Antioksidansi i oplodni potencijal sjemena

dr.sc. Miro Šimun Alebić dr. med

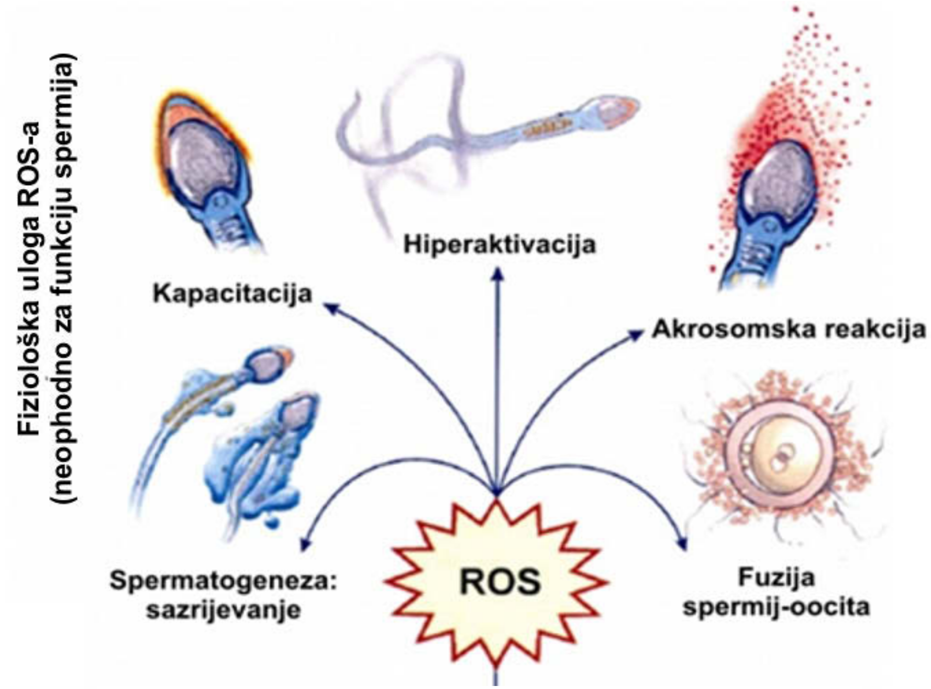
Cilje

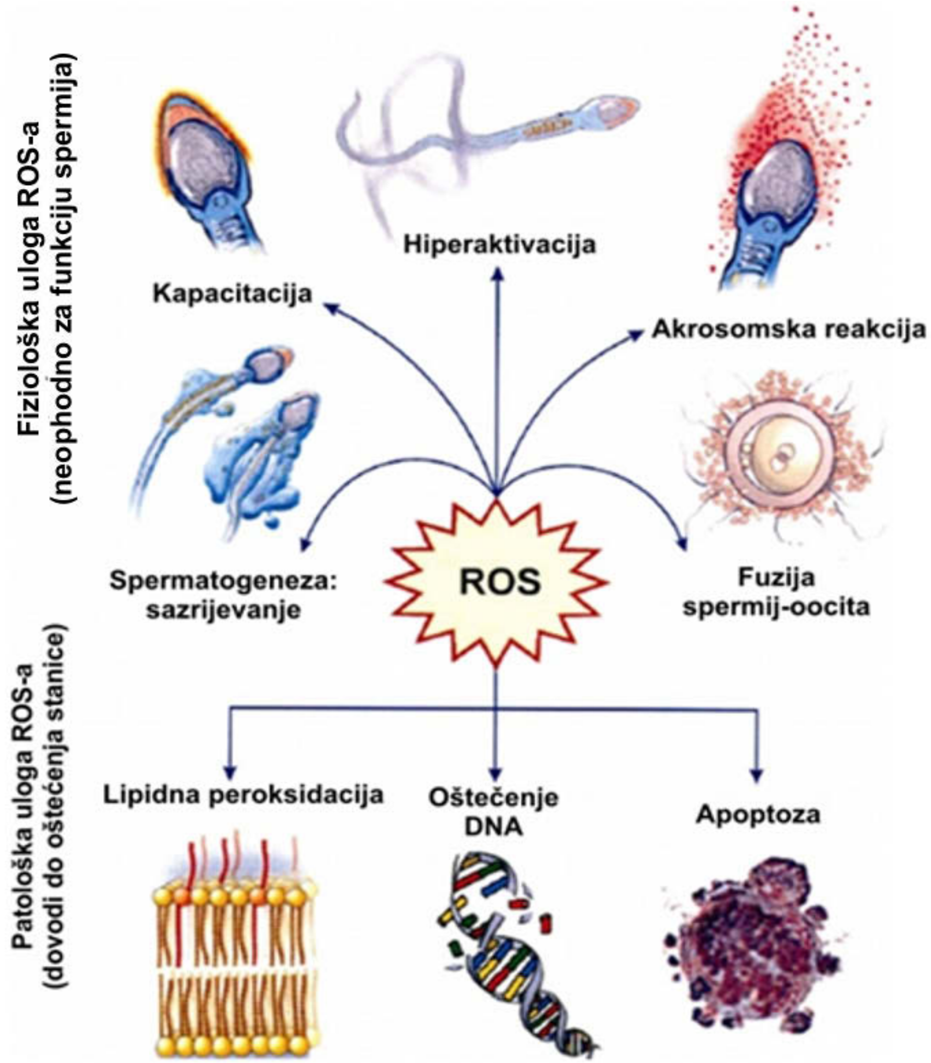
1. razjasniti
   - ulogu slobodnih radikala u fiziologiji reprodukcije i patofiziologiji muške neplodnosti
   - povezanost razine antioksidativnih molekula i smanjenog oplodnog potencijala sjemena

2. predstaviti
   - uloga suplemenata antioksidansa u liječenju muške neplodnosti

3. pokazati
   - vlastita iskustva

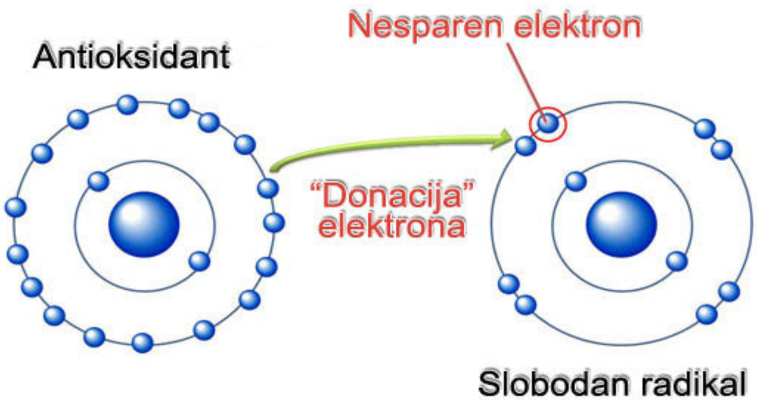
Repet

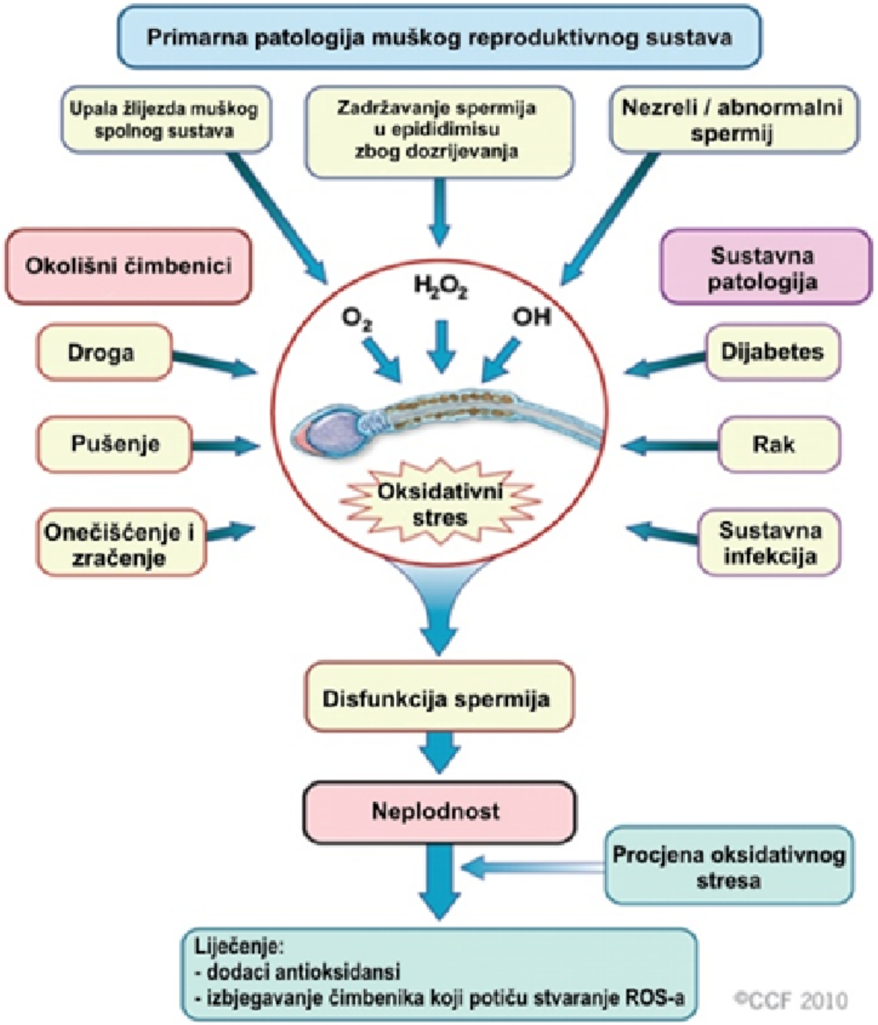

Fiziološka uloga ROS-a
(neophodno za funkciju spermija)
Kapacitacija
Hiperaktivacija
Akrosomska reakcija
Spermatogeneza:
sazrijevanje
ROS
Fuzija
spermij-oocita



antioksidacijski enzimski sustavi:

1. superoksidna dismutaza (prostata),
2. katalaza (spermiji, prostata)
3. glutation peroksidaza (prostata)





Izvor: Agarwal et al. 2010, P.21

OKSIDATIVNI STRES

30-80% uzroka muške neplodnosti

Tremellen, 2008

K l i n i č

Agarwal et al. Reproductive Biology and Endocrinology 2014, 12:126
http://www.rbej.com/content/12/1/126

RB&E REPRODUCTIVE BIOLOGY AND ENDOCRINOLOGY

RESEARCH Open Access

Reactive oxygen species and sperm DNA damage in infertile men presenting with low level leukocytospermia

Ashok Agarwal[1,3*], Aditi Mulgund[1], Saad Alshahrani[1,2], Mourad Assidi[3,4], Adel M Abuzenadah[3,4], Rakesh Sharma[1] and Edmund Sabanegh[1]

Conclusions: Infertile men, irrespective of their clinical diagnoses, have reduced semen parameters and elevated ROS levels compared to proven fertile men who have established a pregnancy recently or in the past. Reactive oxygen species are negatively correlated with traditional semen parameters such as concentration, motility and morphology. Measuring ROS levels in the seminal ejaculates provides clinically-relevant information to clinicians.

Table 1 - Reactive oxygen species (ROS) levels in donors and male factor infertility (MFI) patients and its correlation with semen quality score

Study population	SQ score	P value[a]	ROS levels	P value[b]	Correlation coefficient	P value[c]
Donors	97.07 ± 10.76 (n = 91)	-	1.20 ± 0.80[d] (n = 76)	-	- 0.45	< .001
MFI patients	75.56 ± 18.55 (n = 133)	< .001	2.29 ± 1.05 (n = 121)	< .001	- 0.36	< .001
Oligozoospermic	64.70 ± 14.93 (n = 61)	< .001	2.70 ± 1.18 (n = 57)	< .001	- 0.17	.20
Asthenozoospermic	70.55 ± 18.00 (n = 96)	< .001	2.30 ± 1.10 (n = 90)	< .001	- 0.39	.0002
Teratozoospermic	66.82 ± 16.50 (n = 69)	< .001	2.40 ± 1.19 (n = 66)	< .001	- 0.39	< .001
OAT	56.01 ± 12.69 (n = 38)	< .001	2.82 ± 1.21 (n = 35)	< .001	- 0.16	0.32

*Values are mean ± SD; OAT = oligoasthenoteratozoospermic; SQ = semen quality; [a]$P < .05$ was considered significant comparing SQ score between donors and different groups of infertile patients; [b]$P < .05$ was considered significant comparing ROS levels between donors and different groups of infertile population; [c]$P < .05$ was considered significant using Pearson correlation coefficient between SQ score and ROS levels; [d]Log (ROS+1) were used

Koncentracija slobodnih radikala u sjemenoj tekućini je veća u neplodnih muškaraca neovisno o njihovoj kliničkoj dijagnozi.

Table 4 The value of Pearson's correlation coefficients calculated between the antioxidant parameters and sperm criteria

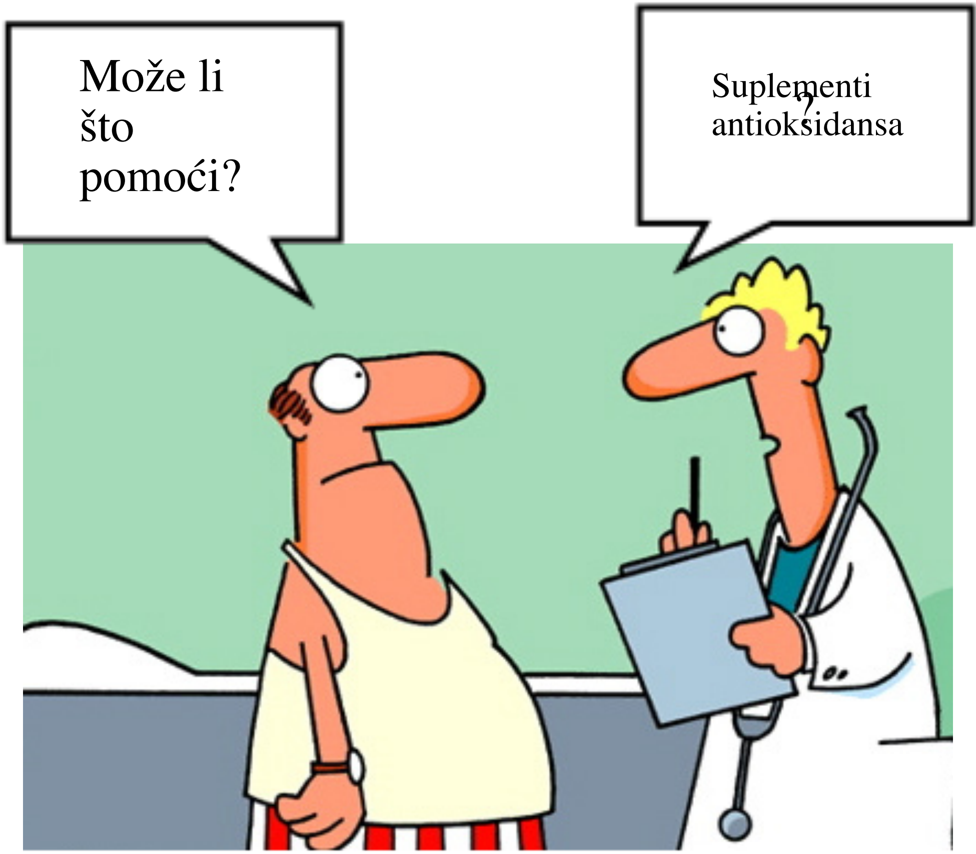
Elements	Motility	Sperm count	Atypical forms
Zinc	**0.29***	**0.49****	NS
Selenium	**0.36****	NS	NS
GSHt	**0.13***	NS	NS
GSSG	**0.22****	NS	**-0.39***
GSHr	**0.42****	**0.11***	**NS**
MDA	**-0.24***	**-0.35***	**0.19***

Note: GSHt = Total glutathione, GSSG = Oxidized glutathione, MDA = Malondialdehyde acid, NS = Not significant. * Significant correlation $P < 0.05$, ** Correlation strongly significant $P \leq 0.001$.

Atig et al., 2014

Koncentracija antioksidativnih molekula je u korelaciji s oplodnim značajkama sjemena

Kliničke implikacije nakupljanja slobodnih radikala u sjemenoj tekućini



OKSIDATIVNI STRES čini 30-80% uzroka muške neplodnosti

Tremellen, 2008

Koncentracija slobodnih radikala u sjemenoj tekućini je veća u neplodnih muškaraca neovisno o njihovoj kliničkoj dijagnozi.

Agarwal, 2010

Koncentracija antioksidativnih molekula je u korelaciji s oplodnim značajkama sjemena.

Atig, 2012

Suplem

Antioxidants for male subfertility (Review)

Showell MG, Mackenzie-Proctor R, Brown J, Yazdani A, Stankiewicz MT, Hart RJ

THE COCHRANE COLLABORATION®

Live birth: antioxidants may have increased live birth rates (OR 4.21, 95% CI 2.08 to 8.51, P< 0.0001, 4 RCTs, 277 men, I2= 0%, low quality evidence).
This suggests that if the chance of a live birth following placebo or no treatment is assumed to be 5%, the chance following the use of antioxidants is estimated to be between 10% and 31%.
However, this result was based on only 44 live births from a total of 277 couples in four small studies.

e-SPEN Journal 7 (2012) e50–e53
Contents lists available at SciVerse ScienceDirect
e-SPEN Journal
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Original article

Improvement of sperm quality after micronutrient supplementation

Martin Imhof[a,b,*], Jakob Lackner[a,c], Markus Lipovac[a,b], Peter Chedraui[d], Claus Riedl[e]

European Journal of Endocrinology (2012) 166 765–778 ISSN 0804-4643

REVIEW
MECHANISMS IN ENDOCRINOLOGY

Vitamin D and fertility:

Elisabeth Lerchbaum and Barbara Oberm
Division of Endocrinology and Metabolism, Department

Human Reproduction, Vol.26, No.7 pp. 1628–1640, 2011
Advanced Access publication on May 5, 2011 doi:10.1093/humrep/der132

human reproduction REVIEW Andrology

The role of sperm oxidative stress in male infertility and the significance of oral antioxidant therapy

Parviz Gharagozloo[1,*] and R. John Aitken[2]
[1]CellOxess LLC, 16 Blue Spruce Drive, Pennington, NJ 08534, USA [2]Priority Research Centre in Reproductive Science, Discipline of Biological Sciences, University of Newcastle, Callaghan, NSW 2308, Australia

Human Reproduction Vol.20, No.4 pp. 1006-
Advance Access publication January 21, 2005

Antioxidant intak
in healthy men

oter[2,3], G.Block[1] and A.J.Wyrobek[2]

J Assist Reprod Genet (2013) 30:593–599
DOI 10.1007/s10815-013-9961-9

GONADAL PHYSIOLOGY AND DISEASE

Effects of supplement therapy on s
protamine content and acrosomal i
of varicocelectomized subjects

Gholamabbas Azizollahi · Saeid Azizollahi · Homayoon Baba
Mohammadali Kianinejad · Mohammad Reza Baneshi ·
Seyed Noureddin Nematollahi-mahani

Mar. Drugs **2013**, *11*, 1909-1919; doi:10.3390/md11061909
OPEN ACCESS
Marine Drugs
ISSN 1660-3397
www.mdpi.com/journal/marinedrugs

Article

Effect of Astaxanthin on Human Sperm Capacitation

Gabriella Donà [1], Ivana Kožuh [1], Anna Maria Brunati [1], Alessandra Andrisani [2], Guido Ambrosini [2], Guglielmo Bonanni [3], Eugenio Ragazzi [4], Decio Armanini [3], Giulio Clari [1] and Luciana Bordin [1,*]

Suple

Table 2 Study outcomes involving oral supplementation of various antioxidants in men

Antioxidant	Study type	Patient population	Intervention (daily dose x duration)	Control group (daily dose)	Study outcome (effect on sperm parameters)	Reference
Vitamin E	Double blind, placebo cross-over, RCT	Healthy men with high seminal ROS levels (n = 30)	600 mg vitamin E x 3 months (n = 15)	Placebo (n = 15)	Improved *in vitro* sperm function (improved zona-binding assay)	Kessopoulou et al. [101]
Vitamin E	Double blind, placebo-controlled	Men with (n = 110) asthenozoospermia or oligoasthenozoospermia	300 mg vitamin E x 6 months (n = 52)	Placebo (n = 55)	1. Reduced MDA concentration (less LPO in spermatozoa) 2. Improved sperm motility 3. 20% of those on therapy achieved pregnancy	Suleiman et al. [102]
Vitamin E + Anti-Estrogen (Clomiphene citrate)	Prospective, placebo-controlled RCT	Infertile men with idiopathic oligozoospermia (n = 60)	400 mg vitamin E +25 mg clomiphene citrate x 6 months (n = 30)	Placebo (n = 30)	1. Improved sperm count and progressive motility 2. Partners had higher incidence of pregnancy	Ghanem et al. [103]
Vitamin E + selenium	Open, randomized	Volunteers and infertile men (n = 54)	400 mg vitamin E +225 μg selenium x 3 months (n = 28)	4.5 g vitamin B x 3 months (n = 26)	1. Reduced MDA concentration (less LPO in spermatozoa) 2. Improved sperm motility	Keskes-Ammar et al. [104]
Vitamin E + selenium	Observational study	Infertile men with idiopathic asthenozoospermia (n = 690)	400 IU vitamin E +200 μg selenium x 100 days	None	1. Improvement in sperm motility/morphology or both (53%) 2. Increased spontaneous pregnancy rates (11%)	Moslemi & Tavanbakhsh [105]
Vitamin E + Vitamin C	Double blind, placebo-controlled, RCT	Men (n = 31) with asthenozoospermia or moderate oligoasthenozoospermia	1000 mg vitamin C +800 mg vitamin E x 8 weeks (n = 15)	Placebo (n = 16)	No improvement in sperm parameters No improvement in 24 h sperm survival rate	Rolf et al. [106]
Vitamin E + Vitamin C	Observational study, double-blind	Men with elevated sperm DNA fragmentation (≥15%) who have unexplained infertility	1000 mg vitamin C +1000 mg vitamin E x 2 months (n = 32)	Placebo (n = 32)	Reduced percentage of DNA-fragmented sperm (TUNEL test)	Greco et al. [107]
Vitamin E + Vitamin C	Observational study involving assisted conception treatment	Men with elevated sperm DNA fragmentation (≥15%) who failed their 1st ICSI attempt	1000 mg vitamin C +1000 mg vitamin E x 2 months (n = 38)	None	1. Reduced percentage of DNA-fragmented sperm (TUNEL test) 2. Marked improvement in implantation and clinical pregnancy rates in the 2nd ICSI attempt vs 1st attempt	Greco et al. [108]
Vitamin C		Men with sperm agglutination (>25%) (n = 30)	200 mg vitamin C or 1000 mg vitamin C	Placebo	Improved sperm motility, viability, morphology after 4 weeks (more prominent improvement in 1000 mg vitamin C vs. 200 mg vitamin C)	Dawson et al. [109]
Vitamin C		Men who are heavy smokers (n = 75) with normal reproductive function	200 mg vitamin C or 1000 mg vitamin C	Placebo	1. Improved sperm agglutination 2. Improved 24 h viability 3. Improved sperm morphology	Dawson et al. [110]
Folic acid + zinc sulphate	Double blind, placebo-controlled, RCT	Fertile (n = 108) and subfertile men (n = 103)	5 mg folic acid, 66 mg zinc sulphate or 5 mg folic acid +66 mg zinc sulphate x 26 weeks	Placebo or placebo + placebo	Increased sperm concentration in subfertile and fertile males after combined treatment	Wong et al. [111]
Folic acid + zinc sulphate	Double blind, placebo-controlled	Fertile (n = 47) and subfertile men (n = 40)	5 mg folic acid +66 mg zinc sulphate x 26 weeks	Placebo	Increased sperm concentration in infertile males, but not fertile males	Ebisch et al. [112]
Folic acid + zinc sulphate	Double blind, placebo controlled, RCT	Subfertile men with OAT (n = 83)	5 mg folic acid +220 mg zinc sulphate x16 weeks	Placebo	Zinc sulfate + folic acid did not improve sperm quality in men with OAT (severely compromised sperm parameters)	Raigani et al. [113]
Folic acid + zinc sulphate	Prospective, randomized controlled	Men with palpable varicocele (grade III) who underwent surgical repair of varicocele (n = 160)	5 mg folic acid (n = 26), 66 mg zinc sulphate (n = 32) or 5 mg folic acid +66 mg zinc sulphate (n = 29) x 6 months	Placebo (n = 25)	1. Zinc sulfate + folic acid improved sperm parameters and improved varicocelectomy outcomes 2. Improved protamine content and halo formation rate	Azizollahi et al. [114]
Coenzyme Q_{10}	Systematic review and meta-analysis (3 RCTs)	Infertile men	CoQ_{10} (n = 149)	Controls (n = 147)	1. Improved seminal CoQ_{10} levels 2. Increased sperm concentration 3. Increased sperm motility 4. No increase in pregnancy rates 5. Data on live births were lacking	Lafuente et al. [115]
Coenzyme Q_{10}	Double blind, placebo-controlled, RCT	Men with IOT (n = 60)	200 mg CoQ_{10} x 3 months (n = 30)	Placebo (Lactose) (n = 30)	1. Increased levels of CoQ_{10} in seminal plasma 2. Decreased 8-isoprostane levels (biomarker of LPO) (attenuation of OS in seminal plasma) 3. Increased sperm forward and total motility 4. Increased catalase, SOD activity	Nadjarzadeh et al. [116]
Coenzyme Q_{10}	Double blind, placebo-controlled, RCT	Men with IOAT (n = 47)	200 mg CoQ_{10} x 12 weeks	Placebo	1. Reduced TBARS (reduced plasma MDA levels) 2. Increased TAC in seminal plasma	Nadjarzadeh et al. [117]
Coenzyme Q_{10}	Double blind, placebo-controlled, RCT	Men with IOAT (n = 228)	200 mg ubiquinol x 26 weeks (n = 114)	Placebo (n = 114)	Improved sperm quality (density, motility, normal strict morphology)	Safarinejad et al. [118]
Coenzyme Q_{10}	Double blind, placebo-controlled, RCT	Men with idiopathic infertility (n = 60)	200 mg CoQ_{10} x 6 months	Placebo	1. Increase in CoQ_{10} and ubiquinol in seminal plasma and spermatozoa 2. Increase in spermatozoa motility	Balercia et al. [119]
Coenzyme Q_{10}	Prospective	Men with IOAT (n = 212)	300 mg CoQ_{10} x 26 weeks (n = 106)	Placebo (n = 106)	1. Improved sperm density, motility, normal strict morphology 2. Improved acrosome reaction	Safarinejad [120]
Coenzyme Q_{10}	Open-label, prospective	Men with IOAT (n = 287)	600 mg CoQ_{10} x 12 months (n = 106)	None	1. Improved sperm quality (concentration, progressive motility, normal morphology) 2. Improved pregnancy rates	Safarinejad [121]

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Table I Study characteristics and the effect of oral antioxidants on semen parameters and 2° outcomes.

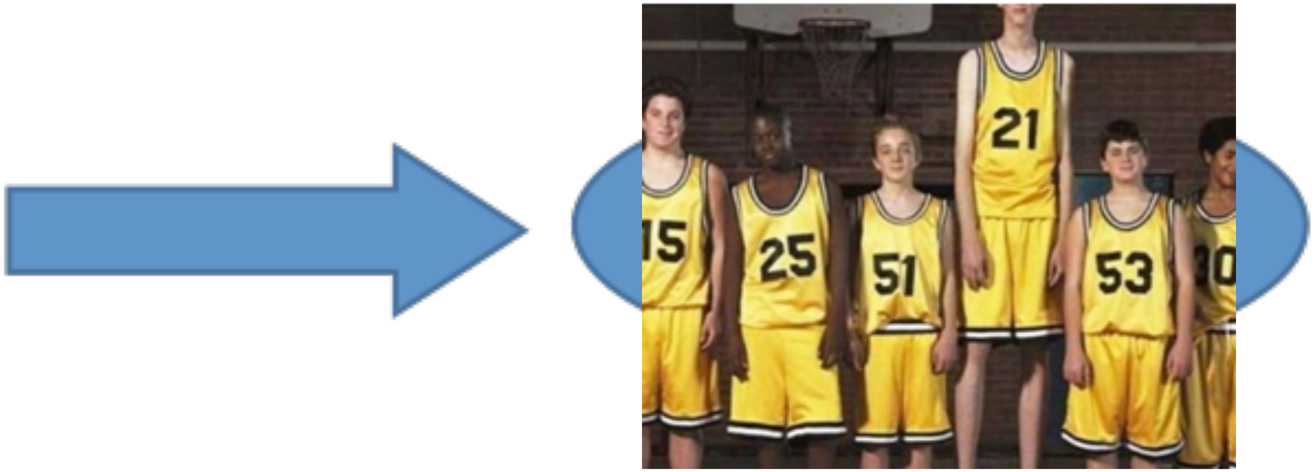
Author	C	M	Morph	OS/DFI	2° outcomes	Target patient	Active (n)	Placb (n)	Dose/day	Duration	Comment
Vitamin C											
Fraga et al. (1991)	ND	ND	ND	↑, 8-OHdG	ND	Smokers, controlled environment	10	NA	5–250 mg	15 weeks	Depletion/ repletion
Vitamin E											
Suleiman et al. (1996)	ND	60/11	ND	↑, MDA	9 versus 0 live birth	Asthenospermic (motility <40%)	52	35	3 × 100 mg	6 months	
Geva et al. (1996)	ND	ND	NE	↑, MDA	Fert. rate 19.3 → 29.1	Fertile normospermic with low FR	15	NA	200 mg	3 months	Prospective
Zinc											
Omu et al. (2008)	NE	↑	NE	↑, MDA, TAC, DFI	ND	Asthenozoospermia ≥ 40% immotile	11	8	2 × 200 mg	3 months	
L-Carnitine											
Balercia et al. (2005)	NS	↑	ND	↑, TOSC	Two pregnancy versus three placebo	Idiopathic asthenozoospermia	15	15	3 g	6 months	Detailed motility
Acetyl-L-carnitine											
Balercia et al. (2005)	↑	↑	ND	↑, TOSC	Three pregnancy versus three placebo	Idiopathic asthenozoospermia	15	15	3 g	6 months	Detailed motility
Astaxanthine											
Comhaire et al. (2005)	NS	NS	NE	↑, ROS counts	Pregnancy (54.5 versus 10.5%)	Infertile	11	19	16 mg	3 months	IUI
N-acetyl-L-cysteine											
Ciftci et al. (2009)	NE	↑	NE	↑, OSI	ND	Idiopathic with normal sperm parameters	60	60	600 mg	3 months	Also improvement in volume/viscosity
Vitamins C and E											
Greco et al. (2005a)	NS	NE	NE	↑, TUNEL	ND	Idiopathic non-smokers, DFI ≥ 15%	32	32	2 × 0.5 g each	2 months	
Greco et al. (2005b)	NE	NE	NE	↑, TUNEL	2/29 versus 14/29 clinical pregnancies	DFI ≥ 15%, ICSI patients (1 before and 1 after antioxidant treatment)	38	NA	2 × 0.5 g each	2 months	29 antioxidant responders
Vitamins E and Se											
Keskes-Amma et al. (2003)	NE	↑	NE	↑, MDA	ND	Infertile men	12	8 (Vit B 4.5 g)	2 × 200 mg and 3 × 75 μg	3 months	Active control (vitamin B)
Vitamins E and Zn											
Omu et al. (2008)	NE	↑	NE	↑, MDA, TAC, DFI	ND	Asthenozoospermia ≥40% immotile	12	8	2 × 10 mg, 2 × 200 mg	3 months	
Vitamins C, E and Zn											
Omu et al. (2008)	NE	↑	NE	↑, MDA, TAC, DFI	ND	Asthenozoospermia ≥40% immotile	14	8	2×5 mg, 2 × 10 mg 2 × 200 mg	3 months	
Vitamins C, E, glutathione											
Kodama et al. (1997)	↑	NE	NE	↑, 8-OHdG	ND	Infertile men	14	NA	200 mg, 200 mg, 400 mg	2 months	
Vitamins C, E, Zn and b-carotene											
Ménézo et al. (2007)	ND	ND	ND	↑, SCSA DFI	ND	2-failed IVF/ICSI cycles and DFI > 15%	58	NA	400 mg, 400 mg, 1 μmol, 500 μmol, 18 mg	90 days	Sperm decond. observed
Vitamins C, E, Se, Zn, folic acid and garlic											
Tremellen et al. (2007)	ND	ND	ND	TUNEL	38.5 versus 16% pregnancy rate at 13 weeks gestation	IVF-ICSI patients TUNEL + ve >25% and poor morphology or low motility	36	16	100 mg, 400 IU, 26 μg, 25 mg, 0.5 mg, 6 mg, 1 g	3 months	Patent reports no TUNEL effect [125]
Tunc et al. (2009)	NE	NE	NE	↑, TUNEL, ROS	ND	Men exhibiting oxidative stress	50	NA	100 mg, 400 IU, 26 μg, 25 mg, 0.5 mg, 6 mg, 1 g	3 months	
L-carnitine and acetyl-L-carnitine											
Vicari et al. (2001)	NE	↑	NE	↑, ROS	11.7 % pregnancy versus 0%	PVE infertile patients 34 normal WBC, 20 abnormal	54	NA	2 × 1 g, 2 × 0.5 g	3 months	
Vicari et al. (2002)	NE	NS	NE	fMLP ROS (NS)	No pregnancy	PVE and abnormal WBC	30	NA	2 × 1 g, 2 × 0.5 g	4 months	Improved viability
Balercia et al. (2005)	↑	↑	ND	↑, TOSC	Five pregnancy versus three placebo	Idiopathic asthenozoospermia	14	15	2 g and 1 g	6 months	Detailed motility

C, concentration; DFI, DNA fragmentation index; M, motility; MDA, malondialdehyde; Morph, morphology; ND, not determined; NE, no effect; NS; not significant; 2° Outcomes; fertilization rate (FR) or pregnancy; OS, oxidative stress; Placb, Placebo; PVE, prostatovesiculoepididymitis; TAC, total antioxidant capacity; WBC, white blood cell.

Gharagozloo and Aitken **Human Reproduction, Vol.26, No.7 pp. 1628–1640, 2011**

Razlozi nekonzistentnosti zaključaka studija o suplementima antioksidansa kao pomoći u liječenju muške neplodnosti:

- različite pripravci (pojedinačni/kombinirani pripravci
- različite populacije ispitanika
- različite kontrolne skupine
- različiti ishodi



Vlasti

e-SPEN Journal 7 (2012) e50–e53

Contents lists available at SciVerse ScienceDirect

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Original article

Improvement of sperm quality after micronutrient supplementation

Martin Imhof[a,b,*], Jakob Lackner[a,c], Markus Lipovac[a,b], Peter Chedraui[d], Claus Riedl[e]

Each capsule of the active compound (PROfertil®) contained:

- L-carnitine (440 mg),
- L-arginine (250 mg),
- zinc (40 mg),
- vitamin E (120 mg),
- glutathione (80 mg),
- selenium (60 mg),
- coenzyme Q10(15 mg),
- folic acid (800 mg)

participation, leaving 132 subjects who completed 3 months of treatment and provided data for full analysis. The control group included 73 sub-fertile men. Mean age of men taking the active compound was 34 years (min/max: 18–43 years) whereas in the control group this was 38 years (min/max: 22–52 years).

Table 1
Semen analysis data among studied groups (active treatment group and controls).

	Ejaculatory volume (ml)		Sperm cell density (million/ml)		Progressive motility (%)		Total motility (%)		Normal morphology (%)	
	Treatment	Control[c]	Treatment	Control	Treatment	Control	Treatment	Control	Treatment	Control
WHO lower limits	2		20		25		50		30	
Baseline median [IQR]	2.9 [1.5]	3.0 [1.7]	5.0 [6.5]	4.9 [5.8]	30.5 [25]	31 [38.8]	32.5 [23.8]	40.5 [44.8]	29.0 [15.2]	39.0 [38.5]
At three months median [IQR]	3.5 [2.3]	3.2 [1.8]	18.5 [23]	7.5 [9.0]	49 [32]	44.0 [47.2]	47.0 [26.0]	50.0 [40.1]	40.0 [17.5]	35.5 [42.3]
p value[a]	0.0001	0.46	0.0001	0.01	0.0001	0.06	0.0001	0.06	0.0001	0.95
Median % change from baseline	+33.3%	+3.7%[b]	+215.5%	+46.4%[b]	+83.1%	+44.0%[b]	+36.4%	+33.9[b]	+23.0%	−2.4%[b]

Note: Lower limit values for each semen parameter are provided in accordance to the WHO.[16]

[a] p values when comparing 3 months with baseline using Wilcoxon rank test; IQR: interquartile range.

[b] $p < 0.05$ when treatments are compared using Mann Whitney test.

[c] Controls were sub-fertile men ($n = 73$) who did not receive active compound.

PROfertil®

Peroralni kombinirani suplement antioksidansa:

- L-karnitin - pozitivno korelira sa brojem i pokretljivošću spermija
- L-arginin - povezan je s pokretljivošću spermija
- cink - povezan sa duljinom životnog vijeka ejakuliranih spermija
- vitamin E - sudjeluje u očuvanju funkcionalnih značajki spermija
- glutation - ključna uloga sintezi DNA i bjelančevina spermija
- selen - esencijalna komponenta glutation-peroksidaze
- koenzim Q10 - uključen u održavanje energetske ravnoteže
- folna kiselina

- ishodi
- koncentracija, broj progresivno pokretnih
- ispitanici
- isključeni pacijenti s azoospermijom

N=27 **1.8 caps/dne** **2.7 mjeseci**	**Prije**		**Poslije**		**P vrijednost**
	Median	IQR	Median	IQR	
Koncentracija (M/ml)	34.5	15.9-57.3	46.8	37.0-68.0	**0.051**
prog. pokretnih (x106)	7.1	2.6-14.7	15.6	6.9-38.4	**0.049**

Zaklj

Može li što pomoći?

Za početak, pokušat ćemo s PROfertilom®