

## The association between dietary antioxidant intake and semen quality in infertile men

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### Abstract

**Background:** Oxidative stress is detrimental to semen quality and has a significant role in the etiology of male subfertility.

**Methods:** Dietary intake of antioxidants were compared between thirty two men with oligoastheno/ teratazoospermic (cases) and 32 normospermic volunteers (controls) attending fertility clinic in Mirza Koochak-khan Hospital in Tehran, Iran. All participants were nonsmokers and matched according their age and Body Mass Index (BMI). Nutrient consumption was calculated using a semi- quantitative food frequency questionnaire. Semen samples were collected and were assessed by measuring volume, concentration, motility and morphology.

**Results:** infertile subjects had a significantly lower intake of zinc and folate compare to control ones ( $p < 0.001$ ). Dietary intake of vitamin C and E was lower than recommended values in 59.4% of case group that was significantly different from control ones ( $p < 0.05$ ). In control group, 36.4 and 40.9% of participants had insufficient dietary intake of vitamin C and E, respectively. Significant correlations were found between folate ( $r = 0.5$ ,  $p < 0.001$ ), zinc ( $r = 0.6$ ,  $p < 0.001$ ) and percentage of motility and also between vitamin E and morphology ( $r = 0.3$ ,  $p = 0.03$ ), zinc and concentration ( $r = 0.4$ ,  $p = 0.004$ ) in all participants.

**Conclusion:** In summary, a low intake of folate, zinc, and vitamin E were related to poor sperm concentration and motility.

**Keywords:** Dietary antioxidant, Male infertility, Oligastheno-teratozoospermia.

### Introduction

Approximately 30% of all couples in Iran experience infertility in their reproductive years (1). Male infertility accounts for 30 to 50 percent of infertility cases (2). But identification of the causes of male infertility is difficult. Due to a high content of polyunsaturated fatty acids within the plasma membranes of spermatozoa and a low concentration of antioxidant enzymes within their cytoplasm, spermatozoa are prone to

oxygen induced damage (3). Studies have generated definitive evidence that oxidative injuries imposed on spermatozoa, have a key role in the etiology of male infertility (4-5). Oxidative stress can reduce sperm motility and also interferes with sperm-oocyte binding and fusion (6-7).

There is some evidence showing that oxidative stress induced by high levels of reactive oxygen species (ROS) or low levels of antioxidants in seminal plasma play a major role in causing abnormal sperm parameters

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(6, 8-11).

Some research have shown that 40 to 88% of nonselected infertile men have high levels of seminal reactive oxygen species (12). Furthermore, studies have shown that antioxidant intake is associated with better semen quality and sperm chromatin stability in healthy nonsmoking men (13-14). Although high dose of antioxidant supplementation may improve semen quality, few studies have examined the association between dietary intakes of antioxidants with semen quality among infertile men (15). Furthermore, most of the observational studies have been done in unselected infertile men.

Thus, the purpose of the present study was to examine the association between dietary intake of antioxidant (Vitamins C, E,  $\beta$  carotene, folate, selenium and zinc) with semen quality. In addition, we aimed to determine the difference in antioxidant intake between infertile men with oligo/astheno/teratozoospermia and normospermic ones.

### Methods

This case- control study was conducted between November 2006 and December 2007 in the outpatient clinic of infertility at Mirza Koochak Khan Hospital in Tehran, Iran.

The case group consisted of 32 men (20-40 years) with primary infertility due to idiopathic oligo and/or astheno and/or teratozoospermia (WHO 1999) and 32 age-matched normal healthy donors who referred for premarital tests and considered as control ones. The diagnosis of primary infertility was made after medical assessment which included: medical history, clinical examination, semen analysis, semen culture for Mycoplasma Urelicum and Chlamydia detection, FSH, LH, testosterone,  $E_2$  and prolactin assay and sonography of genitalia. Infertility is defined as the inability to conceive after 12 months of unprotected intercourse.

Exclusion criteria were: smoking, alcohol consumption, occupational chemical expo-

sure, history or presence of endocrine disorders, testicular disease such as cryptorchidism, orchitis and varicocele, infectious genital disease, treatment with drugs or using antioxidant supplements within the 3 months before enrollment, leucocytospermia seminal white blood cells (WBC)  $> 1 \times 10^6/\text{ml}$  and azoospermia.

The study has been performed in accordance with ethical standards laid down by the appropriate version of the 1964 declaration of Helsinki and the study protocol was approved by faculty of public health, Tehran University of Medical Sciences. All participants were given written informed consent.

Two questionnaires were completed for each person. The first questionnaire ascertained sociodemographic characteristics and anthropometric data. Anthropometric assessment included measurements of height and weight. BMI was calculated as weight (Kg) divided, height (squared, meter,  $\text{m}^2$ ).

Dietary information was collected by using a semiquantitative food- frequency questionnaire (FFQ) with 116 food items. Participants were asked to state the portion size of given food and how often they had consumed each of the foods and beverages included in the FFQ during the previous year. The questionnaire had 9 options for frequency of intake, ranging from  $< 1$  time per month to  $\geq 6$  times per day. Nutrient intakes were estimated by summing the nutrient contribution of all food items in the questionnaire. Then the average for daily energy and nutrient intakes was calculated by using food processor software version II (ESHA Research, 1999, Salem, OR).

Semen samples were obtained by masturbation after 48-72 hours of abstinence. Samples were collected into sterile containers and allowed to liquefy at  $37^\circ\text{C}$  for 20 minutes, and evaluated immediately according to WHO recommendation (ejaculate volume, pH, sperm concentration, motility and morphology) (WHO 1999). Sperm concentration was expressed as  $10^6$  per milliliter of semen, in which motility and morphology expressed as percentage. Sperm concen-

tration  $\geq 20 \times 10^6$  per milliliter of semen, motility  $\geq 50\%$  and normal forms  $\geq 30\%$  were considered as normal sperm parameters according to WHO criteria (16).

**Statistical methods:** Statistical analyses were performed using SPSS 16.0 for Windows statistical software (SPSS Inc. Chicago, IL, USA). Differences between control and infertile groups were assessed using independent t- test. The correlations between sperm parameters and antioxidant nutrient intakes were evaluated by the Pearson correlation coefficient. Results were given as mean  $\pm$  SD, and correlation coefficients, and  $p < 0.05$  considered statistically significant. All p-values presented were two-sided.

## Results

The mean of age in case and control group were  $32.3 \pm 5.2$  and  $32.2 \pm 4.4$  years, respectively. The duration of infertility in case group ranged between 2 and 10 years.

Selected characteristics of the study population are shown in table 1. Infertile and healthy donors did not differ significantly in age and BMI. Patients and donors had body weight of  $74.6 \pm 11.8$  Kg (mean  $\pm$  SD) and  $74.9 \pm 8.07$  Kg and heights of  $172 \pm 7.08$  cm and  $174 \pm 5.72$  cm, respectively. The control subjects, in comparison to the infertile patients had significantly lower sperm concentration, motility, and morphology.

Pertinent antioxidant intake data are shown in table 2. Infertile men and donors did not differ significantly in vitamin E, beta - carotene, vitamin C and selenium

intake. 59.4% of infertile men and 36.4% of donors consumed less than 90 mg vitamin C per day (the recommended daily allowance (RDA) for men). More than a half of infertile men (59.4 percent) and 40.9 percent of donors consumed less than the RDA of 15 mg vitamin E per day (Institute of Medicine, 2000). Consumption less than 55 microgram of selenium was seen in 15.6 and 18.2 percent of infertile and healthy donors respectively.

The correlations between antioxidant intakes and sperm parameters are shown in table 3. Significant correlations were observed between folate, zinc and sperm motility. Sperm concentration had direct correlation with zinc intake and an inverse correlation with beta-carotene.

## Discussion

Our study suggests that poor semen quality may be associated with a lower intake of zinc, folate and vitamin E. This study showed that a low intake of some antioxidants may have a negative effect on sperm motility and morphology. Eskenazi et al. (13) found an association between antioxidant intake and sperm numbers and motility in a healthy population of nonsmoking men.

Similarly, Mendiola et al found a positive association between semen quality and vitamin C intake (15, 17). There was a significant difference in zinc and folate intake between oligo/ astheno/ teratozoospermic and healthy donors ( $p < 0.000$ ). He also found a positive association between folate intake and semen quality. Likewise, in Young et al. study healthy nonsmoker men with high

Table 1. Characteristics of the infertile patients and control subjects. The data are presented as mean  $\pm$  SD

|  | Infertile patients (n=32) | Control subjects (n=32) | p value |
|--|---------------------------|-------------------------|---------|
| Age (years)                              | $32.28 \pm 5.2$           | $32.23 \pm 4.43$        | 0.7     |
| Body Mass Index (Kg/m <sup>2</sup> )     | $25.02 \pm 3.4$           | $24.7 \pm 2.2$          | 0.9     |
| Abstinence (days)                        | $2.56 \pm 0.51$           | $2.46 \pm 0.52$         | 0.7     |
| Duration of infertility (years)          | $3.6 \pm 1.2$             |                         |         |
| Semen volume (ml)                        | $3.44 \pm 1.62$           | $3.85 \pm 1.01$         | 0.3     |
| Sperm concentration ( $\times 10^6$ /ml) | $28.58 \pm 29.32$         | $50.77 \pm 19.07$       | 0.002   |
| Motility (%)                             | $29.53 \pm 15.9$          | $59.36 \pm 4.88$        | <0.001  |
| Morphology (%)                           | $38.4 \pm 21.17$          | $50.9 \pm 14.38$        | 0.01    |

Table 2. Comparison of dietary antioxidant intake between infertile patients and control subjects. The data are presented as mean  $\pm$  SD

|                      | Infertile patients (n=32) | Control subjects (n=32) | p value |
|----------------------|---------------------------|-------------------------|---------|
| Vitamin A (RE)       | 1133.7 $\pm$ 1050         | 1007.2 $\pm$ 752        | 0.7     |
| Beta- carotene (mcg) | 6536.7 $\pm$ 567          | 8647.8 $\pm$ 639        | 0.08    |
| Vitamin E (mg)       | 10.93 $\pm$ 6.23          | 14.53 $\pm$ 3.81        | 0.053   |
| Vitamin C (mg)       | 91.6 $\pm$ 43.9           | 98.67 $\pm$ 34          | 0.1     |
| Folate (mcg)         | 214.2 $\pm$ 100           | 427.7 $\pm$ 130         | <0.001  |
| Selenium (mcg)       | 88.58 $\pm$ 31.5          | 89 $\pm$ 30.5           | 0.8     |
| Zinc (mg)            | 6.41 $\pm$ 2.4            | 12.1 $\pm$ 3.4          | <0.001  |

Table 3. Correlations between sperm parameters and antioxidant intake

| Sperm parameter                    | Beta carotene (mcg) | Vitamin C (mg) | Vitamin E (mg)  | Folate (mcg)     | Zinc (mg)        | Selenium (mcg) |
|------------------------------------|---------------------|----------------|-----------------|------------------|------------------|----------------|
| Concentration ( $\times 10^6$ /ml) | r=-0.4<br>p=0.003   | r=-0.1<br>N.S  | r=-0.1<br>N.S   | r=0.1<br>N.S     | r=0.4<br>p=0.004 | r=0.1<br>N.S   |
| Motility (%)                       | r=0.15<br>N.S       | r=0.18<br>N.S  | r=0.2<br>N.S    | r=0.5<br>p<0.001 | r=0.6<br>p<0.001 | r=0.01<br>N.S  |
| Morphology (%)                     | r=-0.06<br>N.S      | r=0.04<br>N.S  | r=0.3<br>p=0.03 | r=0.1<br>N.S     | r=0.1<br>N.S     | r=0.08<br>N.S  |

folate intake ( $\geq 75$ th percentile) had lower frequencies of sperm aneuploidy compared to men with lower intake ( $\leq 25$ th percentile) (18). Some interventional studies showed that oral supplementation with vitamin E, folate and zinc sulfate improve semen quality in infertile patients (19-20).

Folate is essential micronutrient for DNA synthesis and repair. Inadequate 5, 10-methylenetetrahydrofolate has been shown to cause massive misincorporation of uracil into human DNA (21).

Moreover we did not find any association between selenium and semen quality, which is in agreement with Hawkes and Iwanier et al., in which selenium supplementation did not improve semen quality in healthy and subfertile men (22-23).

Finally there was a significant negative correlation between sperm concentration and beta-carotene. Since we did not assess the concentration of this nutrient in body fluids, our data were not enough to explain the effect. This unexpected result implies that the effect of a single food, nutrient, or food group is not always clear; foods and nutrients are consumed in combination and as a result may have a synergistic effect (24). Analysis of overall dietary patterns may provide a comprehensive correlation with their overall effects on oxidation, in-

flammation, and disease risk. In spite of the fact that the main sources of beta-carotene and vitamin C are fruit and vegetables, the quantification of antioxidant consumption may be further complicated by food storage, handling, processing, and preparation (25). Water-soluble antioxidants such as vitamin C are released into high temperature cooking water and discarded. It has been suggested that high levels of beta-carotene might induce DNA damage due to oxidative stress (26). Van Helden et al. demonstrated that the anti or pro oxidant effect of beta carotene, is dependent on the type of radicals involved. In their study showed that beta carotene is an anti-oxidant against vitamin C and it can significantly reduces the M<sub>1</sub>dG levels in vitro as well as in vivo. But, it was not capable to scavenge. The OH agent and even resulted in an increased ROS production in lung epithelial cells (27).

This study had some limitations. First, only one semen sample was taken from each participant. We knew that collecting several semen samples over 1 to 2 weeks was ideal, but this may reduce the number of participants and thus decreased the power of this study. Also, some associations could have remained unrecognized owing to sample size insufficiency. We assessed nutrient

intake from a FFQ. But, responses on a FFQ may not reflect the concentration of nutrients in blood, testicular or seminal plasma. Measurement of relevant antioxidants in seminal plasma may be preferable. However a single measurement may not reflect the nutrient exposures during the whole period of spermatogenesis. Single measurements may show some other factors such as variation in absorption, metabolism and so on. The strength of the present study was the control for potential confounders such as age, BMI, smoking, alcohol intake and occupational factors.

Our study might be compatible with the hypothesis that oxidative stress related to high production of reactive oxygen species and low antioxidant capacity is negatively correlated with sperm concentration, motility, and morphology in infertile men.

### Conclusion

The results of this case-control study suggest that the risk of poor sperm concentration, motility and morphology are associated with low intake of some antioxidant agents in a group of Iranian oligo/ astheno/ teratozoospermic men.

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