Western Dietary Pattern Reduced Male Fertility: A Systematic review and Meta-analysis of Observational Studies

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ABSTRACT

Background: Previous studies have investigated the associations between dietary patterns and male infertility, but found contradictory results. So, this meta-analysis was conducted to examine the association between dietary patterns and male infertility. Methods: An electronic search was conducted to identify the studies over association between dietary patterns and male infertility. The western and healthy dietary patterns were selected in this regard. The random-effect model was used to compute the summary risk estimates. Results: We found eight articles, of which five (n = 7679 participants) were included in our meta-analysis. This meta-analysis determined that the western dietary patterns containing high levels of processed, meat, high-fat dairy, and low levels of vegetables, fruit, and whole grains could significantly reduce the sperm concentration (MD = -0.07, P = 0.01). However, no significant relationship was observed between this dietary pattern and the sperm motility and morphology (MD = 0.01, P = 0.71, MD = 0.2, P = 0.39, respectively). Moreover, regarding the healthy dietary pattern, which contains high level of fruit, vegetables, whole grain, low-fat dairy, as well as low level of red meat and processed food, no significant relationship was observed (sperm concentration: MD = 0.11, P = 0.11, morphology: MD = -0.02, P = 0.42 and motility: MD = 0.35, P = 0.09). Conclusions: Findings of our meta-analysis suggested that the western dietary patterns could decrease the sperm concentration and reduce male fertility. Further prospective studies are required to confirm this result.

Keywords: Infertility; Dietary pattern; Semen quality; Meta-analysis; Systematic review

Introduction

Infertility is a disability in the reproductive system, which can be defined as one year or more of unprotected intercourse without pregnancy (Boivin et al., 2007). Several factors can lead to men infertility, especially decrease of the semen quality which allocated 25 percent of infertility to itself (Evers, 2002). Furthermore, other factors such as environmental (Merzenich et al., 2010), genetic (Wong et al., 2000b), endocrine disorder (Wong et al., 2000b), age (Wong et al., 2000b), smoking (Wong et al., 2000a), heavy alcohol use (Homan et al., 2007, Wong et al., 2000b), and nutrition (Vujkovic et al., 2009a) may affect semen quality.

In the past years, we observed a decline in the trend...
of semen quality as a result of nutrition transition (Pastuszak and Lipsultz, 2012). Studies established that the prevalence of unhealthy diets, characterized by low intakes of fruits and vegetables and high intakes of foods rich in saturated fats, has increased in men within the reproductive age (Jurewicz et al., 2016b, Vujkovic et al., 2007). High consumption of red meat, processed meat, full-fat dairy, sweets, trans fatty acid, saturated fatty acid, and soy foods were associated with lower sperm quality (Cutillas-Tolín et al., 2015). In return, high intakes of fruits, vegetables, skim milk, seafood, and cereal were associated with healthy semen quality (Gaskins et al., 2012). In addition, some studies investigated the effects of individual nutrients such as folate (Wong et al., 2002), zinc (Wong et al., 2002), vitamin C (Piomboni et al., 2008), carotenoids (Eskenazi et al., 2005), vitamin E (Suleiman et al., 1996), and food groups (Attaman et al., 2012, Mendiola et al., 2009a) on semen quality.

Since nutrients and food groups are not consumed alone, considering the overall diet in which the combined effects of foods and nutrients are examined, can give us better and more comprehensive view of the relationship between diet and the disease (Hu et al., 1999). However, we found few studies in relation to dietary patterns and semen quality (Salas-Huetos et al., 2017). The results of these studies are contradictory, for example some studies indicated that the western dietary pattern which contains high fat dairy, processed meat, refined grains, pizza, and fried food could decrease the sperm concentration (Cutillas-Tolín et al., 2015, Liu et al., 2015b), sperm motility (Eslamian et al., 2016b), and sperm morphology (Liu et al., 2015b); whereas, this result was not found in the other study (Oostingh et al., 2017b). We also observed conflicting results regarding the healthy dietary patterns (Eslamian et al., 2016a, Gaskins et al., 2012) such as prudent (Gaskins et al., 2012) and Mediterranean (Karayiannis et al., 2016) dietary patterns, which are determined by high level of low fat dairy, poultry, fish, vegetables, whole grains, and fruits. Some studies established that healthy dietary patterns could improve the semen quality (Cutillas-Tolín et al., 2015, Jurewicz et al., 2016c), but others could not show the same result (Karayiannis et al., 2016, Vujkovic et al., 2009b).

Several mechanisms are responsible for this result. For instance, healthy dietary patterns with high levels of antioxidants and carotenoids could overcome the stress oxidative and inflammatory marker, so they improved the male fertility (Eskenazi et al., 2005, Mínguez-Alarcón et al., 2012). However, the western dietary pattern may have adverse effects on male fertility due to the saturated and trans fatty acids (Afeiche et al., 2014a, Mendiola et al., 2009a).

The relationship of dietary patterns and nutrient with male infertility was investigated in only one review without meta-analysis. This study focused on individual nutrients, but not the dietary pattern (Salas-Huetos et al., 2017). To the best of our knowledge, the present study is the first systematic review and meta-analysis on the relationship between dietary patterns and semen quality.

Materials and Methods

We conducted this systematic review and meta-analysis according to the MOOSE (Meta-Analysis of Observational Studies in Epidemiology). Moreover, this review was registered in the PROSPERO (http://www.crd.york.ac.uk/PROSPERO), an international prospective registration website of systematic reviews with the code number of CRD42017068581 (Hosseinzadeh and Hasanizadeh, 2017).

We conducted an electronic search throughout the PubMed, Web of Science, Scopus, and Google Scholar databases to obtain the articles published up to 22 May 2017. To identify the relevant articles, we used medical subject heading terms (MeSH) and non MeSH terms including: “dietary pattern”, “food pattern”, “eating pattern”, “dietary habit”, “eating habit”, “dietary behavior”, “fertility”, “infertility”, “fecundability”, “abnormal sperm”. Moreover, we did not limit our search by language.

Study selection: The process of study selection is summarized in Figure 1. First, we selected the relevant studies on the basis of articles’ titles and abstracts. In the next step, the full text of all related
Western dietary pattern and male fertility

articles was considered by reviewers to extract the studies on dietary pattern and semen quality parameters. All the steps were performed by two authors independently (Hasanizadeh H and Hosseinzadeh M) and any disagreements were discussed and resolved by consensus with the third researcher (Salehi–Abarghuei A).

Finally, we entered the studies that met the following criteria into our meta-analysis: 1) examined dietary patterns using standard instruments such as a 24-h dietary recall, food record, and frequency questionnaire (FFQ); 2) calculated scores of dietary patterns or identified dietary patterns using a statistical method such as principal component analysis, and 3) calculated the mean value or median value for semen quality parameters in each quartile. Studies that considered only nutrients, food groups, or supplements rather than the dietary patterns were excluded.

Data extraction: The following information was obtained from each study: author’s family name, publication year, country where the study was conducted, age of participants, sample size, design, dietary assessment tools, race/ethnicity, obtained dietary pattern, adjusted mean with standard deviation (SD), adjusted median with range, adjusted odds ratio/relative risk with confidence interval (CI), adjusted β coefficient with CI, and confounding factors adjusted in the analysis.

In order to enhance the comparability of results included in the meta-analysis, we selected the results based on the models with the most number of confounding variables. In addition, we only extracted the patterns of dietary intake identified in multiple studies such as the western and healthy dietary patterns. We also ensured that the selected dietary patterns were similarly based on the loading of food groups.

Quality assessment: In this study, the Newcastle–Ottawa scale was used to assess the quality of studies (Lo et al., 2014). In this scale a “star system” is used to compare the studies. Studies are given star based on three sections: 1) selection of the study groups, 2) comparability of the groups, and 3) ascertainment of either the exposure or outcome of interest. Then, if the study acquired 3 or 4 stars in the selection part, 1 or 2 stars in the comparability part, and 2 or 3 stars in the outcome/exposure part, it was considered as a high quality study (Lo et al., 2014).

Data synthesis and analysis: Statistical analyses were conducted by STATA software, version 11.2 (STATA Corp, College Station, TX). Mean value and SD were also calculated as the effect size of different dietary patterns and sperm concentration, motility, and morphology. We selected the mean value that compared the highest and the lowest categories of adherence to the dietary patterns. In addition, to incorporate the between-study variation, a random effects model was applied to combine the effect sizes. This model takes the study heterogeneity into account. I² and Q statistic was used to evaluate the statistical heterogeneity among studies (Higgins and Thompson, 2002). For the Q statistic, a p-value of < 0.1 was considered as statistically significant heterogeneity (Higgins and Thompson, 2002). Publication bias was evaluated by examination of the funnel plot (Egger et al., 2008). Sensitivity analysis was also performed to identify whether a specific study or a particular group of studies affected the conclusions (Egger et al., 2008). P-values less than 0.05 was considered significant.

Results

Literature search: A total of 1313 studies were identified by our electronic search throughout PubMed, Web of Science, Scopus, and Google Scholar prior to 22 may 2017. After removing the duplicates and screening the titles and abstracts of the studies, 53 full-text articles were reviewed. Of these, 44 studies were excluded. Therefore, 9 studies met the inclusion criteria and were included in our systematic review. For the meta-analysis, only 5 (n = 7679 participants) of the 9 articles were included. Of the four excluded studies, two studies had different categories of dietary patterns (Jurewicz et al., 2016a, Jurewicz et al., 2016b) and two calculated the odds ratio (Eslamian et al., 2016a) and Beta coefficient
(Oostingh et al., 2017a). So, to obtain more reliable results, we pooled the results from 5 cross-sectional studies involving 7679 participants. **Figure 1** shows the flow chart of the study selection process.

**Study Characteristics:** Characteristics of all included studies are represented in Table 1. Studies were conducted in different continents: one study was performed in USA (Cutillas-Tolín et al., 2015), three in Europe (Gaskins et al., 2012, Karayiannis et al., 2016, Vujkovic et al., 2009a), and one in Asia (Liu et al., 2015a). All the selected studies had a cross-sectional design. Studies were published during 2009 - 2016. Confounding factors such as total energy intake, body mass index, age, ethnicity, and smoking were adjusted in most of the studies. Dietary consumptions were determined by validated FFQ. In most studies, the western and healthy dietary patterns were derived according to the similarity of foods and food groups highly loaded on each pattern.

According to the Newcastle–Ottawa quality assessment scale, the score quality of the included studies was 9 to 10 and all the studies had a high quality based on the: 1) selection of the study groups, 2) comparability of the groups, and 3) ascertainment of either the exposure or outcome of interest.

**Healthy dietary patterns and semen quality parameters:** The healthy dietary patterns are highly loaded with vegetables, fruits, whole grains, poultry, and low-fat dairy. Our pool analysis demonstrated no significant relationship between the healthy dietary patterns and the semen quality parameters (**Figure 2**) such as sperm concentration (MD = 0.11, P = 0.11), morphology (MD = -0.02, P = 0.42) and motility (MD = 0.35, P = 0.09). Pooled analysis on 5 studies (n = 7679 participants) found a substantial relationship between healthy dietary patterns and sperm motility. However, the sensitivity analysis indicated that results of the sperm concentration and motility could change in the case that we remove Liu et al. study (Liu et al., 2015a) (MD = 0.23, P = 0.01, MD = 0.48, P = 0.03, respectively). In regard to sperm morphology, no heterogeneity was observed among the studies (P = 0.7, I^2 = 0), whereas, moderate heterogeneity was recognized for sperm concentration (P = 0.19, I^2 = 33.91). However, high heterogeneity was reported for sperm motility (P < 0.001, I^2 = 90.1).

**Figure 2** Forest plot demonstrates mean differences (represented by the black square) and 95% confidence interval (represented by a horizontal line) for sperm parameters, such as a) concentration, b) morphology, c) motility in participants with the highest and lowest adherence to “Healthy “dietary patterns. Weights are from random effects analysis. The area of the black square is proportional to the specific study weight to the overall meta-analysis. The center of the diamond displays the pool mean differences and its width shows the pooled 95% CI.

**Western dietary patterns and semen quality parameters:** The western dietary patterns are determined by high levels of red and processed meat, refined grains, high-fat dairy products, and low intakes of fruits and vegetables. Our meta-analysis showed that higher adherence to the western dietary patterns could significantly reduce the sperm concentration (MD = -0.079, P = 0.015, **Figure 3**). However, we found no significant relationship between this dietary pattern and sperm morphology (MD = 0.2, P = 0.396, **Figure 3**) and motility (MD = 0.01, P = 0.716, **Figure 3**). In terms of sperm concentration and morphology, sensitivity analysis, we found that the results changed significantly by excluding the study conducted by Liu et al. (Liu et al., 2015a), but exclusion of this study did not have any effect on sperm motility. Moreover, no heterogeneity was observed among the studies conducted on the relationship of this dietary pattern with sperm concentration and motility (P = 0.68 I^2 = 0 and P = 0.94 I^2 = 0, respectively). However, high heterogeneity was found among studies in relation to sperm morphology (P < 0.001, I^2 = 86.99).
Table 1. Characteristics of all included studies

<table>
<thead>
<tr>
<th>Author name and year</th>
<th>Country</th>
<th>Study design</th>
<th>Age (years)</th>
<th>Sample size(n)</th>
<th>Outcome</th>
<th>Dietary pattern</th>
<th>Dietary assessment tool</th>
<th>Adjusted effect size</th>
<th>Adjustment score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gaskin et al. 2012</td>
<td>USA</td>
<td>Cross-sectional</td>
<td>18-22</td>
<td>188</td>
<td>semen quality</td>
<td>FFQ</td>
<td>Western</td>
<td>Adjusted means (95% CI), p-value</td>
<td>Concentration: 50.7(33.1-77.5), 0.55 Motility:58.6(51.9-64.9), 0.48 Morphology:9.6(7.5-11.6), 0.59 Prudent</td>
</tr>
<tr>
<td>Vujkovic et al. 2009</td>
<td>Netherlands</td>
<td>Cross-sectional</td>
<td>35.3</td>
<td>161</td>
<td>semen quality</td>
<td>FFQ</td>
<td>Health Conscious</td>
<td>median with range, p-value concentration:48.2(33-1-63.2),0.89 motility:37(32-42),0.06 Morphology:5(4-6),0.74 Traditional Dutch</td>
<td>concentration:61.7(44.5-78.9),0.01 motility:37(31-43),0.98 Morphology:6(5-7),0.34</td>
</tr>
<tr>
<td>Liu et al. 2015</td>
<td>Taiwan</td>
<td>Cross-sectional</td>
<td>31</td>
<td>7282</td>
<td>Semen Quality</td>
<td>FFQ</td>
<td>Healthy</td>
<td>Concentration: OR=1.028(0.77,1.36) Motility: OR=1.25(1.84,1.85) Morphology: OR=1.85(0.51,6.66) Western</td>
<td>Concentration: OR=1.04(0.82,1.31) Motility: OR=0.093(0.67,1.30) Morphology: OR=1.32(0.64,2.71) Concentration: OR=0.97(0.76,1.25) High carbohydrate</td>
</tr>
<tr>
<td>Study</td>
<td>Country</td>
<td>Design</td>
<td>Age (Yrs)</td>
<td>Sample Size</td>
<td>FFQ Method</td>
<td>Diet Pattern</td>
<td>Means (95% CI)</td>
<td>p-value</td>
<td>BMI (kg/m²), smoking (current smoker versus not current smoker) and ejaculation abstinence time (hours), total calorie intake</td>
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<tr>
<td>Cutillas-Tolín et al. 2015</td>
<td>USA</td>
<td>Cross-sectional</td>
<td>18-23</td>
<td>215</td>
<td>FFQ</td>
<td>Mediterranean Western pattern</td>
<td>Concentration: 44.2 (33.1, 59.0), 0.34</td>
<td>Motility: 56.1 (52.9, 59.2), 0.68</td>
<td>Morphology: 8.17 (6.77, 9.87), 0.28</td>
</tr>
<tr>
<td>Karayiannis et al. 2016</td>
<td>Greece</td>
<td>Cross-sectional</td>
<td>26-55</td>
<td>225</td>
<td>FFQ</td>
<td>Mediterranean</td>
<td>Concentration: OR=2.69 (1.05, 6.90)</td>
<td>Motility: OR=2.88 (1.23, 6.78)</td>
<td>Morphology: OR=1.56 (0.64, 3.5)</td>
</tr>
</tbody>
</table>

OR: Odds Ratio, CI: confidence interval, FFQ: Food frequency questionnaire, BMI: body mass index, EN: energy Intake
Western dietary pattern and male fertility

Forest plot (Figure 2) demonstrates mean differences (represented by the black square) and 95% CI (represented by a horizontal line) for sperm parameters such as concentration, morphology, and motility in participants with the highest and lowest adherence to “Western” dietary patterns. Weights are from random effects analysis. The area of the black square is proportional to the specific study weight to the overall meta-analysis. The center of the diamond displays the pool mean differences and its width shows the pooled 95% CI.

Publication bias: Publication bias was found using the funnel plots for the effect of western dietary pattern on sperm morphology and the effect of healthy dietary patterns on sperm concentration and motility. The findings showed no relationship between healthy dietary pattern and sperm morphology. Furthermore, western dietary pattern had no association with sperm concentration and motility.

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**Figure 1. Flow chart of study selection process**

- Excluded (n = 1236)
  - Without relevant title and abstract (n = 938)
  - Without abstract (n = 13)
  - Women were assessed (n = 285)

- 45 did not meet inclusion criteria:
  - Examined nutrients (n = 9)
  - Examined single food groups or diet (n = 22)
  - Examined Supplement (n = 13)

- 3 did not meet inclusion criteria:
  - Different categories of dietary patterns (n = 1)
  - Calculated OR with CI (n = 1)
  - The calculated Beta coefficient with CI (n = 1)

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**Figure 2. Forest plot for sperm parameters in participants with highest v. lowest adherence to healthy dietary patterns.**
Discussion

This study investigated the effect of dietary patterns on sperm concentration, morphology, and motility. The results of our meta-analysis showed that western dietary patterns reduced the sperm concentration significantly; whereas, this significant relationship was not found between healthy dietary patterns and sperm parameters. As far as we know, this was the first meta-analysis that evaluated the relationship of dietary patterns with sperm concentration, morphology, and motility. Previous systematic reviews examined the relationship of foods, nutrients, and dietary patterns with the male fertility parameters and fecund ability. In this systematic review, we used five studies that focused on food and nutrients rather than dietary patterns and contained no meta-analysis (Salas-Huetos et al., 2017).

As semen quality is one of the most important factors in male infertility (Evers, 2002) and dietary pattern as a modifiable factor can affect the semen quality, it is necessary to identify the dietary patterns that improve the semen parameters such as motility, morphology, and concentration.

Recent studies revealed that the western dietary patterns have a conflicting effect on sperm concentration, motility, and morphology. Similar to our meta-analysis, a study was conducted on healthy men and claimed that high adherence to western dietary pattern decreased the sperm concentration and increased the percentage of normal morphology sperm among the overweight or obese men (Cutillas-Tolín et al., 2015). Moreover, in another study, the western dietary pattern could reduce the concentration and percentage of normal morphology sperm (Liu et al., 2015a). Previous studies on food group and semen quality showed the same results. One study revealed that high intake of lipophilic foods such as meat products or milk could negatively effect on the semen quality in oligoasthenoteratospermic patients (Mendiola et al., 2009b). In addition, a case-control study conducted on 72 asthenozoospermic men and 169 normozoospermic men determined that high levels of processed meat and sweets could significantly increase the risk of asthenozoospermic (Eslamian et al., 2012). According to findings from these studies, western dietary pattern can have adverse effect on semen quality parameters in several ways. High calorie content can result in weight gain and affects the molecular and physical structure of sperm (Palmer et al., 2012). It is contained in trans and saturated fatty acids, especially in red meat, high fat dairy, and fast food. So, consumption of western dietary pattern that is highly loaded with these materials can lead to impairment in spermatogenesis (Mendiola et al., 2009a). Furthermore, western pattern contains refined carbohydrate that is high in potato, white bread, and sweets. Refined carbohydrate can increase the insulin resistance and oxidative stress by increasing the reactive oxygen species (ROS) production (Park et al., 2009).

However, in other two studies, no significant relationship was found between western dietary
pattern and sperm parameters (Gaskins et al., 2012, Oostingh et al., 2017a). Finally, our meta-analysis represented that western dietary pattern led to a significant decrease in sperm concentration. As mentioned, exclusion of the study conducted by Liu et al. (Liu et al., 2015a) from our meta-analysis could lead to a change in the results of the concentration and morphology of the sperm. This study was conducted in Taiwan and investigated the relationship between dietary patterns and semen quality parameters in healthy men (Liu et al., 2015a). Furthermore, in this study, components of the western dietary pattern were somewhat different from other western dietary patterns; salads and seafood were also included as components of the western dietary pattern (Liu et al., 2015a). In addition, high heterogeneity observed among the studies regarding the relationship between the western dietary patterns and sperm morphology can be attributed to participants' different race, ethnicity, and age, as well as the consumed food groups' loading in various studies. However, we categorized dietary patterns as much as possible according to the similarity of food groups.

Regarding the healthy dietary pattern, several studies examined the relationship between healthy dietary patterns and sperm parameters but the results were not constant (Karayiannis et al., 2016, Oostingh et al., 2017a). Oostingh et al. revealed that the healthy dietary pattern containing high levels of vegetables, legumes, cereals, fruits, and low levels of eggs, meat, sweets, and solid fat could increase the sperm concentration and motility in men with poor semen quality (Oostingh et al., 2017a). Furthermore, Karayiannis et al. showed that adherence to the Mediterranean diet (high intake of olive oil, fish vegetables, fruit, and legumes) significantly raised the semen quality parameters (concentration, motility and morphology of sperm) in male partners (Karayiannis et al., 2016).

Studies on food groups also reported this protective effect on sperm parameters.

Findings of a prospective study showed that high intake of fish could improve the sperm count and percentage of morphologically normal sperm (Afeiche et al., 2014b). Another study expressed that higher intake of skimmed milk, shellfish, fruits, tomatoes, and lettuce may improve the semen quality (Mendiola et al., 2009b). These benefits from healthy dietary patterns may be due to high levels of antioxidants and carotenoids in fruits and vegetables (Eskenazi et al., 2005), which could prevent oxidation of sperm membranes by ROS (Agarwal and Sekhon, 2010). On the other hand, Cutillas-Tolin et al. found that Mediterranean dietary pattern (characterized by intake of vegetables, fruits, olive oil and seafood) did not have any effect on the concentration, motility, and morphology of the sperm in healthy men (Cutillas-Tolin et al., 2015). They also indicated no relationship between healthy dietary pattern (contained the high level of light color vegetables, dark color vegetables and fruits) and semen quality parameters in healthy men (Liu et al., 2015a). These contradictory results can be due to the presence of pesticides and chlorinated pollutants in fruits and vegetables that can reduce their antioxidant effect (Meeker and Hauser, 2010, Rozati et al., 2002).

Eventually, our meta-analysis showed no significant relationship between healthy dietary patterns and sperm parameters such as concentration, motility, and morphology. Furthermore, we found that the western dietary pattern, excluding Liu et al. study (Liu et al., 2015a), could significantly change the results of sperm concentration and motility. The various findings can be justified by differences in data analysis methods, race and age of participants, the status of men (healthy or subfertile) who participated in the study, and components of dietary patterns. For instance, considering the differences in the components of dietary patterns we can mention the study of Cutillas-Tolin (Cutillas-Tolin et al., 2015), in which high levels of wine and potato were included in the healthy dietary patterns.

The strengths of our study are as follows: we selected patterns across different studies based on the authors’ identified name or similarities among
the food groups to reduce the exposure misclassification. Moreover, we included studies that used validated FFQ to obtain the dietary intake. However, we were also faced with some limitations. First, limitations of the study designs and measurement errors of the assessment tools might affect our findings. Second, misclassification may exist within the investigated dietary patterns. Third, the number of studies included in the meta-analysis was low. Fourth, the studied populations in various articles were heterogeneous. Finally, the risk estimates were adjusted for different potential confounders among the included studies. Further studies are consequently required to examine the relationship of dietary patterns with sperm concentration, motility, and morphology using both methods in the populations from different ethnicities.

**Conclusion**

This meta-analysis revealed that the western dietary patterns are associated with sperm concentration; whereas, no significant association was observed between healthy dietary patterns and sperm parameters. Our meta-analysis shows that the role of dietary patterns on male infertility is not confirmed. In this respect, further studies especially more intervention and prospective cohort studies are required to clarify the true causal relation between dietary patterns and semen quality parameters.

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**Authors’ contributions**

Hasanizadeh H and Hosseinazadeh M made substantial contributions to manuscript conception, design, data interpretation, as well as intellectual content. Salehi - Abarghuei A contributed to statistical analyses and data interpretation. All authors approved the final manuscript for submission.

**Conflict of interest**

None

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