

A low intake of antioxidant nutrients is associated with poor semen quality in patients attending fertility clinics

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Objective: To compare specific nutrient intake between normospermic and oligoasthenoteratospermic patients attending infertility clinics in two Mediterranean provinces of Spain.

Design: Case-control study.

Setting: Private fertility clinics in southeastern Spain.

Patient(s): Thirty men with poor semen quality (case subjects) and 31 normospermic control subjects of couples attending our fertility clinics.

Intervention(s): We recorded dietary habits and nutrient consumption using a food frequency questionnaire adapted to meet specific study objectives.

Main Outcome Measure(s): We calculated nutrient intakes by multiplying the frequency of use for each food by the nutrient composition of the portion size specified on the food frequency questionnaire and by addition across all foods to obtain a total nutrient intake for each individual. Semen quality was assessed by measuring volume, concentration, motility, and morphology. Hormones levels were also analyzed in case and control subjects.

Result(s): In the logistic regression, control subjects had a significantly higher intake of carbohydrates, fiber, folate, vitamin C, and lycopene and lower intakes of proteins and total fat.

Conclusion(s): A low intake of antioxidant nutrients was associated with a poor semen quality in this case-control study of Spanish men attending infertility clinics. (*Fertil Steril*® 2010;93:1128–33. ©2010 by American Society for Reproductive Medicine.)

Key Words: Food frequency, male infertility, antioxidants, vitamins

Several studies have suggested that human semen quality and fertility have been declining during the last decades (1–6). Deterioration in seminal samples has been related to environmental and occupational pollutants, changes in lifestyles, exposure to toxics, and dietary habits (7–9).

Concerning dietary factors, a lower intake of some antioxidant nutrients, such as vitamins A, C, and E, carnitines, folate, zinc, and selenium, has been associated with male infertility (10–14). In a cross-sectional study of 97 healthy

male volunteers, a higher nutrient intake of vitamins C, E and β -carotene was associated with a higher sperm count number and motility (15). Lewis et al. (16) found that men with the lowest intake of dietary antioxidants had less sperm motility; the consumption of <5 servings/day of fruits and vegetables as well as the vitamin C intake were significantly lower among infertile men than among control subjects.

In a prior analysis comparing intakes of food items in the same study population, we found that control subjects had a higher intake of skim milk, shellfish, tomatoes, and lettuce, and case subjects consumed more yogurt, meat products, and potatoes. In the logistic regression model, case subjects had lower intake of lettuce, tomatoes, and fruits (apricots and peaches) and significantly higher intake of dairy and meat products (17). However, in that study we could not attribute those differences to specific nutrients.

The aim of the present study was to compare specific nutrient intake between normospermic and oligoasthenoteratospermic patients attending infertility clinics in two Mediterranean provinces of Spain.

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MATERIALS AND METHODS

Design and patients

The men of couples attending the three infertility centers of the Instituto Bernabeu in Murcia and Alicante (southeastern Spain) between 2005 and 2007 were classified into two groups on the basis of seminal quality following World Health Organization (WHO) criteria (18): 1) case subjects ($n = 30$; 17 from Alicante and 13 from Murcia) composed of men with severe or moderate oligozoospermia (<5 million spermatazoa (spz)/mL or 5–20 million spz/mL, respectfully) and severe teratozoospermia [$<6\%$ normal forms strict criteria (19)]; and 2) control subjects ($n = 31$, 18 from Alicante and 13 from Murcia) composed of normospermic patients (≥ 20 million spz/mL, $\geq 50\%$ motile sperm, and $\geq 14\%$ normal forms strict criteria). Another ten patients and twelve controls that were invited to participate refused to be included in the study; therefore, there were no significant differences between the refusal rates in case and control subjects. Subjects provided at least two semen samples. Abstinence time ranged from 3 to 5 days without significant differences between case and control subjects. Following WHO recommendations, the interval between the two collections was between 7 days and 3 weeks (18).

Semen analyses were performed by the same technician, which was blind for other clinical data. Semen parameters evaluated included: ejaculate volume, sperm concentration, percentage of motile sperm (grades a and b according to WHO criteria), and percentage of normal forms following Kruger's strict criteria (19). To assess motility, a 5- μ L aliquot was added to a Makler chamber (Sefi-Medical Instruments, Haifa, Israel) and sperm was visualized by phase-contrast microscope (Olympus CX21; Olympus, Tokyo, Japan) at $\times 200$ magnification. A 20- μ L semen aliquot smear was dried at room temperature and stained with Papanicolau stain (20). Methods used in this study have been previously described (17). This study was approved by the Institutional Review Board of our clinics, and patients were included in the study after giving informed written consent.

Data collection

Study participants were interviewed by the same trained fieldworker, who was blinded regarding case-control patient status, using structured questionnaires to collect information for sociodemographic characteristics, tobacco use, and dietary assessment. At the clinical examination, the fieldworker followed the same standard protocol in all centers to obtain anthropometric measurements. Height was measured to the nearest 0.1 cm with subjects standing without shoes and with their backs to the stadiometer. Body weight was measured in light clothes to the nearest 0.1 kg on a digital scale which was placed on a firm flat surface. The body mass index (BMI) was calculated from weight and height measurements as kg/m^2 . We also collected information on occupation history using a questionnaire adapted from the one developed by the U.S. Agency for Toxic Substances and Disease Registry in cooperation with the National Institute for Occupational Safety and Health (21). Past exposure to chemicals

was assessed from the occupational history as reported by case and control subjects. It was considered that the person had a previous occupational toxic exposure if he answered that he had been exposed to any of the chemicals that were listed. Detailed information of this study methodology may be found elsewhere (22).

Dietary assessment

A semiquantitative food frequency questionnaire (FFQ) of 93 food items was used to assess usual dietary intake. The FFQ was a modified version of the Harvard questionnaire (23) that was previously validated and adapted for adult Spanish populations (24, 25). When comparing the nutrient intakes from the FFQ with those from four 1-week dietary records, the average correlation coefficients for 1-year validity and reproducibility of nutrient intakes were 0.47 and 0.40, respectively, a similar range to other established diet questionnaires (26). For a similar version of this FFQ adapted for an elderly population in the same area of Spain, we observed satisfactory correlation coefficients between plasma concentration and the nutrient intake of carotenoids and vitamin C: 0.20 and 0.36, respectively (27).

Participants in the present study were asked how often, on average, they had consumed each type item during the previous year. Standard units or serving sizes were specified for each food item in the FFQ. The questionnaire had nine possible responses, ranging from "never or less than once per month" to "six or more times per day." The response to each food item was converted to average daily intake for each individual participant. The average daily intakes for each fruit and vegetable were summed to compute the total fruit and vegetable intake. Nutrient values were primarily obtained from food composition tables from the United States Department of Agriculture and other published sources (28). We calculated nutrient intakes by multiplying the frequency of use for each food by the nutrient composition of the portion size specified on the FFQ and by addition across all foods to obtain a total nutrient intake for each individual. None of the patients had dietary or vitamin supplements during the total duration of study. Alcohol consumption was assessed within an FFQ including specific items on wine, beer, and liquor and specifying standard portions for all of them. We calculated ethanol intake by multiplying the frequency of consumption of each beverage by the alcohol content of the specified portion size and summing across beverages.

Analysis

Statistical analysis was performed with the statistical package SPSS 15.0 (SPSS, Chicago, IL). Results are presented as percentage and mean with standard deviation (SD). Descriptive means are presented using untransformed data. We used unpaired Student *t* tests for means comparison of continuous variables for case and control subjects. χ^2 tests were used for categoric variables. The level of statistical significance was set at .05, and all tests were two-tailed. Statistical tests for nutrient intakes were made with calorie-adjusted values by calculating the residuals from a linear

TABLE 1**Personal characteristics, semen quality parameters, and nutrient intakes among case and control subjects.**

Variables	Case subjects (n = 30)	Control subjects (n = 31)
Age (y)	34.2 ± 3.7	32.8 ± 3.9
Body mass index (kg/m ²)	23.2 ± 1.0	23.5 ± 1.1
Current smoker	8 (26.7%)	11 (35.5%)
Current alcohol drinking	14 (46.7)	18 (58.1%)
Hormonal levels		
FSH (mIU/mL)	6.4 ± 2.1	6.5 ± 1.4
LH (mIU/mL)	4.2 ± 1.2	4.1 ± 1.4
T (ng/mL)	5.3 ± 1.6	5.4 ± 1.3
Semen characteristics ^a		
Volume (mL)	3.8 ± 1.2	3.5 ± 1.4
Concentration (million/mL)	3.5 ± 2.1	39.5 ± 14.6
Sperm motility (grades a + b, %)	27.4 ± 18.6	52.2 ± 12.3
Normal morphology ^b (%)	3.7 ± 1.5	22.3 ± 4.5
Nutrient intakes		
Protein (g)	91.8 ± 33.9	94.4 ± 33.3
Carbohydrates (g)	132.2 ± 46.6	156.7 ± 44.1 ^c
Total fat (g)	94.1 ± 23.3	99.0 ± 27.9
Fiber (g)	11.8 ± 4.4	14.2 ± 3.9 ^c
Folate (μg)	217.6 ± 76.6	269.5 ± 74.8 ^c
Vitamin C (mg)	45.5 ± 20.9	57.9 ± 21.8 ^c
Vitamin E (mg)	9.8 ± 2.1	10.9 ± 2.3
Lycopene (μg)	2,890.5 ± 1,529.9	4,238.5 ± 2,044.9 ^c
β-Carotene (μg)	3,647.3 ± 2,311.1	4,351.4 ± 2,008.8
Selenium (mg)	108.6 ± 41.0	115.4 ± 39.0
Kilocalorie intake/d	1,767.7 ± 484.1	1,921.5 ± 530.7

^a Mean of the two samples.^b Normal morphology defined according to the strict criteria by Kruger et al. (19).^c Control subjects presented significantly higher intakes of the following nutrients: carbohydrates, fiber, folate, vitamin C, and lycopene ($P < .05$).

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regression with the log e of the nutrient modeled as the dependent variable and the log e of total energy intake as the independent variable (26).

Adjusted odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using unconditional logistic regression. To explore the association between semen quality (normospermia yes/no) and nutrients intake we used the lowest tertile of every nutrient intake adjusted for total energy as the reference category. Tertiles of nutrient intakes were estimated based on the whole distribution of case and control subjects. The following potential confounders were included in the models: age (years), total energy intake (kcal), tobacco smoking (no/yes), and previous occupational toxic exposures (no/yes). Tests for trend in the ORs across exposure strata were calculated for nutrient intakes by using logistic models that included categorical terms as continuous variables in a model with all of the potential confounders. For trend tests, we used the likelihood ratio test statistic with one degree of freedom.

RESULTS

Table 1 shows the distribution of cases and controls according to personal characteristics, hormone levels, and semen parameters. The distribution of age, BMI, and hormone levels were similar between case and control subjects. Control subjects had higher absolute intakes of carbohydrates, fiber, folate, vitamin C, and lycopene ($P < .05$).

Table 2 shows risk estimates according to nutrient intakes by tertiles using logistic regression models. Control subjects presented significantly higher intakes of carbohydrates, fiber, folate, vitamin C, and lycopene ($P < .05$) and lower intakes of proteins and total fat ($P < .05$). For these nutrients, statistically significant trends were observed ($P < .05$).

Significant differences also existed between case and control subjects in occupational exposure. A multiple logistic regression model was used incorporating the nutrients that had significantly different intakes between case

TABLE 2

Adjusted odds ratios (ORs) for poor/low semen quality according to usual nutrient intake.

Nutrient intake in tertiles	Case subjects	Control subjects	OR ^a (95% CI)	P value	OR ^b (95% CI)	P value	OR ^c (95% CI)	P value	Test for trend, P value
Protein									
Low	8	12	1		1		1		
Medium	6	14	0.66 (0.17–2.54)	.55	0.83 (0.20–3.45)	.80	1.27 (0.25–6.41)	.77	
High	16	5	4.92 (1.23–19.62)	.02	5.25 (1.29–21.52)	.02	8.27 (1.58–43.34)	.01	.011
Carbohydrates									
Low	14	6	1		1		1		
Medium	8	12	0.32 (0.08–1.22)	.10	0.36 (0.09–1.44)	.15	0.32 (0.06–1.65)	.17	
High	8	13	0.26 (0.07–0.99)	.05	0.21 (0.05–0.85)	.03	0.09 (0.02–0.59)	.01	.001
Total Fat									
Low	7	13	1		1		1		
Medium	9	11	1.67 (0.44–6.28)	.45	2.61 (0.61–11.22)	.20	4.91 (0.86–28.17)	.07	
High	14	7	3.46 (0.93–12.85)	.06	5.04 (1.21–20.95)	.03	11.81 (1.87–74.65)	< .01	.009
Fiber									
Low	13	7	1		1		1		
Medium	11	9	0.68 (0.18–2.63)	.58	0.84 (0.20–3.56)	.82	0.87 (0.17–4.43)	.87	
High	6	15	0.19 (0.05–0.74)	.02	0.14 (0.03–0.64)	.01	0.13 (0.02–0.68)	.01	.018
Folate									
Low	13	7	1		1		1		
Medium	10	10	0.53 (0.14–1.97)	.34	0.60 (0.15–2.43)	.48	0.44 (0.09–2.18)	.31	
High	7	14	0.23 (0.06–0.88)	.03	0.16 (0.04–0.73)	.02	0.13 (0.02–0.71)	.02	.024
Vitamin C									
Low	14	6	1		1		1		
Medium	10	10	0.37 (0.09–1.47)	.16	0.35 (0.08–1.50)	.16	0.27 (0.05–1.40)	.12	
High	6	15	0.14 (0.03–0.57)	< .01	0.11 (0.02–0.52)	< .01	0.09 (0.02–0.51)	< .01	.008
Vitamin E									
Low	13	5	1		1		1		
Medium	8	14	0.20 (0.05–0.80)	.02	0.16 (0.04–0.71)	.02	0.22 (0.04–1.11)	.07	
High	9	12	0.32 (0.08–1.26)	.10	0.36 (0.09–1.55)	.17	0.52 (0.11–2.49)	.41	
Lycopene									
Low	12	8	1		1		1		
Medium	11	9	0.77 (0.20–3.05)	.72	0.68 (0.16–2.81)	.59	0.66 (0.13–3.37)	.62	
High	7	14	0.29 (0.08–1.09)	.07	0.25 (0.06–1.00)	.05	0.16 (0.03–0.82)	.03	.034
β-Carotene									
Low	13	7	1		1		1		
Medium	6	13	0.22 (0.05–0.92)	.04	0.26 (0.06–1.12)	.07	0.20 (0.04–1.10)	.06	
High	11	11	0.44 (0.12–1.61)	.21	0.41 (0.11–1.60)	.20	0.53 (0.12–2.34)	.40	
Selenium									
Low	11	9	1		1		1		
Medium	6	14	0.30 (0.07–1.18)	.08	0.27 (0.07–1.17)	.08	0.45 (0.10–2.11)	.31	
High	13	8	1.48 (0.40–5.52)	.56	1.52 (0.40–5.90)	.54	2.04 (0.45–9.21)	.36	

^a OR adjusted for age and total energy intake.

^b OR adjusted for age, total energy intake, BMI, and smoking.

^c OR adjusted for age, total energy intake, BMI, smoking, and previous occupational toxic exposures.

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and control subjects in the univariate analyses and occupational exposure. In the logistic regression, control subjects had a significantly higher intake of carbohydrates, fiber, folate, vitamin C, and lycopene ($P < .05$) and lower intakes of proteins and total fat, and had been exposed less frequently to occupational toxics than case subjects ($P < .05$).

DISCUSSION

Our study suggests that poor semen quality may be associated with a lower intake of carbohydrates, fiber, folate, vitamin C,

and lycopene and a higher intake of protein and total fat. Overall, a low intake of antioxidant nutrients might seem to have a negative effect on semen quality.

A higher antioxidant dietary intake has been associated with higher sperm numbers and motility in healthy nonsmoking men (15). A recent review of intervention studies about the effect of antioxidants on semen parameters concluded that vitamins A, C, E, carnitine, and glutathione improve semen quality in male factor infertility (14). Oral supplementation with vitamin C or E and folic acid plus zinc sulfate has

also been shown to improve sperm counts, motility, and morphology in infertile patients (11, 29, 30).

In the present study, we have found a positive association between semen quality and vitamin C intake. The association between vitamin E and β -carotene was not significant once we adjusted by previous occupational toxic exposures. We did not find an association between selenium intake and semen quality, which is in agreement with recent intervention studies in which selenium supplements did not improve semen quality (31, 32). We also found a positive association between folate intake and semen quality, which has been supported in intervention studies that have shown that supplementation with folic acid significantly increased sperm quality in subfertile males (11, 33).

Moreover, in a recent article, Young et al. (34) found in a healthy population of nonsmoking men that men with high folate intake (>75th percentile) had lower frequencies of sperm with X, 21, sex nullisomy, and a lower aggregate measure of sperm aneuploidy ($P \leq .04$) compared with men with lower intakes. Finally, we found that lycopene intake was positively associated with semen quality. Lycopene is a powerful carotenoid antioxidant, and therefore, the effect we found is consistent with the hypothesis discussed earlier. We have found only an experimental study in which the semen quality of 30 men with idiopathic nonobstructive oligo/astheno/teratozoospermia improved after receiving 2,000 μg lycopene (35). However that study was not randomized.

As in other case-control studies, the present study may present limitations common to observational studies. Besides, some real associations could have remained undetected owing to sample size limitations. However, the strength of the associations, the existence of a dose response, the control for other potential confounders such as age, tobacco smoking, and occupational factors, and the consistency with other studies in humans and other animals support that the study results are real, and that nutrient intake, particularly low intake of antioxidant nutrients, is likely to be causally related to poor semen quality (15, 16, 36). Our findings might be compatible with the hypothesis that oxidative stress associated with increased reactive oxygen species generation and reduced antioxidant capacity is negatively correlated with sperm concentration, motility, and morphology in infertile men (37–40).

In conclusion, the present case-control study suggests that the risk of poor semen quality is associated with a low intake of some antioxidant nutrients in a Mediterranean population of Spanish men.

REFERENCES

1. Carlsen E, Giwercman A, Keiding N, Skakkebaek NE. Evidence for decreasing quality of semen during the past 50 years. *BMJ* 1992;305:609–13.
2. Irvine S, Cawood E, Richardson D, MacDonald E, Aitken J. Evidence of deteriorating semen quality in the United Kingdom: birth cohort study in 577 men in Scotland over 11 years. *BMJ* 1996;312:467–71.
3. Swan SH, Elkin EP, Fenster L. Have sperm densities declined? A reanalysis of global trend data. *Environ Health Perspect* 1997;105:1228–32.
4. Aitken RJ, Koopman P, Lewis SE. Seeds of concern. *Nature* 2004;432:48–52.
5. Skakkebaek NE, Jørgensen N, Main KM, Rajpert-De Meyts E, Leffers H, Andersson AM, et al. Is human fecundity declining? *Int J Androl* 2006;29:2–11.
6. Swan SH. Does our environment affect our fertility? Some examples to help reframe the question. *Semin Reprod Med* 2006;24:142–6.
7. Tielemans E, Burdorf A, te Velde ER, Weber RF, van Kooij RJ, Veulemans H, et al. Occupationally related exposures and reduced semen quality: a case-control study. *Fertil Steril* 1999;71:690–6.
8. López-Tejón M, Garcia F, Serra O, Moragas M, Rabanal A, Olivares R, et al. Semen quality in a population of volunteers from the province of Barcelona. *Reprod Biomed Online* 2007;15:434–44.
9. Chavarro JE, Toth TL, Sadio SM, Hauser R. Soy food and isoflavone intake in relation to semen quality parameters among men from an infertility clinic. *Hum Reprod* 2008;23:2584–90.
10. Wong WY, Thomas CM, Merkus JMW, Zielhuis GA, Steegers-Theunissen RPM. Male factor subfertility: possible causes and impact of nutritional factors. *Fertil Steril* 2000;73:435–42.
11. Wong WY, Merkus HM, Thomas CM, Menkveld R, Zielhuis GA, Steegers-Theunissen RP. Effects of folic acid and zinc sulfate on male factor subfertility: a double-blind, randomized, placebo-controlled trial. *Fertil Steril* 2002;77:491–8.
12. Comhaire FH, Mahmoud A. The role of food supplements in the treatment of the infertile men. *Reprod Biomed Online* 2003;7:385–91.
13. Agarwal A, Said TM. Carnitines and male infertility. *Reprod Biomed Online* 2004;8:376–84.
14. Agarwal A, Nallella KP, Allamaneni SSR, Said TM. Role of antioxidants in treatment of male infertility: an overview of the literature. *Reprod Biomed Online* 2004;8:616–27.
15. Eskenazi B, Kidd SA, Marks AR, Slotter E, Block G, Wyrobek AJ. Antioxidant intake is associated with semen quality in healthy men. *Hum Reprod* 2005;20:1006–12.
16. Lewis V, Kochman L, Herko R, Brewer E, Andolina G, Song G. Dietary antioxidants and sperm quality in infertile men. *Proceedings of the 62nd American Society of Reproductive Medicine Annual Meeting, New Orleans, USA, October 21–25: P-623. Fertil Steril* 2006; 86:s364.
17. Mendiola J, Torres-Cantero AM, Moreno-Grau JM, Ten J, Roca M, Moreno-Grau S, et al. Food intake and its relationship with semen quality: a case-control study. *Fertil Steril*. Published online 1 March 2008 [Epub ahead of print].
18. World Health Organization. WHO laboratory manual for the examination of human semen and sperm–cervical mucus interaction. 4th ed. Cambridge: Cambridge University Press, 1999.
19. Kruger TF, Menkveld R, Stander FS, Lombard CJ, Van der Merwe JP, van Zyl JA, et al. Sperm morphologic features as a prognostic factor in in vitro fertilization. *Fertil Steril* 1986;46:1118–23.
20. Vazquez-Levin MH, Goldberg SI, Friedmann P, Des Jarlais DC, Nagler HM. Papanicolaou and Kruger assessment of sperm morphology: thresholds and agreement. *Int J Androl* 1998;21:327–31.
21. Agency for Toxic Substances and Disease Registry. Case studies in environmental medicine: taking an exposure history. 1992. Department of Health and Human Services. Atlanta, United States. Available at: www.atsdr.cdc.gov/HEC/CESEM/exphistory/index.html. Last accessed 5 October 2005.
22. Mendiola J, Torres-Cantero AM, Moreno-Grau JM, Ten J, Roca M, Moreno-Grau S, et al. Exposure to environmental toxins in males seeking infertility treatment: a case-controlled study. *Reprod Biomed Online* 2008;16:842–50.
23. Willett WC, Sampson L, Stampfer MJ, Rosner B, Bain C, Witschi J, et al. Reproducibility and validity of a semiquantitative food frequency questionnaire. *Am J Epidemiol* 1985;122:51–65.
24. Vioque J, Gonzalez L. Validity of a food frequency questionnaire (preliminary results). *Eur J Cancer Prev* 1991;1:19–20.
25. Vioque J. Validez de la evaluación de la ingesta dietética. In: Serra-Majem L, Aranceta-Bartrina J, eds. *Nutrición y salud pública. Métodos, bases científicas y aplicaciones*. Barcelona: Mansson-Elsevier, 2006: 199–210.

26. Willett W. Reproducibility and validity of food-frequency questionnaires. In: Willett WC, ed. *Nutritional epidemiology*. New York: Oxford University Press, 1998:101–47.
27. Vioque J, Weinbrenner T, Asensio L, Castelló A, Young IS, Fletcher A. Plasma concentrations of carotenoids and vitamin C are better correlated with dietary intake in normal weight than overweight and obese elderly subjects. *Br J Nutr* 2007;97:977–86.
28. U.S. Department of Agriculture. Composition of foods raw, processed, prepared, USDA national nutrient database for standard reference, release 18. 2005. Available at: <http://www.nal.usda.gov/fnic/foodcomp/search>. Last accessed 5 October 2005.
29. Keskes-Ammar L, Feki-Chakroun N, Rebai T, Sahnoun Z, Ghozzi H, Hammami S, et al. Sperm oxidative stress and the effect of an oral vitamin E and selenium supplement on semen quality in infertile men. *Arch Androl* 2003;49:83–94.
30. Akmal M, Qadri JQ, Al-Waili NS, Thangal S, Haq A, Saloom KY. Improvement in human semen quality after oral supplementation of vitamin C. *J Med Food* 2006;9:440–2.
31. Hawkes WC, Turek PJ. Effects of dietary selenium on sperm motility in healthy men. *J Androl* 2001;22:764–72.
32. Iwanier K, Zachara BA. Selenium supplementation enhances the element concentration in blood and seminal fluid but does not change the spermatozoal quality characteristics in subfertile men. *J Androl* 1995;16:441–7.
33. Ebisch IM, Pierik FH, DE Jong FH, Thomas CM, Steegers-Theunissen RP. Does folic acid and zinc sulphate intervention affect endocrine parameters and sperm characteristics in men? *Int J Androl* 2006;29:339–45.
34. Young SS, Eskenazi B, Marchetti FM, Block G, Wyrobek AJ. The association of folate, zinc and antioxidant intake with sperm aneuploidy in healthy nonsmoking men. *Hum Reprod* 2008;23:1014–22.
35. Gupta NP, Kumar R. Lycopene therapy in idiopathic male infertility—a preliminary report. *Int Urol Nephrol* 2002;34:369–72.
36. Dabrowski K, Ciereszko A. Ascorbic acid protects against male infertility in a teleost fish. *Experientia* 1996;52:97–100.
37. Saleh RA, Agarwal A. Oxidative stress and male infertility: from research bench to clinical practice. *J Androl* 2002;23:737–52.
38. Agarwal A, Saleh RA, Bedaiwy MA. Role of reactive oxygen species in the pathophysiology of human reproduction. *Fertil Steril* 2003;79:829–43.
39. Pasqualotto FF, Sharma RK, Nelson DR, Thomas AJ, Agarwal A. Relationship between oxidative stress, semen characteristics, and clinical diagnosis in men undergoing infertility investigation. *Fertil Steril* 2000;73:459–64.
40. Agarwal A, Sharma RK, Nallella KP, Thomas AJ Jr., Alvarez JG, Sikka SC. Reactive oxygen species as an independent marker of male factor infertility. *Fertil Steril* 2006;86:878–85.