higher fertilization potential did not show higher ATP levels compared to sperm with lower fertilization potential.

Their conclusion is that ATP levels are not predictive for male infertility (Vigue et al, 1992). The data published by Megory, however, was confirmed in our study - higher value of ATP was observed in patients with higher sperm count. Similar results were obtained from Irvine and Aitken, who detected a positive correlation between sperm concentration and ATP levels (Irvine and Aitken, 1985).

Our results demonstrate a positive relationship between ATP and the motile sperm percent - class A ($R = 0.456$, $P = 0.001$) and class B ($R = 0.393$, $P = 0.004$); and negatively correlated with the immotile sperm percentage - class D ($R = -0.432$, $P = 0.009$). The relationship is statistically significant. Direct correlation between sperm motility and ATP levels has been reported by other authors: (Chan and Wang, 1987; Irvine and Aitken, 1985). Despite reporting positive relationship between the concentration of sperm in the ejaculate and sperm ATP levels, Levin et al. found no such relation between motility and ATP production (Levin et al, 1981). Similar results were published by Pousette and colleagues (1986), but for unprocessed ejaculate. Performing this experiment with density gradient purified sperm, they showed a strong positive correlation ($R = 0.88$) between motility and ATP (Pousette et al, 1986).

There is not enough data in the literature in terms of morphological assessment of sperm and its relation with ATP production. We found a weak positive correlation between these two parameters ($R = 0.245$, $P = 0.048$), while Megory‘s team found no such relation (Megory et al, 1987).

Our data demonstrated a negative correlation between the ejaculate‘s viscosity and ATP levels in spermatozoa. So far, there are no published data describing this type of relationship. Further studies are needed to confirm or refute the correlation between these two sperm parameters.

CONCLUSIONS

In conclusion, ATP levels have significant positive and negative correlations with several important seminal parameters (concentration, motility, morphology assessment and viscosity), which makes the ATP molecule a suitable candidate for the evaluation and selection of sperm in the field of assisted reproductive technology.

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