DOI: 10.4149/BLL_2013_129

Bratisl Lek Listy 2013; 114 (11)
607 – 609

PERSPECTIVES

Importance of the assessment of coenzyme Q_{10}, alpha-tocopherol and oxidative stress for the diagnosis and therapy of infertility in men

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Abstract: Male infertility is one of the most stressful factors of couples, being present in about 40% cases. It is usually caused by a low number of sperm (oligozoospermia) or poor sperm motility (asthenozoospermia). The sperm motility is used as an indicator of semen quality and male infertility. To the impairment of male reproduction health can contribute genetic, nutritional and environmental factors, smoking and drugs. It is well documented that excessive reactive oxygen species (ROS) production decreases sperm motility, impairs sperm function, damages the morphology of spermatozoa (1, 2). To the decreased sperm motility contribute also disturbances of sperm mitochondrial function and energy production, low levels of coenzyme Q_{10} and carnitine, as well as sperm mitochondrial deoxyribonucleic acid (DNA) defects. The origin of sperm dysfunction, however, is not well understood.

Background: Oxidative stress has been established as a major factor in the pathogenesis of male infertility. Low level of coenzyme Q_{10} contributes to the decreased sperm motility, which plays a vital role in sperm mitochondrial energy production and neutralization of reactive oxygen species (ROS).

The aim of the present study was to find out, if an assessment of coenzyme $Q_{10\text{-TOTAL}}$ ($CoQ_{10\text{-TOTAL}}$), α-tocopherol, γ-tocopherol and oxidative stress could contribute to the diagnosis of infertility in men.

Subjects and methods: Two groups of infertile men, according to sperm motility (a+b and b+c) were included in the study. $CoQ_{10\text{-TOTAL}}$, α-tocopherol, γ-tocopherol in plasma and seminal fluid, and parameter of oxidative stress (thiobarbituric acid reactive substances - TBARS) in plasma were determined.

Results: Higher sperm density and decreased sperm pathology were found in group a+b vs b+c (class a and b - fast and weak forward motility, class c - nonprogressive motility). Concentrations of $CoQ_{10\text{-TOTAL}}$ and α-tocopherol were significantly increased in seminal fluid of groups a+b vs b+c, opposite results were estimated in plasma. Concentrations of γ-tocopherol in plasma and seminal fluid of both groups were similar. Plasmatic TBARS concentrations were increased in both groups of infertile men.

Conclusion: We suppose that incorporation of oxidative stress assessment, $CoQ_{10\text{-TOTAL}}$ and α-tocopherol concentrations in seminal fluid and plasma into routine andrology can play an important role for the diagnosis and targeted therapy of male infertility *(Tab. 1, Ref. 16)*. Full Text in PDF *www.elis.sk*.

Key words: infertility, coenzyme Q_{10}, alpha-tocopherol, oxidative stress, diagnosis.

The aim of the present study was to find out, if an assessment of CoQ_{10}, α-tocopherol, γ-tocopherol and oxidative stress in plasma and seminal fluid can contribute to the diagnosis and targeted therapy of male infertility.

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Acknowledgements: This study was supported by grants of Ministry of Education, Slovakia, VEGA, 1/0614/12 and 1/0686/10. Technical assistance: A. Štetková.

Material and methods

Infertile men (n=37), aged 28–38 years, were included in this study. Subjects were divided into two groups according to sperm motility: a+b motility (n=18), b+c motility (n=19). Seminal fluid was collected after 3–5 days of sexual abstinence. Seminal fluid analysis, as sperm concentration, motility, and morphology in male subjects was assessed in accordance with WHO criteria (3). Motility was graded as follows: class a and b – fast and weak forward motility, class c – nonprogressive motility. Each subject signed an agreement with incorporation into the study. Plasma and seminal fluid concentrations of $CoQ_{10\text{-TOTAL}}$ (ubiquinol – $CoQ_{10\text{-REDUCED}}$ + ubiquinone – $CoQ_{10\text{-OXIDIZED}}$), α-tocopherol, γ-tocopherol were determined by HPLC method using UV detector (4, 5) with minor modifications as follows: 1.0 mL seminal fluid + 200 µL p-benzoquinone of 18.5 mmol/L + 2.0 mL methanol + 1.0 mL 0,1

Tab. 1. Antioxidants and TBARS in seminal fluid and plasma of infertility in men.

	SEMINAL FLUID (µg/ml)			PLASMA (µmol/l)	
	Groups		Reference	Groups	
Parameters	a+b	b+c	values	a+b	b+c
$CoQ_{10\text{-TOTAL}}$					
Mean	**0.147**	**0.094**↓	0.4–1.0	**0.434**	**0.606**↑
SD	0.048	0.041		0	0.145
±	0.012	0.010		0.025	0.037
P	**0.003**			**0.00066**	
α-tocopherol					
Mean	**0.669**	**0.448**↓	15–40	**24.44**	**32.73**↑
SD	0.343	0.151		5.6	6.10
±	0.086	0.038		1.26	1.58
P	**0.025**			**0.00028**	
γ-tocopherol					
Mean	0.039	0.042	2–7	2.080	2.171
SD	0.022	0.015		0.946	0.765
±	0.006	0.004		0.223	0.180
P	**NS**			**NS**	
TBARS					
Mean	9.188	8.979	≤4.5	5.467	5.354
SD	1.657	1.616		1.097	0.707
±	0.460	0.392		0.257	0.171
P	**NS**			**NS**	

a+b – fast and weak forward sperm motility, b+c – weak, nonprogressive sperm motility, $CoQ_{10\text{-T OTAL}}$ – Coenzyme $Q_{10\text{-TOTAL}}$ α-tocopherol – alpha-tocopherol, γ-tocopherol – gamma-tocopherol, TBARS – thiobarbituric acid reactive substances, Mean – mean of numbers, SD – standard deviation, ± – standard error, p – statistical significance

M sodium dodecyl sulphate, vortexed 1 min, with 3.0 mL and 2.0 mL hexane, vortexed 5 min, centrifuged for 10 min, hexane layer dried under gas N_2, residue dissolved in 50 µL ethanol, and 20 µL used for HPLC analysis. Thiobarbituric acid reactive substances (TBARS) – parameter of oxidative stress - was determined spectrophotometrically (6). Statistical analysis of the data was made using unpaired Student´s t-test between groups: a+b and b+c, statistical significance was at $p<0.05$.

Results

Significant differences were detected in *sperm density* between groups a+b and b+c (24.58 ± 2.17 x 10^6/ml vs 13.53±1.76x10^6/ml), $p<0.0003$, in *sperm pathology* a+b (33.75±1.25 %) vs b+c (51.11±2.54 %), $p<0.000001$. *In seminal fluid* concentrations of coenzyme $Q_{10\text{-TOTAL}}$ and α-tocopherol were significantly higher in groups a+b vs b+c (0.147±0.012 µg/ml vs 0.094±0.010 µg/ml, $p<0.003$, resp. 0.669±0.086 µg/ml vs 0.448±0.038 µg/ml, $p<0.025$). Concentrations of γ-tocopherol and TBARS in seminal fluid were similar in both groups. *In plasma* of infertile men concentrations of coenzyme $Q_{10\text{-TOTAL}}$ and α-tocopherol were significantly lower in groups a+b vs b+c (0.443±0.025 µmol/l vs 0.606±0.037 µmol/l, $p<0.00066$, resp. 24.44±1.26 µmol/l vs 32.73±1.58 µmol/l, $p<0.00028$). In both groups concentrations of γ-tocopherol and TBARS in plasma were without significant differences, however TBARS in both groups of infertile men was increased (5.467±0.257 µmol/l and 5.354±0.171 µmol/l) in comparison with reference values, ≤4.50 µmol/l (Tab. 1).

Discussion

Oxidative stress has been established as a major factor in the pathogenesis of male infertility. It can be caused by infection or inflammation in the genital tract, stimulation of leukocytes activity and depressed seminal antioxidant capacity of enzymes (superoxide dismutase, glutathione peroxidase and catalase). While baseline ROS concentration is essential for sperm function, excessive ROS production decreases sperm function, mitochondrial activity and DNA integrity (7, 8, 9). The etiology of sperm DNA damage in male infertility is multifactorial, such as nuclear protein deficiency, apoptosis, drugs, cigarette smoking, leukocytospermia, varicocelle, chemotherapy and radiotherapy (10).

Plasma membrane of spermatozoa contains high concentrations of polyunsaturated fatty acids (PUFA) and low concentrations of scavenging enzymes in their cytoplasm (11). Oxidative deterioration PUFA is known as peroxidation of lipids. Other metabolic changes can play a role in male infertility , as decreased ω-3-PUFA concentration and increased ω-6/ω-3 PUFA ratio (12).

Lipoperoxidation (TBARS) is one parameter of oxidative stress. Our results confirmed high levels of plasmatic lipoperoxidation in both groups (with sperm motility a+b and b+c) of infertile men in comparison with reference values. TBARS of seminal fluid was similar in both groups (Tab. 1).

To the decreased sperm motility contributes also to disturbances of sperm mitochondrial function and energy production, low levels of coenzyme Q_{10} and carnitine. A vital role plays CoQ_{10} in sperm mitochondrial energy production and neutralization of ROS. CoQ_{10} biosynthesis is also present in testis, its reduced form with higher protective role as a scavenger of ROS is present in high levels in semen (4). Reduced levels of CoQ_{10} in the seminal plasma and sperm cells of infertile men with idiopathic and varicocelle-associated asthenozoospermia were demonstrated (13). In our study, concentrations of $CoQ_{10\text{-TOTAL}}$ and α-tocopherol in seminal fluid were significantly higher in group a+b (with higher motility) vs group b+c (with lower motility). Opposite results were found in plasma, significantly lower concentration of antioxidants ($CoQ_{10\text{-TOTAL}}$ and α-tocopherol) were in group with higher sperm motility (Table 1). In agreement with authors (1), lower CoQ_{10} plasmatic levels do not indicate the tissue deficiency of CoQ_{10}, but suggest a defect in energy metabolism. Diminished concentrations of $CoQ_{10\text{-TOTAL}}$ and α-tocopherol in seminal fluid contribute to the decreasing sperm quantity, function and to the increasing pathology. There is a rationale supporting the use of antioxidants in infertile men with elevated ROS levels and low antioxidant defenses.

Several treatments are used for improving sperm function, as lipophilic (vitamin E, CoQ_{10}, carotenoids) and hydrophilic antioxidants (carnitine, vitamin C). At present it is not recommended which of antioxidants, their daily doses and combinations should be more effective and safe (7). Vitamin E is a major chain-breaking antioxidant in the sperm membranes and it appears to have a dose-dependent protective effect. Treatment with 300 mg vitamin E daily, significantly reduced oxidative stress, and improved sperm motility. Vitamin C level in plasma of about 1000 microrgam/L positively influenced the motility of spermatozoa. Higher con-

centration of vitamin C reduced the motility of the spermatozoa, doses under 200 mg of vitamin C did not prove any benefit. Carnitine plays an important role in sperm maturation and development. CoQ_{10} protects lipids against peroxidative damage, it is a key component for sperm mitochondrial energy production. Large doses of antioxidants should be avoided because of negative effects, such as selenium in higher doses significantly reduces the number of motile spermatozoa in fertile men (14).

A consensus is still required on the type and dosage of antioxidants to be used for improving sperm parameters of infertile men. Previously, supplementary therapy with combination of ubiquinone, carnitine, vitamin E and vitamin C was found to be more effective on sperm count and motility of male infertility (15, 16) in comparison with single antioxidant (13, 16).

These results suggest an important role of CoQ_{10}, vitamin E and oxidative stress in sperm function. We suppose that supplementary therapy with stronger antioxidant - ubiquinol in combination with other antioxidants may reduce oxidative stress and could be beneficial for improving sperm function in male infertility.

Conclusion

Our results show the importance of assessing oxidative stress, $CoQ_{10\text{-TOTAL}}$ and α-tocopherol concentrations in seminal fluid and in plasma for the diagnosis of male infertility. We suppose that incorporating these metabolical estimation into routine andrology practice is important for diagnosis and targeted antioxidant and energy therapy of male infertility. Supplementary therapy with optimal combination of ubiquinol with other antioxidants is warranted.

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Received May 30, 2012.
Accepted August 18, 2013.