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# Dietary Fats, Minerals and Semen Quality and Quantity among Men Attending a Fertility Clinic: A Cross-Sectional Study in Iran

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***ABSTRACT***

**Background**: At least 50% of infertile couple's problems are related to male factor infertility and in many patients; nutritional problems have been related to decreased sperm quality. Thus, the present study aims to examine the association of dietary fat and mineral intake with semen quantity and quality. **Methods**: This cross-sectional study was conducted on 175 infertile men in Isfahan Infertility and Fertility Center, Iran. Dietary intake of all patients was evaluated by validated Food Frequency Questionnaire (FFQ) and semen analysis was performed by standard protocol. Linear and quartile regression were used to determine the association of dietary fat and mineral intake with semen related factors. **Results**: There was a positive association between the sperm total count with iron (Fe) ($P_{trend}$ = 0.03), zinc (Zn) ($P_{trends}$= 0.02), and selenium (Se) ($P_{trend}$ = 0.001). Men in the highest quartile of Zn and alpha-linolenic acid (ALA) had higher sperm density ($P$ < 0.05). Levels of Zn and docosahexaenoic acid (DHA) in participant's diet were also negatively related to DNA damage ($P_{trend}$ = 0.01 for Zn and $P_{trend}$ = 0.04 for DHA). Higher intake of saturated fatty acid (SFA) was associated with lower sperm density ($P_{trend}$ = 0.02) and higher intake of monounsaturated fatty acids (MUFAs) was associated with higher sperm totality ($P_{trend}$ = 0.02). **Conclusion:** In this preliminary cross-sectional study, diets containing higher amounts of Fe, Zn, Se, MUFA, and DHA and lower amounts of SFA were associated with more favourable semen quality parameters.

**Keywords:** *Male infertility; Semen quality, Minerals, Fats*

## Introduction

Over the past years, one of the concerns of health professionals has been increasing the incidence of infertility in both genders (Irvine *et al.*, 1996, Zou *et al.*, 2011). More than 70 million couples suffer from infertility worldwide. More than 50% of infertility is associated with male disorders (Mehra *et al.*, 2018). Contradictory results have been reported regarding the decrease

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in the quality and quantity of sperm in young men (Auger *et al.*, 1995, Rolland *et al.*, 2012). Researchers have suggested that reducing sperm quality is more affected by environmental factors. Some factors, such as radiation, smoking, varicocele, infection, and urinary tract infection contribute to the etiology of male infertility. Nevertheless, regional differences also affect the results of various studies (Jorgensen *et al.*, 2001, Lipshultz, 1996, Paulsen *et al.*, 1996, Wong *et al.*, 2000), so that, infertility rates are higher in developed and industrialized countries (Danielewicz *et al.*, 2018). One of the environmental factors affecting the quality of sperm is individuals' diets. Little is known of how diet may affect male reproductive potential. Emerging literature supports the hypothesis that specific nutritional factors can affect semen quality. Previous studies have shown that some food components, such as antioxidants, fruits, and vegetables can affect the quality of sperm (Afeiche *et al.*, 2013, Eslamian *et al.*, 2012). Other factors, such as the high consumption of alcoholic beverages, obesity, coffee, processed meat, and fast foods can also reduce sperm quality (Salas-Huetos *et al.*, 2017, Sermondade *et al.*, 2013).

Dietary fats are one of the essential dietary ingredients. Cholesterol as a type of dietary fat plays an important role in the structure of mammalian membranes (Diaz-Fontdevila and Bustos-Obregon, 1993). The sperm membrane is affected by the type of dietary fat (Saez Lancellotti *et al.*, 2010). Overconsumption of dietary saturated and Trans fatty acids by increasing inflammation in the body may affect sperm quality (Dadkhah *et al.*, 2017). On the other hand, previous studies have shown that there is an important association between the intake of certain dietary minerals (Ammar *et al.*, 2019, Fallah *et al.*, 2018, Moslemi and Tavanbakhsh, 2011), zinc (Zn), iron (Fe), and semen quality.

Although some previous studies have evaluated the association between the dietary components and quality of sperm, the results of existing studies are contradictory. Therefore, this study aims to investigate the relation between dietary fats dietary fats and minerals with factors related to semen quantity and quality.

## Materials and Methods

*Study design:* The present study was conducted using a case-control design on 175 infertile participants referred to Isfahan Infertility and Fertility Center, Iran, in 2018. The study participants were infertile men. The inclusion criteria consisted of adults aged 20-45 years, sperm counts lower than 20 million, motility lower than 60%, damaged morphology greater than 65%. The exclusion criteria included frequent consumption of omega 3, vitamin E and other antioxidant supplements, history of reproductive disorders or vasectomy, smoking, urinary tract infection, testicular surgery, testicular tumor, anatomical abnormalities of the genital area (Complete information has been previously published (Rahimlou *et al.*, 2019)).

*Measurements*: A bioelectrical impedance analysis (Omron BF511; Omron Healthcare, Osaka, Japan) was employed to measure weight with minimum clothes and without shoes. Height was measured using a Seca stadiometer with the precision of 0.1 cm in a standing position next to a wall, without shoes, and with relaxed shoulders. Then body masas index (BMI) was computed by dividing the weight (kg) by the square of height ($m^2$).

For evaluation of dietary intake, a validated detailed food frequency questionnaire (FFQ) was used (Esmaillzadeh *et al.*, 2007). This questionnaire contains 168 food items that are consumed more by the Iranian population. The participants were asked to report on average, the frequency of food intake variation from never or less than once/month to 12 times or more per day, during the previous year. Eventually, the amount of consumption of each item of food was computed and converted to grams per day using household measures (Ghaffarpour *et al.*, 1999) (Complete information has been previously published (Rahimlou *et al.*, 2019)).

*Semen analysis:* Semen samples were collected from all of the participants by masturbation. Of all




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the participants, two samples of sperm were taken and if the difference between the samples was more than 20%, the third sample was taken. The samples were held at 37 °C to liquefy. After liquefaction, the samples were analyzed according to the World Health Organization guideline (Künzle *et al.*, 2003). Semen counts and motility percentage were evaluated using computer-aided semen analysis (CASA) (Hamilton-Thorne Version 10HTM-IVOS) using 2 Chamber Lejaw slides (Leja, The Netherlands). Morphology was established according to the Kruger parameters. Finally, sperm DNA damage was assessed by terminal deoxynucleotidyl trans-ferase dUTP nick end labeling (TUNEL) and the sperm chromatin structure assay (SCSA).

*Ethical considerations:* The study was approved by the Ethics Committee from Esfahan University of Medical Sciences with the code of IR.MUI.RESEARCH.REC.1397.267 and informed consent was obtained from all the participants. Prior to data collection, the purpose of the study was explained to the participants and their informed consent was recorded.

*Data analysis:* Sperm samples were taken from 175 infertile men. These men were divided into four groups according to quartile of intake of total major fat and minerals categories. Participants with the lowest intake of each fats or minerals were considered as the reference group. Linear regression was used to examine the association of fats or minerals intake with semen quality parameters. Analysis of covariance (ANCOVA) was used for calculating adjusted semen parameters for each nutrient quartile by relevant covariates. Multivariate ANCOVA models were created with continuous semen parameters as dependent variables, and fats and minerals covariates as independent variables. For statistical analyses, SPSS® 20 (IBM Corp, Armonk, NY) was used and P-value < 0.05 was considered significant.

## Results

A total of 175 participants aged from 20 to 45 years were included in this cross-sectional study. Baseline characteristics of the participants are summarized in **Table 1**. The participants were infertile men with mean age of 32.19 ± 2.34 year and BMI of 26.86 ± 1.60 kg/m$^2$. Thirty-seven percent of the participants were smokers, and five percent consumed alcohol. All of the participants performed semen analysis and semen analysis parameters, including the mean sperm concentration was 10.39 ± 32.5 mill/mL; seminal volume, 4.20 ± 1.89 ml, and total sperm count was 42.91 ± 30.91 million. Dietary intake of the participants is shown in the **Table 1**. The mean daily total intake was 2183.56 ± 202.81 kcal/day for total energy, 153.23 ± 11.85 g/day for carbohydrate, 88.21 ± 10.40 g/day for protein, and 75.34 ± 13.41 g/day for total fat. The mean caffeine intake was 65.45 ± 46.01 mg/day.

The correlation between sperm related parameters with dietary components is shown in **Table 2**. Based on the table, there was no significant association between dietary intake of carbohydrate, protein, total fat, and caffeine with none of the sperm related factors ($P > 0.05$).

The mean daily intake of dietary minerals was 1212.49 ± 432.45 mg/day for calcium, 16.5 ± 4.32 mg/day for Fe, 425.86 ± 114.28 mg/day for magnesium, 14.35 ± 4.41 for Zn, 1.93 ± 0.58 µg/day, and 101.52 ± 26.58 µg/day for selenium (Se). According to **Table 3**, after adjusting for covariates, the sperm total count had linear association with Fe ($P_{trend}$ = 0.03), Zn ($P_{trends}$ = 0.02) and Se intake ($P_{trend}$ = 0.001). The participants in the higher quartile of Zn consumption had higher sperm density ($P_{trend}$ = 0.03). Moreover, a linear association was found between Se intake with sperm total motility ($P_{trend}$ = 0.01) and an inverse association between Zn intake with sperm DNA damage ($P_{trend}$ = 0.01).

The mean daily intake of dietary fats was 338.19 ± 221.46 mg/day for total cholesterol, 28.17 ± 11.13 mg/day for saturated fatty acid (SFA), 28.38 ± 11.91 mg/day for monounsaturated fatty acid (MUFA), 16.75 ± 7.66 mg/day for poly unsaturated fatty acid (PUFA), 14.04 ± 6.99 mg/day for linoleic acid, 1.27 ± 0.67 mg/day for alpha-linolenic acid (ALA), 0.07 ± 0.5 mg/day for




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ecosapantaenoic acid (EPA), and 0.25 ± 0.23 mg/day for docosahexaenoic acid (DHA).

**Table 4** reveals that there was an inverse significant association between dietary intake of SFA and semen density ($P_{trend}$ = 0.02). A significant linear association was also found between MUFA intake and sperm total motility ($P_{trend}$ = 0.02), ALA with sperm density ($P_{trend}$ = 0.04) and sperm total motility ($P_{trend}$ = 0.01), and between dietary DHA with sperm density ($P_{trend}$ = 0.03). Finally, the subjects in the higher quartile of dietary intake of DHA had lower percentage of DNA damage ($P_{trend}$ = 0.04) and the participants in the higher quartile of PUFA had lower percentage of DNA fragmentation index (DFI) ($P_{trend}$ = 0.03).

**Table 1.** Baseline characteristics of the study population.

| Characteristics | Total (n = 57) |
|---|---|
| Age (year) | 32.19 ± 2.34[a] |
| Height (cm) | 178.17 ± 4.12 |
| Weight (kg) | 85.28 ± 5.51 |
| Body mass index ( kg/m$^2$) | 26.86 ± 1.60 |
| MET ( MET-h/week ) | 30.91 ± 1.81 |
| **Dietary intake** | |
| Energy (kcal/day) | 2183.56 ± 202.81 |
| Carbohydrate (g/day) | 153.23 ± 11.85 |
| Protein (g/day) | 88.21 ± 10.40 |
| Fat (g/day) | 75.34 ± 13.41 |
| Caffeine (mg/day) | 65.45 ± 46.01 |
| **Semen parameters** | |
| Seminal volume (ml) | 4.20 ± 1.89 |
| Sperm concentration (mill/ml) | 10.39 ± 32.50 |
| Total sperm count (mill) | 42.91 ± 30.91 |
| Motile sperm (PR+NP) (%) | 25.65 ± 16.76 |
| Normal morphology (%) | 2.31 ± 1.32 |
| DNA fragmentation index (%) | 18.57 ± 6.94 |

[a]: Mean ± SD

**Table 2.** Correlation between sperm related parameters with dietary components.

| Sperm parameters | Energy (kcal/day) | Protein (g/day) | Carbohydrate (g/day) | Fat (g/day) | Caffeine (mg/day) |
|---|---|---|---|---|---|
| Total sperm count | 0.08 (0.27) | 0.9 (0.23) | 0.15 (**0.04**) | -0.017 (0.82) | -0.07 (0.32) |
| Sperm density ($10^6$/ml) | 0.012 (0.87) | -0.013 (0.86) | 0.09 (0.23) | -0.07 (0.33) | -0.04 (0.16) |
| Total motility (%) | 0.07 (0.34) | 0.04 (0.6) | 0.13 (0.07) | 0.01 (0.87) | - 0.076 (0.55) |
| DNA damage (%) | -0.08 ( 0.26) | -0.08 (0.24) | -0.1 (0.17) | -0.04 (0.59) | -0.02 ( 0.17) |
| DNA fragmentation index (%) | -0.09 (0.22) | -0.08 (0.26) | -0.12 (0.1) | -0.04 (0.52) | -0.05 (0.48) |




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**Table 3.** Multivariate-adjusted model of dietary intake of minerals and semen parameters.

| Variables | Sperm total count | | | | Sperm density | | | | Total motility | | | | DNA damage | | | | DNA Fragmentation Index | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Beta | SE | $P^a$ | $P^b$ | Beta | SE | $P^a$ | $P^b$ | Beta | SE | $P^a$ | $P^b$ | Beta | SE | $P^a$ | $P^b$ | Beta | SE | $P^a$ | $P^b$ |
| **Calcium** | | | | | | | | | | | | | | | | | | | | |
| Q1 | | | | | | | | | | | | | | | | | | | | |
| Q2 | -0.77 | 6.61 | 0.9 | 0.69 | 1.55 | 1.27 | 0.23 | 0.24 | 1.70 | 3.57 | 0.62 | 0.81 | -0.98 | 1.44 | 0.49 | 0.92 | -0.76 | 1.48 | 0.6 | 0.95 |
| Q3 | 8.13 | 6.57 | 0.21 | 0.55 | 2.1 | 1.28 | 0.1 | 0.18 | 4.79 | 3.59 | 0.18 | 0.57 | 0.96 | 1.45 | 0.5 | 0.13 | 0.89 | 1.49 | 0.54 | 0.17 |
| Q4 | 7.34 | 6.54 | 0.26 | 0.7 | 0.68 | 1.29 | 0.59 | 0.69 | 2.61 | 3.61 | 0.46 | 0.89 | -0.7 | 1.46 | 0.62 | 0.47 | -0.75 | 1.48 | 0.63 | 0.64 |
| $P_{trend}$ | | | 0.17 | | | | 0.38 | | | | 0.39 | | | | 0.34 | | | | 0.46 | |
| Fe | | | | | | | | | | | | | | | | | | | | |
| Q1 | | | | | | | | | | | | | | | | | | | | |
| Q2 | 3.69 | 6.41 | 0.56 | 0.69 | -2.46 | 1.24 | 0.04 | 0.21 | 2.55 | 3.53 | 0.47 | 0.57 | -2.29 | 1.44 | 0.62 | 0.66 | -1.41 | 1.47 | 0.33 | 0.31 |
| Q3 | 0.75 | 6.38 | 0.9 | 0.85 | 0.56 | 1.25 | 0.65 | 0.44 | -0.93 | 3.51 | 0.78 | 0.7 | - 1.52 | 1.43 | 0.28 | 0.44 | -1.95 | 1.46 | 0.17 | 0.27 |
| Q4 | 15 | 6.41 | 0.01 | 0.18 | 1.26 | 1.26 | 0.31 | 0.2 | 6.58 | 3.53 | 0.06 | 0.41 | -0.7 | 1.44 | 0.11 | 0.42 | -2.74 | 1.47 | 0.06 | 0.26 |
| $P_{trend}$ | | | 0.03 | | | | 0.24 | | | | 0.08 | | | | 0.15 | | | | 0.22 | |
| **Zn** | | | | | | | | | | | | | | | | | | | | |
| Q1 | | | | | | | | | | | | | | | | | | | | |
| Q2 | 2.45 | 4.28 | 0.32 | 0.42 | 0.76 | 2.75 | 0.56 | 0.63 | 1.19 | 2.16 | 0.2 0 | 0.36 | -0.28 | 1.34 | 0.84 | 0.58 | 0.19 | 1.47 | 0.59 | 0.45 |
| Q3 | 3.17 | 4.28 | 0.09 | 0.12 | 1.32 | 2.73 | 0.25 | 0.27 | 1.65 | 2.17 | 0.08 | 0.12 | -0.94 | 1.34 | 0.32 | 0.22 | - 1.26 | 1.47 | 0.19 | 0.08 |
| Q4 | 6.47 | 4.29 | 0.03 | 0.04 | 3.63 | 2.76 | 0.04 | 0.05 | 1.88 | 2.19 | 0.12 | 0.24 | -1.68 | 1.35 | 0.09 | 0.04 | -1.82 | 1.48 | 0.11 | 0.032 |
| $P_{trend}$ | | | 0.02 | | | | 0.03 | | | | 0.16 | | | | 0.01 | | | | 0.04 | |
| **Cu** | | | | | | | | | | | | | | | | | | | | |
| Q1 | | | | | | | | | | | | | | | | | | | | |
| Q2 | -1.97 | 1.26 | 0.11 | 0.3 | 7.69 | 3.52 | 0.02 | 0.007 | -4.96 | 7.08 | 0.48 | 0.11 | -1.18 | 1.66 | 0.46 | 0.3 | -1.80 | 1.46 | 0.21 | 0.43 |
| Q3 | 0.67 | 1.25 | 0.59 | 0.41 | 1.69 | 3.50 | 0.62 | 0.16 | 0.26 | 7.84 | 0.97 | 0.5 | -1.87 | 1.77 | 0.28 | 0.12 | -2.80 | 1.47 | 0.05 | 0.21 |
| Q4 | 1.06 | 1.23 | 0.4 | 0.21 | 0.31 | 3.51 | 0.92 | 0.27 | 11.74 | 9.69 | 0.22 | 0.4 | -0.87 | 2.19 | 0.69 | 0.19 | -1.99 | 1.48 | 0.17 | 0.79 |
| $P_{trend}$ | | | 0.18 | | | | 0.73 | | | | 0.44 | | | | 0.16 | | | | 0.58 | |
| **Se** | | | | | | | | | | | | | | | | | | | | |
| Q1 | | | | | | | | | | | | | | | | | | | | |
| Q2 | 1.66 | 6.51 | 0.37 | 0.77 | -0.45 | 1.29 | 0.72 | 0.53 | 0.81 | 3.42 | 0.00 | 0.006 | -0.39 | 1.44 | 0.23 | 0.08 | - 0.7 | 1.47 | 0.59 | 0.22 |
| Q3 | 5.83 | 6.44 | 0.04 | 0.08 | 0.12 | 1.27 | 0.92 | 0.73 | 2.42 | 3.38 | 0.08 | 0.12 | - 1.63 | 1.43 | 0.24 | 0.78 | - 1.66 | 1.48 | 0.25 | 0.75 |
| Q4 | 10.33 | 6.55 | <0.001 | 0.001 | 0.27 | 1.28 | 0.83 | 0.94 | 5.42 | 3.44 | 0. 03 | 0.048 | - 1.39 | 1.43 | 0.78 | 0.51 | - 1.85 | 1.45 | 0.97 | 0.47 |
| $P_{trend}$ | | | 0.001 | | | | 0.77 | | | | 0.01 | | | | 0.35 | | | | 0.44 | |

$P^a$: crude p- value, $P^b$: adjusted p-value, tests for linear trend were performed using the median value for each quartile. Multivariate model adjusted for BMI, energy intake and physical activity.





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**Table 4.** Multivariate-adjusted model of dietary intake of fats and semen parameters

| Median for each quartile | Sperm total count | | | | Sperm density | | | | Total motility | | | | DNA damage | | | | DNA Fragmentation Index | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Beta | SE | P[a] | P[b] | Beta | SE | P[a] | P[b] | Beta | SE | P[a] | P[b] | Beta | SE | P[a] | P[b] | Beta | SE | P[a] | P[b] |
| **Cholesterol** | | | | | | | | | | | | | | | | | | | | |
| Q1 | | | | | | | | | | | | | | | | | | | | |
| Q2 | -3.76 | 7.08 | 0.59 | 0.72 | -0.63 | 1.27 | 0.61 | 0.76 | -2.77 | 3.50 | 0.42 | 0.61 | -0.71 | 1.58 | 0.65 | 0.48 | -2.17 | 1.47 | 0.14 | 0.2 |
| Q3 | -12.59 | 7.63 | 0.1 | 0.32 | -2.12 | 1.26 | 0.94 | 0.37 | -9.63 | 3.48 | 0.006 | 0.03 | 0.69 | 1.70 | 0.68 | 0.73 | -1.22 | 1.46 | 0.4 | 0.56 |
| Q4 | - 11.52 | 9.00 | 0.201 | 0.46 | -1.44 | 1.27 | 0.25 | 0.16 | -1.27 | 3.50 | 0.71 | 0.48 | -2.68 | 1.44 | 0.06 | 0.24 | 0.31 | 1.47 | 0.83 | 0.87 |
| $P_{trend}$ | | 0.38 | | | | 0.28 | | | | 0.4 | | | | 0.53 | | | | 0.16 | | |
| **Saturated fatty acid (SFA)** | | | | | | | | | | | | | | | | | | | | |
| Q1 | - | | | | | | | | | | | | | | | | | | | |
| Q2 | -0.62 | 6.57 | 0.43 | 0.51 | -0.6 | 1.28 | 0.63 | 0.24 | -5.12 | 3.56 | 0.15 | 0.06 | 0.01 | 1.45 | 0.99 | 0.41 | 0.16 | 1.47 | 0.91 | 0.37 |
| Q3 | 0.97 | 6.57 | 0.88 | 0.05 | -1.15 | 1.25 | 0.12 | 0.21 | -2.49 | 3.56 | 0.48 | 0.16 | -1.15 | 1.45 | 0.42 | 0.68 | -2.02 | 1.47 | 0.17 | 0.99 |
| Q4 | -5.11 | 6.53 | 0.43 | 0.69 | -1.90 | 1.17 | 0.03 | 0.04 | -1.48 | 3.54 | 0.67 | 0.27 | 0.15 | 1.44 | 0.91 | 0.2 | 0.29 | 1.46 | 0.84 | 0.72 |
| $P_{trend}$ | | 0.13 | | | | 0.02 | | | | 0.93 | | | | 0.91 | | | | 0.85 | | |
| **Mono unsaturated fatty acid (MUFA)** | | | | | | | | | | | | | | | | | | | | |
| Q1 | | | | | | | | | | | | | | | | | | | | |
| Q2 | 2.57 | 7.16 | 0.72 | 0.9 | -0.76 | 1.32 | 0.56 | 0.4 | 1.84 | 3.70 | 0.3 | 0.06 | -1.68 | 1.50 | 0.26 | 0.72 | -0.95 | 1.54 | 0.53 | 0.96 |
| Q3 | -2.13 | 7.13 | 0.76 | 0.89 | -2.16 | 1.38 | 0.11 | 0.25 | 4.45 | 3.87 | 0.25 | 0.02 | -1.26 | 1.57 | 0.41 | 0.85 | -0.9 | 1.61 | 0.57 | 0.68 |
| Q4 | 2.5 | 6.82 | 0.7 | 0.27 | -1.24 | 1.39 | 0.37 | 0.82 | 5.28 | 3.89 | 0.29 | 0.04 | -0.73 | 1.57 | 0.64 | 0.59 | -0.5 | 1.61 | 0.75 | 0.51 |
| $P_{trend}$ | | 0.63 | | | | 0.69 | | | | 0.02 | | | | 0.41 | | | | 0. 29 | | |
| **Poly unsaturated fatty acid (PUFA)** | | | | | | | | | | | | | | | | | | | | |
| Q1 | | | | | | | | | | | | | | | | | | | | |
| Q2 | 1.32 | 6.43 | 0.01 | 0.05 | -1.39 | -0.5 | 1.27 | 0.69 | 4.16 | 3.54 | 0.24 | 0.32 | -2.27 | 1.43 | 0.11 | 0.08 | 0.47 | 1.46 | 0.09 | 0.06 |
| Q3 | 5.51 | 6.50 | 0.001 | 0.001 | -0.54 | 1.15 | 1.29 | 0.9 | -0.57 | 3.56 | 0.87 | 0.85 | -1.59 | 1.44 | 0.27 | 0.11 | -1.62 | 1.47 | 0.27 | 0.09 |
| Q4 | 11.43 | 6.43 | 0.001 | 0.001 | 0.2 | 1.12 | 1.27 | 0.37 | 3.12 | 3.54 | 0.37 | 0.38 | -2.55 | 1.43 | 0.07 | 0.04 | -2.88 | 1.46 | 0.04 | 0.02 |
| $P_{trend}$ | | 0.001 | | | | 0.15 | | | | 0.2 | | | | 0.14 | | | | 0.03 | | |
| **Linoleic acid** | | | | | | | | | | | | | | | | | | | | |
| Q1 | | | | | | | | | | | | | | | | | | | | |
| Q2 | 3.09 | 6.84 | 0.65 | 0.34 | -1.03 | 1.31 | 0.43 | 0.83 | 0.76 | 3.57 | 0.83 | 0.9 | -2.72 | 1.43 | 0.05 | 0.05 | -2.59 | 1.47 | 0.07 | 0.05 |
| Q3 | 5.70 | 8.21 | 0.48 | 0.57 | 0.57 | 1.58 | 0.75 | 0.5 | - 2.04 | 3.55 | 0.5 | 0.62 | -2.08 | 1.43 | 0.14 | 0.11 | -1.72 | 1.46 | 0.23 | 0.18 |
| Q4 | -3.34 | 10.64 | 0.75 | 0.4 | 0.4 | 2.05 | 0.87 | 0.74 | 1.15 | 3.57 | 0.74 | 0.74 | -2.10 | 1.43 | 0.14 | 0.13 | - 2.34 | 1.47 | 0.11 | 0.12 |
| $P_{trend}$ | | 0.97 | | | | 0.99 | | | | 0.88 | | | | 0.95 | | | | 0.62 | | |




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**Table 4.** Multivariate-adjusted model of dietary intake of fats and semen parameters

| Median for each quartile | Sperm total count | | | | Sperm density | | | | Total motility | | | | DNA damage | | | | DNA Fragmentation Index | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Beta | SE | $P^a$ | $P^b$ | Beta | SE | $P^a$ | $P^b$ | Beta | SE | $P^a$ | $P^b$ | Beta | SE | $P^a$ | $P^b$ | Beta | SE | $P^a$ | $P^b$ |
| **Alpha-linolenic acid** | | | | | | | | | | | | | | | | | | | | |
| Q1 | | | | | | | | | | | | | | | | | | | | |
| Q2 | 3.53 | 6.55 | 0.14 | 0.09 | 0.07 | 1.28 | 0.95 | 0.36 | 0.43 | 3.58 | 0.32 | 0.61 | -1.77 | 1.42 | 0.21 | 0.18 | -1.83 | 1.46 | 0.21 | 0.16 |
| Q3 | 4.27 | 6.65 | 0.55 | 0.31 | 0.33 | 1.27 | 0.31 | 0.11 | 1.17 | 3.58 | 0.04 | 0.06 | 0.36 | 1.41 | 0.8 | 0.81 | -0.74 | 1.47 | 0.68 | 0.37 |
| Q4 | 4.38 | 6.51 | 0.5 | 0.44 | 1.65 | 1.28 | 0.05 | 0.02 | 2.15 | 3.56 | 0.01 | 0.03 | -2.94 | 1.42 | 0.03 | 0.05 | - 2.77 | 1.47 | 0.05 | 0.08 |
| $P_{trend}$ | | | 0.97 | | | | 0.04 | | | | 0.01 | | | | 0.78 | | | | 0.87 | |
| **Docosapantaenoic acid (DHA)** | | | | | | | | | | | | | | | | | | | | |
| Q1 | | | | | | | | | | | | | | | | | | | | |
| Q2 | -6.67 | 6.48 | 0.30 | 0.24 | -0.59 | 1.27 | 0.64 | 0.58 | 3.14 | 3.53 | 0.37 | 0.56 | 0.58 | 1.43 | 0.68 | 0.41 | 0.59 | 1.47 | 0.68 | 0.78 |
| Q3 | -7.53 | 6.17 | 0.22 | 0.13 | 1.16 | 1.45 | 0.13 | 0.08 | -1.49 | 3.36 | 0.65 | 0.52 | -0.47 | 1.36 | 0.15 | 0.18 | -0.67 | 1.40 | 0.62 | 0.79 |
| Q4 | -0.4 | 7.42 | 0.95 | 0.73 | 1.38 | 1.20 | 0.10 | 0.04 | -1.10 | 4.04 | 0.78 | 0.72 | -1.22 | 1.64 | 0.05 | 0.02 | -1.32 | 1.68 | 0.43 | 0.46 |
| $P_{trend}$ | | | 0.43 | | | | 0.03 | | | | 0.59 | | | | 0.04 | | | | 0.4 | |
| **Ecosapataenoic acid (EPA)** | | | | | | | | | | | | | | | | | | | | |
| Q1 | | | | | | | | | | | | | | | | | | | | |
| Q2 | 1.61 | 6.55 | 0.8 | 0.99 | 0.66 | 1.27 | 0.6 | 0.47 | 1.33 | 3.56 | 0.708 | 0.85 | 0.72 | 1.45 | 0.61 | 0.47 | 0.46 | 1.48 | 0.75 | 0.63 |
| Q3 | -5.85 | 6.55 | 0.37 | 0.33 | 1.97 | 1.27 | 0.12 | 0.15 | 1.13 | 3.56 | 0.246 | 0.31 | 0.03 | 1.45 | 0.98 | 0.83 | -0.31 | 1.48 | 0.83 | 0.9 |
| Q4 | 2.77 | 6.59 | 0.67 | 0.94 | 1.37 | 1.28 | 0.76 | 0.93 | 1.82 | 3.58 | 0.611 | 0.53 | -0.18 | 1.46 | 0.89 | 0.74 | -0.48 | 1.49 | 0.74 | 0.98 |
| $P_{trend}$ | | | 0.56 | | | | 0.42 | | | | 0.61 | | | | 0.57 | | | | 0.78 | |

$P^a$: crude p- value, $P^b$: adjusted p-value, tests for linear trend were performed using the median value for each quartile. Multivariate model adjusted for BMI, energy intake and physical activity.





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## Discussion

In this cross-sectional study, it was found that higher intake of Fe, Zn, and Se were associated with higher sperm total count; Zn, ALA, DHA with semen density, Se, MUFA, and ALA with sperm total motility; DHA, Zn with DNA damage, and finally PUFA with lower percentage of DFI. Higher intake of SFA is inversely associated with sperm density. An increasing number of studies have examined the association between nutrients with semen quality (Cutillas-Tolin *et al.*, 2019, Rahimlou *et al.*, 2019, Ricci *et al.*, 2018). The findings can be compared to some extent with previous studies. Attaman J *et al.*, in a cross-sectional study, examined the association between dietary fat intake and sperm related parameters. They found that higher intake of total fat had a negative association with total sperm count and sperm concentration. Their results revealed a negative association between dietary SFA with sperm concentration and a linear association between dietary omega-3 intake with favorable sperm morphology (Attaman *et al.*, 2012). Mendiola *et al.* reported that higher intake of processed meat as one of the richest sources of SFA is associated with decreased sperm quality (Attaman *et al.*, 2012). Moreover, Safarinejad randomized 238 Iranian oligoasthenoteratospermic (OAT) men to receive a supplement containing 1840 mg EPA and DHA or placebo for 32 weeks and found that omega-3 fatty acid supplementation increased the total percentage of sperm with normal morphology (Safarinejad *et al.*, 2010), which is in line with the findings of the present study. In the present study, high intake of saturated fatty acids was associated with decreased sperm concentration. Lipids are key components of the mammal's semen. They work as structural components of semen membranes, which are precursors of numerous biologically active compounds (eicosanoids) and can be used for energy production. It has been shown that DHA is the most important spermatozoan PUFA in mammals, including man, bull, monkey, ram, and boar (Cerolini *et al.*, 2000, Poulos *et al.*, 1973). Some studies have shown that increased DHA concentrations in semen are associated with better sperm motility (Aksoy *et al.*, 2006, Safarinejad *et al.*, 2010). The study results indicated that the participants in the higher quartile of dietary DHA intake had higher semen concentration and lower sperm DNA damage. Martínez-Soto J *et al.* in their RCT study reported that 1500 mg/day DHA supplementation for 10 weeks led to significant improvement in the seminal antioxidant status and decreased sperm DNA fragmentation (Martínez-Soto *et al.*, 2016). Safarinejad *et al.* randomized 238 Iranian OAT men to receive a supplement containing 1840 mg EPA and DHA or placebo for 32 weeks. They found that omega-3 supplementation caused a significant increase in the sperm total count, concentration, and motility (Safarinejad, 2011). Various mechanisms have been suggested for the beneficial effects of PUFA and MUFA on sperm quality. Adipose tissue depots play a significant role in the development of oxidative stress after a pro-inflammatory disorder that may alter normal reproductive pathways and sperm activity (Bachir and Jarvi, 2014, Morielli and O'Flaherty, 2015). In reality, under stress conditions, mitochondria are likely to trigger a cascade of oxidative damage in the testicular environment, since these organelles play an important role in the ROS production. MUFA and PUFA fatty acids have protective effects against increased ROS levels due to their anti-inflammatory and anti-oxidative properties (Ferramosca *et al.*, 2017, Gürler *et al.*, 2015). Evidence has suggested that these fatty acids may facilitate body fat reduction and losing weight, thereby preventing the development of obesity-related infertility; on the other, they control specific aspects of male fertility (Esmaeili *et al.*, 2015, Wathes *et al.*, 2007). PUFA supplies precursors for eicosanoid production that can attenuate the expression trends of several key enzymes (including cyclooxygenase and lipoxygenase) implicated in both prostaglandin and steroid metabolism These fatty acids are also functional elements of cell membranes, the fluidity of which is needed to facilitate fertilization-related membrane fusion activities (Ferramosca *et al.*,




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2017).

The results of the current study revealed that some minerals included Zn, Fe, and Se had beneficial effects on sperm related factors. Colagar A *et al.* in a cross-sectional study compared the concentrations of some micronutrients in semen and its association with male fertility. They found that the concentration of seminal Zn was significantly higher in the fertile subjects compared to infertile men (Colagar *et al.*, 2009). Zhao *et al.* in a meta-analysis showed that seminal plasma Zn concentrations from infertile patients were significantly lower than the control group (SMD [95% CI]: −0.64 [−1.01, −0.28]). After reviewing various studies, they reported that Zn supplementation significantly improved percentage of normal sperm morphology, sperm motility, and semen volume (Zhao *et al.*, 2016). Acute Zn depletion creates a 50% decline in Zn per ejaculate, resulting in pathozoospermia (Dissanayake *et al.*, 2010). There are several mechanisms by which Zn might interfere with sperm function. Zn as a cofactor for many metalloenzymes involved in various metabolic processes (Adewoyin *et al.*, 2017) affects phospholipases, thereby modifying the flexibility of biological membranes. It has been proposed that perhaps the elimination of Zn from the sperm cell surface disrupts the plasma membrane and plays a major role in the preparation for the completion of capacitation and the acrosome reaction. Some studies have reported that higher Zn concentration in the seminal plasma is associated with better chromatin stability (Kumar and Singh, 2016, Subhani *et al.*, 2019). It has been shown that sperm motility is most affected by Zn concentration. Zn induces stiffening the exterior dense fibers by forming disulfide bridges throughout sperm development in the epididymis, which is an important phase in the development of sperm motility; in particular, progressive motility (Kumar and Singh, 2016). Some previous findings have indicated the importance of Se and Fe in completing sperm maturation and its quality. Se is more involved in the male reproductive performance as selenoproteins. The presence of adequate Se is important for normal spermatogenesis and sperm maturation (Ahsan *et al.*, 2014). Some previous studies have reported an inverse association between high Fe intake and sperm quality, which is not in line with the present study. Fe and copper are essential trace nutrients playing important roles in general health and fertility. However, both elements are highly toxic when accumulating in large quantities. Excessive Fe intake can stimulate the production of oxidative stress and ROS and may provide sperm membrane damage. It has been shown that either Fe excess or deficiency could lead to faulty spermatogenesis, sexual dysfunction, and oxidative damage to testicular tissue and sperm, eventually leading to impaired fertility (Ammar *et al.*, 2019, Tvrda *et al.*, 2015).

The present study had some limitations that must be considered in interpreting the results. First, because the cross-sectional design was unable to explain any causal relationship, further prospective studies are needed to confirm the findings. Second, the plasma or seminal levels of minerals and fatty acids were not measured in the target population in this study. Finally, to record dietary intake of minerals and fatty acids a food frequency questionnaire was used that is dependent on participants' ability to remind, and their education level may increase the risk of recall bias.

## Conclusion

The study results indicated that diets containing higher amounts Fe, Zn, Se, MUFA, and EPA and lower amounts of SFA were associated with more favorable semen quality parameters.

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## Authors' contributions

Rahimlou M and Sohaei S designed the research; Rahimlou M and Nouri M contributed to acquisition of data. Sohaei S and Nouri M performed statistical analyses and interpretation of data. Sohaei S and Salehi-Sahlabadi A wrote the





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manuscript. Rahimlou M and Salehi-Sahlabadi A contributed to critical revision of the manuscript. Each author has participated sufficiently in the study to take public responsibility for appropriate portions of the content.

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