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ORIGINAL ARTICLE

Male Infertility

The effect of healthy dietary patterns on male semen quality: a systematic review and meta-analysis

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The incidence of infertility has recently risen. Semen quality is an important male fertility indicator, and dietary factors can affect semen quality. We conducted this systematic review and meta-analysis to determine the effects of healthy dietary patterns on semen quality. A literature search was conducted in 3 databases (Embase, Web of Science and PubMed) on August 21, 2021. The included cross-sectional studies examined the influence of the Mediterranean, Dietary Approaches to Stop Hypertension (DASH), and prudent diet patterns on semen quality parameters; six studies (1244 subjects) were included. By comparing high consumption with low consumption of healthy dietary patterns, the results of the meta-analysis showed significantly higher sperm concentrations (mean difference [MD] = $6.88 \times 10^6 \text{ ml}^{-1}$, 95% confidence interval [CI]: $1.26 \times 10^6 \text{ ml}^{-1}$ – $12.49 \times 10^6 \text{ ml}^{-1}$; $P < 0.05$), a significant increase in total sperm count (MD = 16.70×10^6 , 95% CI: 2.37×10^6 – 31.03×10^6 ; $P < 0.05$), and a significant increase in progressive sperm motility (MD = 5.85%, 95% CI: 2.59%–9.12%; $P < 0.01$). The sperm concentration, progressive sperm motility, and total sperm count were significantly higher in men with higher versus lower consumption of healthy dietary patterns. However, the results must be interpreted with caution.

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Keywords: cross-sectional study; healthy dietary pattern; meta-analysis; semen quality; systematic review

INTRODUCTION

Infertility has been identified by the World Health Organization (WHO) as a worldwide problem. A study showed that the prevalence of infertility after 12 months of trying to conceive ranges from 3.5% to 16.7% in more developed countries and from 6.9% to 9.3% in less developed countries.¹ It is estimated that 186 million people worldwide are affected by infertility,² which brings adverse social consequences such as marital instability and abuse.³ In the case of infertility, male infertility accounts for 50% of the incidence in couples.⁴ Abnormal semen parameters such as sperm concentration, total motility and morphology, semen volume, and motile sperm count may cause male infertility.⁴ Therefore, the analysis of semen quality is key in assessing male fertility. From 2001 to 2015, a study in China examined semen from 30 636 adult men. It was found that the sperm concentration, normal morphology, sperm progressive motility, and total sperm count decreased during a 15-year observation period, and the proportion of qualified semen donors dropped from 55.78% in 2001 to 17.80% in 2015.⁵ Therefore, the outlook regarding male semen quality is not optimistic. It is important to explore the factors that affect semen quality to improve semen quality.

Studies have found that sperm quality may be related not only to environmental pollution but also to unhealthy lifestyle factors, such as smoking and alcoholism.^{6–8} It has been reported that human sperms are

very sensitive to nutrient flux, whether changes occur in sperm motility or in sperm transfer RNA (tRNA)-derived small RNA.⁹ Diet, as a factor that is closely related to human beings, has a major impact on our lives. In a review, the authors encouraged men to follow a dietary pattern containing seafood, poultry, nuts, whole grains, fruits, and vegetables, which may be beneficial for male fertility.¹⁰ In addition, in a systematic review, the authors concluded that a diet rich in processed meats, soy foods, potatoes, full-fat dairy products, coffee, alcohol, and sugary drinks was associated with decreased semen quality.¹¹ However, a study concluded that the consumption of artificially sweetened beverages and processed red meat and poultry was not closely related to semen quality indicators.¹² Thus, it is important to study the impact of dietary patterns on semen quality; it is also important to study whether the food groups and nutrients included in healthy dietary patterns can have a beneficial effect on improving semen quality.

The traditional Mediterranean diet includes a large intake of fruits, olive oil, grains, nuts, vegetables, and dairy products; moderate consumption of fish and poultry; low consumption of red meat; and small-to-moderate consumption of wine.¹³ One study suggested that this dietary pattern can reduce the risk of cardiovascular disease.¹⁴ Two randomized controlled trial (RCT) studies showed that the consumption of a Mediterranean diet improved semen concentration, sperm count, and other semen parameters;^{15,16} however, a cross-

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sectional study also found that the consumption of a Mediterranean diet only showed a positive association with total sperm count, and no significant association was found with other semen parameters.¹⁷ The Dietary Approaches to Stop Hypertension (DASH) diet, which is based on fruits and vegetables and includes moderate consumption of low-fat dairy products, low consumption of animal protein, and high consumption of plant protein in the form of legumes and nuts, significantly reduced systolic and diastolic blood pressure in people with hypertension and normal blood pressure.¹⁸ The DASH diet not only has a good effect on blood pressure, lowering systolic and diastolic blood pressure in people with hypertension and normal blood pressure, but may also have an effect on semen quality. A study showed that there was a significant positive association between the DASH diet and total motile sperm count, total sperm count, and sperm concentration.¹⁹ A prudent diet is a diet rich in vegetables, legumes, fruits, and whole grains as determined using principal component analysis based on collected Food Frequency Questionnaire (FFQ) data.²⁰ The prudent dietary pattern is characterized by high intakes of vegetables, fruits, legumes, low-fat dairy products, whole grains, fish, and poultry.²¹ Studies have shown that the prudent dietary pattern is associated with reducing the risk of cardiovascular disease and diabetes.^{21,22} A cross-sectional study showed that adherence to the prudent dietary pattern was associated with better semen quality, such as total sperm count and sperm morphology;²³ however, another study did not find a significant association between the prudent dietary pattern and semen parameters.²⁴

Healthy dietary patterns can be defined as being rich in health-promoting foods such as vegetables, fruits, whole grains, and legumes, and limiting the intake of red meat and processed meats, which may have a role in reducing the incidence of noncommunicable diseases and overall mortality.²⁵ The Mediterranean, prudent, and DASH diets have something in common: after factor analysis and major component analysis, they all included the consumption of more fruits, whole grains, legumes, vegetables, and low-fat dairy products, which are part of a healthy dietary pattern. Dietary patterns investigate the effects of overall diet and represent a broader consumption of foods and nutrients that may be better predictors of disease risk than foods and nutrients alone.²⁶ Healthy dietary patterns, as a modifiable factor, have been reported to be associated with male semen parameters; however, the findings about this relationship were not consistent. Therefore, the aim of this analysis was to pool these results to explore the effect of healthy dietary patterns on male semen quality.

MATERIALS AND METHODS

We conducted this systematic review and meta-analysis based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.²⁷ The guidelines are shown in **Supplementary Table 1**. The PICOS (Participants, Intervention, Comparison, Outcomes, and Study design) criteria that were used to structure the research question are shown in **Supplementary Table 2**.

Search strategy

A literature search was conducted in 3 databases (Embase, Web of Science, and PubMed) on August 21, 2021. The keywords for semen quality were “Semen Analysis”, “Semen Analyses”, “Semen Quality Analysis”, “Semen Quality Analyses”, “Semen Quality”, and “Semen Qualities”; the keywords for the healthy dietary pattern were “Diet, Mediterranean”, “Mediterranean Diet”, “Mediterranean Diets”, “Dietary Approaches to Stop Hypertension”, “DASH Diet”, “DASH Diets”, “Diet, DASH”, “Diets, DASH”, “Dietary Approaches to Stop Hypertension

Diet”, “Diet, Healthy”, “Healthy Diet”, “Diets, Healthy”, “Healthy Diets”, “Healthy Eating”, “Eating, Healthy”, “Healthy Nutrition”, “Nutrition, Healthy”, “Prudent Diet”, “Diet, Prudent”, “Diets, Prudent”, “Prudent Diets”, “Healthy Eating Index”, “Healthy Eating Indices”, “Index, Healthy Eating”, and “Indices, Healthy Eating.” We searched by subject, abstract, and keywords. The retrieved documents were imported into Endnote X9 software (Clarivate Analytics, Philadelphia, PA, USA) for screening, and the documents that met the inclusion criteria were screened out.

Selection criteria

The inclusion criteria were as follows: (1) the subjects in all the selected literature were adult males; (2) the research content included the influence of healthy dietary patterns on semen qualities, and according to the consumption, the dietary pattern was scored and grouped; (3) the outcome measures included one or more of the following: sperm concentration, total sperm count, normal sperm morphology, total sperm motility, progressive sperm motility, and semen volume; (4) the results were presented as the median and interquartile or mean and 95% confidence interval (CI) values; and (5) the studies were published in English.

The exclusion criteria were as follows: (1) reviews, animal experiments, systematic reviews, and meta-analyses; (2) the research content was food groups or nutrients; (3) literature quality was assessed as low quality by the Agency for Healthcare Research and Quality (AHRQ)-recommended checklist; or (4) the information provided could not be pooled.

Data extraction

Data were extracted by three reviewers (LLC, JJC, and SJW). The title and abstract screening and full-text review were performed by 2 independent reviewers (LLC and JJC). Through discussion with a third reviewer (SJW), any discrepancies regarding suitability were resolved. An Excel spreadsheet was set up for data extraction. We extracted the following data: author, year, country, survey time, research design, sample size, study population, age, diet assessment method, dietary pattern recognition, and semen parameters.

Quality assessment

We used 11 checklists recommended by the AHRQ to assess the quality of the literature included in this systematic review and meta-analysis. The specific items were as follows: (1) define the source of the information (survey and record review); (2) list the inclusion and exclusion criteria for exposed and unexposed subjects (cases and controls) or refer to previous publications; (3) indicate the time period used for identifying patients; (4) indicate whether or not the subjects were consecutive, if the study was not population-based; (5) indicate if the evaluators of the subjective components of study were masked to other aspects of the status of the participants; (6) describe any assessments undertaken for quality assurance purposes (e.g., test/retest of the primary outcome measurements); (7) explain any patient exclusions from the analysis; (8) describe how confounding was assessed and/or controlled; (9) if applicable, explain how missing data were handled in the analysis; (10) summarize the patient response rates and the completeness of data collection; and (11) clarify what follow-up, if any, was expected and the percentage of patients, for which incomplete data or follow-up was obtained. If the answer was “no” or “unclear”, the item was scored as “0”; if the answer was “yes”, then the item was scored as “1”. The article quality was evaluated by scores as follows: low quality = 0–3; moderate quality = 4–7; and high quality = 8–11.²⁸

Statistical analyses

We used Stata/SE version 15.1 (Stata Corp LLC, College Station, TX, USA) software to perform meta-analysis calculations. Data on the effects of healthy dietary patterns on the six outcomes (sperm concentration, total sperm count, normal sperm morphology, total sperm motility, progressive sperm motility, and semen volume) were pooled. The mean difference (MD) was used as the combined effect size, and its 95% CI was calculated. Statistical significance was set at $P < 0.05$, and the forest plot was mapped at the same time.

The outcome data are presented as the mean \pm standard deviation (s.d.). For studies that did not provide the mean \pm s.d. of the semen parameters, we extracted the data from the articles and transformed them into mean \pm s.d. according to the formulae provided in the Cochrane Handbook or references. If the median and interquartile range were provided, they were converted to the mean \pm s.d. according to Wan *et al.*²⁹ If the means and 95% CIs were provided, we converted them to mean \pm s.d. according to the Cochrane Handbook.³⁰ Cochran's Q test and I^2 statistics were used to assess the heterogeneity between studies, and the significance level was set at $P < 0.10$. Heterogeneity was considered acceptable when $I^2 < 50\%$. In this case, a fixed-effects model was used, and the inverse variance method was used for the pooled data. Otherwise, the heterogeneity was considered significant, and a random-effects model was used. We used Egger's regression test to test whether there was publication bias, and $P < 0.05$ represented a significant publication bias.

RESULTS

Study screening

A total of 490 articles were retrieved from the three databases. After the literature was imported into Endnote software, a total of 123 duplicate studies were removed, leaving 367 studies. After reading the titles and abstracts, a total of 348 articles were deleted because the research type was an animal experiment, systematic review, or review or study on the relationship between specific food and nutrients on semen quality. After full-text readings of the remaining 19 studies, we found 10 studies with no eligible data for meta-analysis; two articles studied food groups rather than dietary patterns, and dietary patterns were not grouped in 1 study. Finally, this systematic review and meta-analysis included a total of 6 articles (Figure 1).^{17,31–35}

Characteristics of the studies

The characteristics of the six studies are shown in Table 1. The six articles were cross-sectional studies involving 1244 subjects from 2009 to 2018. In the meta-analysis, we included 708 subjects with the lowest and highest adherence to a healthy dietary pattern. The included studies were from five countries: two were from Spain and the remaining four were from the USA, Greece, Italy, and Poland, respectively. In addition, the subjects ranged in age from 18 years to 60 years. The health status and semen analysis criteria of the study subjects are shown in Table 2. Four studies examined the effects of a Mediterranean diet on semen quality in men. They were divided into groups based on their scores for the Mediterranean diet from low to high adherence. The effect of the dietary pattern on semen quality was compared by comparing the difference in semen parameters between the high adherence group and the low adherence group. Among them, the subjects of two studies were infertile men,^{33,34} and the subjects of the other two studies were healthy men.^{17,35} The remaining two studies examined the DASH diet and the prudent diet, and the subjects were all healthy men. These studies used different FFQs. In the study of

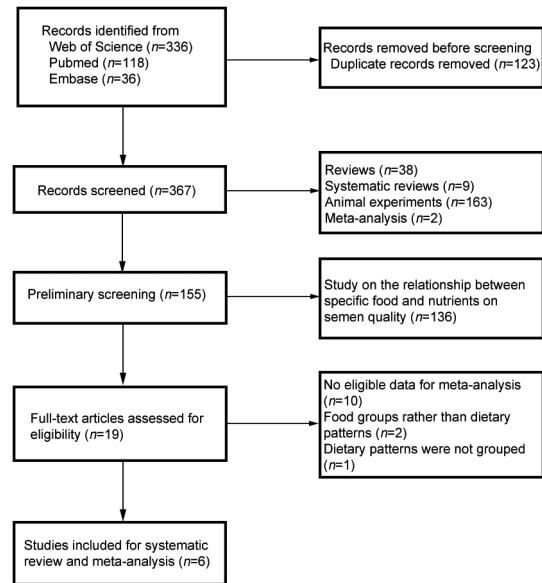


Figure 1: Flowchart of the progress of acquiring the qualified literature and studies included in the systematic review and meta-analysis.

Cutillas-Tolín *et al.*,¹⁷ a semiquantitative FFQ of 101 foods was used to assess daily food and nutrient intake. They estimated 17 foods and food groups by grouping specific foods in the FFQ. This questionnaire had nine possible responses, ranging from “never or less than once per month” to “six or more times per day”. The study of Salas-Huetos *et al.*³⁵ used a validated 143-item semiquantitative FFQ. In this questionnaire, food intake was grouped into 13 food groups. There were nine options for the frequency of food consumption, from never to more than six times per day. The two studies were conducted in Spain, so these FFQs contained foods that were popular in Spain at the time (e.g., kiwi). There were also some foods of interest for the Mediterranean diet (e.g., onion, garlic, olive oil, and wine).

The study of Karayiannis *et al.*³³ used a 75-item FFQ validated for the Greek population. Twelve main food groups (i.e., dairy products, eggs, meat, fish, legumes, vegetables, fruits, sweets, fats, oils, alcohol, and stimulants) were created. This questionnaire had six possible responses, ranging from “never/rarely” to “two or more times per day”. Compared to 101/143-food-item semiquantitative FFQs, although the food groups were similar, the foods included in each group were different (e.g., missing garlic and onion). Ricci *et al.*³⁴ used a previously validated 78-item FFQ; this FFQ was divided into eight sections: bread and cereals; meat and meat substitutes; vegetables; fruits; sweets, desserts, and soft drinks; milk, hot beverages, and sweeteners; alcoholic beverages; and fish. There were no questions about vitamin and mineral supplementation in the FFQ, as they are extremely rare in Italy.

Danielewicz *et al.*³¹ used the validated 165-food-item semiquantitative FFQ. Food items in this FFQ were divided into 23 food groups, and the DASH diet score comprised eight food groups. The food groups included a wide variety of foods but no item for alcohol. Gaskins *et al.*³² used a previously validated 131-item FFQ. The food items in this FFQ were divided into 40 food groups. This questionnaire had nine possible responses, ranging from “almost never” to “six or more times per day”. More details on the outcomes are shown in Supplementary Table 3.

Table 1: The characteristics of 6 studies

Study	Year	Country	Survey time	Study population	Study design	Cases (n)	Age (year), range	Diet assessment method	Dietary pattern identified	Semen parameters
Gaskins <i>et al.</i> ³²	2012	USA	2009–2010	Healthy	CS	188	18–22	131-item FFQ	Prudent pattern, western pattern	Sperm concentration, progressive motility, and morphology
Cutillas-Tolín <i>et al.</i> ¹⁷	2015	Spain	2010–2011	Healthy	CS	209	18–23	101-item FFQ	MED, western diet	Semen volume, sperm concentration, total sperm count, morphologically normal sperm, and total motile sperm
Karayiannis <i>et al.</i> ³³	2017	Greece	2013–2016	Infertility	CS	225	26–55	75-item FFQ	MED	Semen volume, sperm concentration, total sperm count, motility, progressive motility, and morphology
Salas-Huetos <i>et al.</i> ³⁵	2019	Spain	2015–2017	Healthy	CS	106	18–35	143-item FFQ	MED	Semen volume, total sperm count, sperm concentration, vitality, total motility, progressive motility, and morphology
Ricci <i>et al.</i> ³⁴	2019	Italy	2014–2016	Infertility	CS	390	27–60	78-item FFQ	MED	Semen volume, sperm concentration, and total count
Danielewicz <i>et al.</i> ³¹	2019	Poland	2014–2018	Healthy	CS	207	20–55	165-item FFQ	DASH	Semen volume, sperm concentration, sperm count, total motility, progressive motility, and morphology

CS: cross-sectional study; FFQ: Food Frequency Questionnaire; MED: Mediterranean diet; DASH: Dietary Approaches to Stop Hypertension

Table 2: Study population sources and semen analysis methods

Study	Population	Sperm concentration	Total sperm count	Normal sperm morphology	Total sperm motility	Progressive sperm motility	Semen volume
Gaskins <i>et al.</i> ³² 2012	Healthy men from a cross-sectional study recruited at a university to study the effects of environmental pollutants on semen quality	Hemocytometer	Volume × sperm concentration	The slides were Papanicolaou stained and assessed using the Tygerberg strict criteria	The WHO criteria 4 th edition	The WHO criteria 4 th edition	Weighing
Cutillas-Tolín <i>et al.</i> ¹⁷ 2015	Healthy men from a cross-sectional study aimed at studying the influence of environmental and lifestyle factors on semen quality and reproductive hormone levels	Hemocytometer	Volume × sperm concentration	The slides were prepared, air-dried, fixed, Papanicolaou stained, and assessed using the Tygerberg strict criteria	The WHO criteria 5 th edition	NA	Weighing
Karayiannis <i>et al.</i> ³³ 2017	Infertile men from a cross-sectional study conducted at a fertility clinic in Athens, Greece	Hemocytometer	Volume × sperm concentration	The slides were Papanicolaou stained and assessed using the Tygerberg strict criteria	The WHO criteria 5 th edition	The WHO criteria 5 th edition	Weighing
Salas-Huetos <i>et al.</i> ³⁵ 2019	Healthy men from a parallel randomized clinical trial aimed to assess the effects of nut supplementation on sperm quality parameters	Hemocytometer	Volume × sperm concentration	The WHO criteria 5 th edition	The WHO criteria 5 th edition	The WHO criteria 5 th edition	A Pasteur pipette
Ricci <i>et al.</i> ³⁴ 2019	Infertile men were invited to participate in an ongoing prospective cohort study on the role of lifestyle habits and diet on assisted reproduction technologies outcome from an Italian Fertility Clinic	Hemocytometer	Volume × sperm concentration	NA	NA	NA	Weighing
Danielewicz <i>et al.</i> ³¹ 2019	Healthy men from a cross-sectional study who attended the center of reproductive medicine in Olsztyn (Poland) or voluntarily joined the study in 2014–2018	A four-chamber GoldCyto slide (10 µm depth) by applying 4 µl of semen	CASA	The slides were Diff-Quick stained by using WHO criteria 5 th edition	A four-chamber GoldCyto slide (10 µm depth) by applying 4 µl of semen	A four-chamber GoldCyto slide (10 µm depth) by applying 4 µl of semen	The WHO criteria 5 th edition

WHO: World Health Organization; NA: not available; CASA: computer-aided semen analysis

Study quality

A total of six cross-sectional studies^{17,31–35} were included in our systematic review and meta-analysis. We used 11 checklist items recommended by the AHRQ to assess the quality of the literature included in this systematic review and meta-analysis. In the studies we included, two^{32,35} received a score of 7, three^{17,33,34} received a score of 8, and one³¹ received a score of 9. The results of the quality assessments are shown in **Table 3**.

Qualitative analysis of sperm concentration

In the included articles, a total of six studies^{17,31–35} involving 708 subjects reported a healthy dietary pattern relationship with sperm concentration. Four studies^{31,33–35} divided the dietary scores into three groups, and two studies^{17,32} divided the dietary scores into four groups. There are contradictions among the results of the six studies. Karayiannis *et al.*³³ found that in comparison to the men with the highest scores for the Mediterranean diet, among the men

Table 3: Quality rating of included studies using AHRQ

Study	Item											Total score
	1	2	3	4	5	6	7	8	9	10	11	
Gaskins <i>et al.</i> ³² 2012	√	√	√		√	√		√		√		7
Cutillas-Tolín <i>et al.</i> ¹⁷ 2015	√	√	√		√	√	√	√		√		8
Karayiannis <i>et al.</i> ³³ 2017	√	√	√		√	√	√	√		√		8
Salas-Huetos <i>et al.</i> ³⁵ 2019	√	√	√		√	√		√		√		7
Ricci <i>et al.</i> ³⁴ 2019	√	√	√		√	√	√	√		√		8
Danielewicz <i>et al.</i> ³¹ 2019	√	√	√		√	√	√	√	√	√		9

AHRQ: Agency for Health Care Research and Quality

with the lowest scores, there was a higher percentage with a sperm concentration that was lower than the WHO reference value; the results from Danielewicz *et al.*³¹ showed that a higher sperm concentration was associated with adherence to the DASH diet. In the other four studies,^{17,32,34,35} no significant correlation was found.

Qualitative analysis of total sperm count

In the included articles, a total of five studies^{17,31,33–35} involving a total of 614 subjects reported a relationship between a healthy dietary pattern and total sperm count. Four studies^{31,33–35} divided the dietary scores into three groups, and one study¹⁷ divided the dietary scores into four groups. Karayiannis *et al.*³³ found that in comparison to the men with the highest scores for the Mediterranean diet, among the men with the lowest scores, there was a higher percentage with a total sperm count that was lower than the WHO reference value. Cutillas-Tolín *et al.*¹⁷ found that Mediterranean dietary patterns were positively correlated with total sperm count. Danielewicz *et al.*³¹ also showed that a higher total sperm count was associated with adherence to the DASH diet. In the other two studies,^{34,35} no significant correlation was found.

Qualitative analysis of normal sperm morphology

In the included articles, a total of five studies^{17,31–33,35} involving a total of 530 subjects reported a relationship between a healthy dietary pattern and normal sperm morphology. Three studies^{31,33,35} divided the dietary scores into three groups, and two^{17,32} divided them into four groups. Karayiannis *et al.*³³ found that in comparison to the men with the highest scores for the Mediterranean diet, among the men with the lowest scores, the proportion of males with normal sperm morphology lower than WHO reference value was higher. In the other four studies,^{17,31,32,35} no significant correlation was found.

Qualitative analysis of total sperm motility

In the included articles, a total of four studies^{17,31,33,35} involving a total of 436 study subjects reported a relationship between a healthy dietary pattern and total sperm motility. Three studies^{31,33,35} divided the dietary scores into three groups, and one study¹⁷ divided the dietary scores into four groups. Salas-Huetos *et al.*³⁵ found that total sperm motility was statistically higher in those with the highest scores than that in those with the lowest scores for the Mediterranean diet. Karayiannis *et al.*³³ found that in comparison to the men with the highest scores for the Mediterranean diet, among the men with the lowest scores, there was a higher percentage with total sperm motility lower than the WHO reference value. In the other two studies,^{17,31} no significant correlation was found.

Qualitative analysis of progressive sperm motility

Four cross-sectional studies^{31–33,35} involving a total of 425 study subjects reported the relationship between healthy dietary patterns and progressive sperm motility. Three studies^{31,33,35} divided the dietary scores

into three groups, and one study³² divided the dietary scores into four groups. Salas-Huetos *et al.*³⁵ found that progressive sperm motility was statistically higher in those with the highest scores for the Mediterranean diet than that in those with the lowest scores. Karayiannis *et al.*³³ found that in comparison to the men with the highest scores for the Mediterranean diet, among the men with the lowest scores, there was a higher percentage with progressive sperm motility lower than the WHO reference value. Gaskins *et al.*³² found that a prudent diet was positively correlated with progressive sperm motility in a multivariate model. In the one remaining study,³¹ no significant correlation was found.

Qualitative analysis of semen volume

In the included articles, a total of five studies^{17,31,33–35} involving 614 subjects reported on the relationship between a healthy dietary pattern and semen volume. Four studies^{31,33–35} divided the dietary scores into three groups, and one¹⁷ divided them into four groups. None of the five studies^{17,31,33–35} showed any association between healthy dietary patterns and semen volume.

Quantitative analysis of sperm concentration

From the data of the six cross-sectional studies^{17,31–35} that were included, it was concluded that the sperm concentration of the subjects with higher consumption of a healthy dietary pattern was significantly higher than that of the subjects with lower consumption (MD: $6.88 \times 10^6 \text{ ml}^{-1}$, 95% CI: $1.26 \times 10^6 \text{ ml}^{-1}$ – $12.49 \times 10^6 \text{ ml}^{-1}$; $P < 0.05$). Significant heterogeneity did not exist ($I^2 = 0.6\%$, $P > 0.1$), and there was no publication bias in the research (Egger's test, $P = 0.854$; **Figure 2a**).

Quantitative analysis of total sperm count

From the data of five cross-sectional studies,^{17,31,33–35} it was concluded that the total sperm count in the subjects with higher consumption of a healthy dietary pattern was significantly higher than that of the subjects with lower consumption (MD: 16.70×10^6 , 95% CI: 2.37×10^6 – 31.03×10^6 ; $P < 0.05$). Significant heterogeneity did not exist ($I^2 = 48.5\%$, $P > 0.1$), and there was no publication bias in the research (Egger's test, $P = 0.279$; **Figure 2b**).

Quantitative analysis of normal sperm morphology

Data from five cross-sectional studies^{17,31–33,35} showed that there was no significant association between a healthy dietary pattern and normal sperm morphology (MD: 0.28%, 95% CI: -0.33% – 0.90% ; $P > 0.05$). Significant heterogeneity did not exist ($I^2 = 28.3\%$, $P > 0.1$), and there was no publication bias in the research (Egger's test, $P = 0.744$; **Figure 2c**).

Quantitative analysis of total sperm motility

From the data of four cross-sectional studies,^{17,31,33,35} there was no significant correlation between healthy dietary patterns and total sperm motility (MD: 6.86%, 95% CI: -0.25% – 13.96% ; $P > 0.05$). Significant heterogeneity did not exist ($I^2 = 78.6\%$, $P = 0.003$), and there was no publication bias in the research (Egger's test, $P = 0.074$; **Figure 3a**).

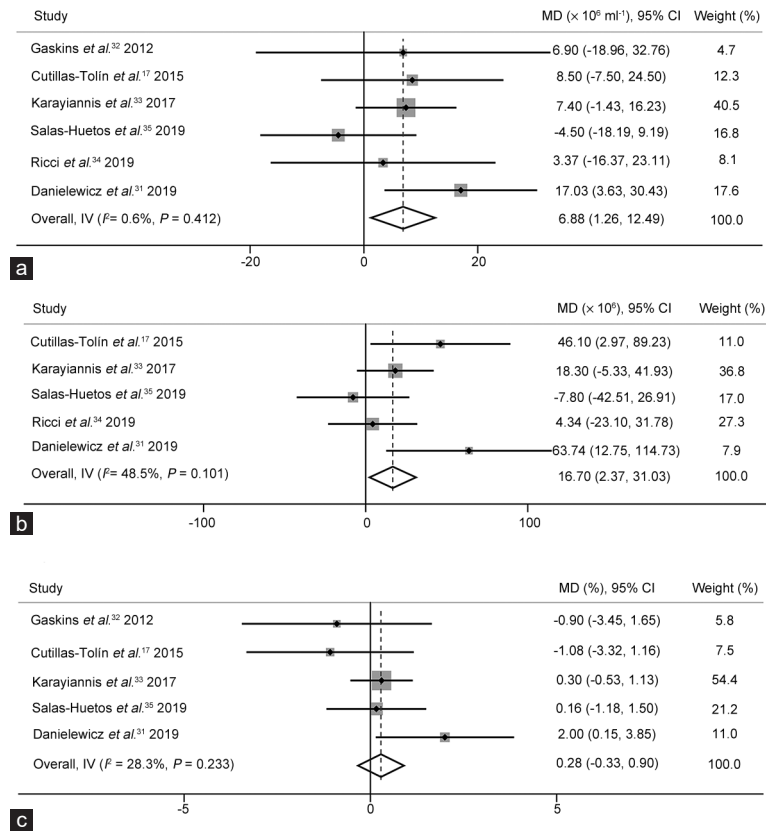


Figure 2: The effects of healthy dietary pattern on semen quality. The forest plots of (a) sperm concentration, (b) total sperm count, and (c) normal sperm morphology use a fixed-effects estimate method. MD: mean difference; CI: confidence interval; IV: Inverse-variance.

Quantitative analysis of progressive sperm motility

Data from four cross-sectional studies^{31–33,35} showed that the men with high consumption of a healthy dietary pattern had significantly higher progressive sperm motility than those with low consumption (MD: 5.85%, 95% CI: 2.59%–9.12%, $P < 0.01$). Significant heterogeneity did not exist ($I^2 = 0.3\%$, $P > 0.1$), and there was no publication bias in the research (Egger's test, $P = 0.193$; **Figure 3b**).

Quantitative analysis of semen volume

Five studies^{17,31,33–35} involving 614 subjects reported healthy dietary patterns and semen volumes. The results showed that there was no significant association between a healthy dietary pattern and semen volume (MD: 0.04 ml, 95% CI: -0.20 ml–0.28 ml; $P > 0.05$). Significant heterogeneity did not exist ($I^2 = 0$, $P > 0.1$), and there was no publication bias in the research (Egger's test, $P = 0.89$; **Figure 3c**).

DISCUSSION

We reviewed and statistically analyzed six published articles to explore the influence of healthy dietary patterns on semen quality. We explored the relationship between healthy dietary patterns and the following six semen parameters: sperm concentration, total sperm count, progressive sperm motility, normal sperm morphology, total sperm motility, and semen volume. From the results, it can be seen that the sperm concentration, progressive sperm motility, and total sperm count were significantly higher in the group with high consumption of a healthy dietary pattern than those in the group with low consumption; however, no clear relationship was found between normal sperm morphology, total sperm motility, and semen volume.

Healthy dietary patterns are rich in plant-based foods, where saturated fats provide only a small percentage of the total energy intake. It has been suggested that a high-fat diet may have an effect on semen quality,³⁶ and some animal experiments have investigated the effects of exercise and diet on semen parameters in mice. In a study of whether a high-fat diet early in life affects sperm quality in adulthood, the results of the study showed that mice fed a high-fat diet had lower sperm motility and viability even into early adulthood.³⁷ In addition, in a systematic review and meta-analysis, strong and consistent evidence was found in animal studies that a high-fat diet is detrimental to male fertility.³⁸ The authors found a higher proportion of sperm with DNA damage and signs of oxidative stress in mice fed a high-fat diet, which affected the metabolic state. There is a link between systemic metabolic status and sperm function, and animal studies have shown that abnormal sperm parameters caused by obesity can be reversed by a low-fat diet.³⁹

Some antioxidants, such as antioxidant vitamins and folic acid, are more abundant in vegetables and fruits. Oxidative stress (OS) has been widely studied and has been shown to have harmful effects on sperm function. Reactive oxygen species (ROS) are oxygen-derived molecules that are formed as intermediary products of cellular metabolism.⁴⁰ At high levels, ROS can influence sperm membranes and alter sperm DNA.⁴¹ Antioxidants, as scavengers of ROS, can ameliorate the adverse effects of high concentrations of active oxygen on semen. From two systematic reviews of studies, we can conclude that the use of antioxidants improves the live birth rate of couples experiencing poor sperm quality and infertility.^{42,43} In a study of healthy men who did not

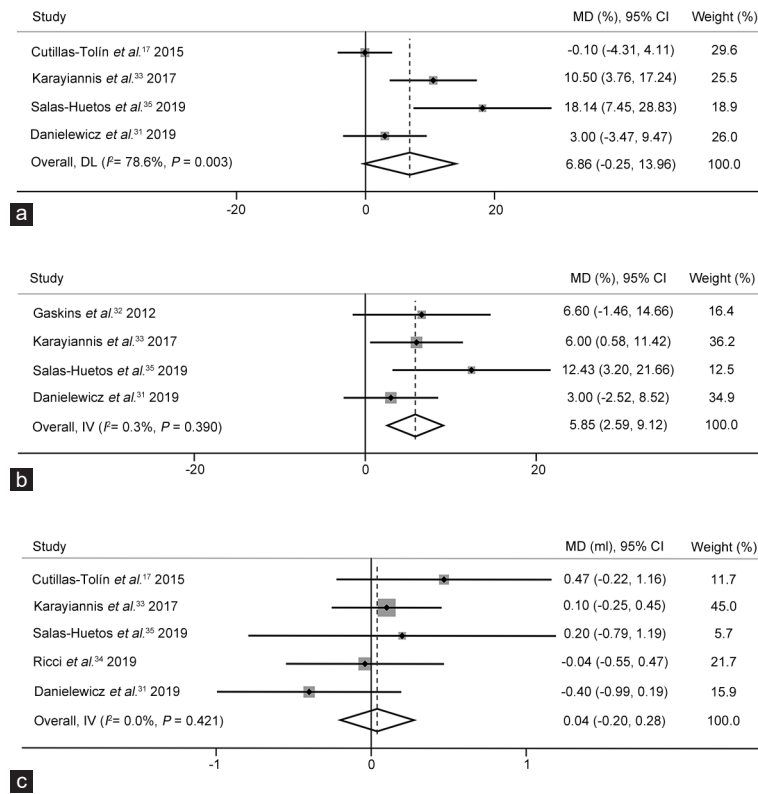


Figure 3: The effects of healthy dietary pattern on semen quality. (a) The forest plots of total sperm motility use a random-effects estimate method. The forest plots of (b) progressive sperm motility and (c) semen volume use a fixed-effects estimate method. MD: mean difference; CI: confidence interval; IV: Inverse-variance; DL: DerSimonian-Laird.

smoke, the results showed that higher antioxidant intake was related to higher total sperm count, sperm concentration, and sperm progressive motility,⁴⁴ which is consistent with our findings. Antioxidants found in fruits and vegetables may influence semen quality.

Folic acid participates in lipid and protein synthesis, removing active oxidative free radicals and aiding in DNA repair. When the intake of folic acid is low, OS will increase accordingly, thereby changing the integrity of DNA and subsequently participating in the molecular processes of spermatogenesis and embryogenesis.⁴⁵ Sevilla y Ruiz *et al.*⁴⁶ showed that excessive estradiol or its metabolites may affect normal sperm production; therefore, reducing plasma estrogen levels may have a beneficial effect on sperm. The major sources of fiber are whole grains, vegetables, and fruits, which bind directly to unbound estrogen, thereby lowering plasma estrogen levels and reducing estrogen absorption.⁴⁷ In our research, the food groups contained in the healthy dietary patterns included vegetables, fruits, legumes, whole grains, and low-fat dairy products. The antioxidants and other substances contained in these food groups are advantageous for semen quality. This may explain the significant increase in sperm concentration, progressive sperm motility, and total sperm count in those with high consumption of a healthy dietary pattern compared with those with low consumption.

In a study of the relationship between the intake of dairy products and semen parameters, it was found that the intake of low-fat dairy foods, especially low-fat milk, had a strong positive correlation with sperm concentration and vitality.⁴⁸ Low-fat milk intake was associated with higher insulin-like growth factor-1 (IGF-1) and circulating insulin levels.⁴⁹ Animal models have shown that insulin increases the total sperm count and sperm motility in rats with type 1 diabetes⁵⁰ and rescues sperm production in mice with type 1 diabetes.⁵¹

Spermatogenesis requires insulin and IGF-1 to bind and activate Leydig cell insulin receptors.⁵² Leydig cells can be affected by insulin and IGF-1, Sertoli cells, or spermatogonia, and the increase in sperm concentration and motility associated with low-fat dairy products may represent a biological effect in humans.⁴⁸

The six studies^{17,31–35} that we included covered a total of five countries, including Spain, the USA, Greece, Poland, and Italy. Because these countries are located in different geographical locations with different population types and cultural beliefs, dietary differences among the populations are relatively large, so when determining dietary patterns, an FFQ must be adapted and validated for each specific population.⁵³ Information about the amount and frequency of consumption of food and food groups and the time of eating is not available to us, and this is a limitation of the FFQ. In our included studies, the FFQs used were validated and appropriate for the study population. Therefore, the dietary patterns obtained by the analysis are locally representative; these long-term dietary patterns are stable and may not have much impact on the analysis of the results. To some degree, different FFQ items could reflect the different dietary habits of people in the included studies. Thus, difference of FFQ items across studies might also increase the heterogeneity of the results.

Of the included studies, two studies^{33,34} investigated infertile men and four studies^{17,31,32,35} investigated healthy men. Due to the different health statuses of the included subjects, the semen quality of infertile men may be poor relative to that of healthy men, which may have an impact on our combined results. In terms of semen analysis, Gaskins *et al.*³² used the 4th edition of the WHO manual,⁵⁴ and the other studies^{17,31,33–35} used the 5th edition of the WHO manual.⁵⁵ In the sperm morphology test, the 4th edition of the WHO manual has no

reference value for the percentage of morphologically normal sperm but considers that the percentage of morphologically normal sperm above 15% does not affect the fertilization rate of *in vitro* fertilization (IVF), while the 5th edition of the WHO manual has a reference value of 4%, which will have an impact on the results of sperm morphology assessment and thus on the reliability of our results. For semen volume measurement, Salas-Huetos *et al.*³⁵ used a Pasteur pipette for measurement, while others used the direct weighing method. Measurement using the Pasteur pipette may lose some semen and therefore underestimate semen volume.⁵⁵ In conclusion, due to the inconsistency of the included studies in various aspects such as study population and methodology, there may be some influence on the reliability of our results.

Limitations and strengths

To better interpret our results, we must acknowledge the limitations of our research. First, the studies we included were all cross-sectional studies, and it is impossible to determine the causal relationship between the high intake of healthy dietary patterns and semen quality. Second, after literature screening, the number of studies we included was small, and there was not enough literature to support us in drawing reliable conclusions. Third, among the outcome variables of our study, total sperm motility had the greatest heterogeneity because the number of studies was small, and no subgroup analysis was performed to analyze the heterogeneity. By reading the literature, we estimate that the source of heterogeneity may be related to the health status of the research subjects, different semen analysis criteria, different dietary patterns, the methods of determining dietary patterns, the confounding factors of adjustment, *etc.*

The main advantages of this study are that we studied six semen parameters, and these semen parameters can better reflect the semen quality of men. The included studies grouped the dietary patterns according to consumption and clearly compared the difference between low consumption and high consumption.

CONCLUSION

Our analysis showed that healthy dietary patterns had beneficial effects on sperm concentration, total sperm count, and progressive sperm motility in males, which affect male fertility. We recommend that dietary interventions should be considered in clinical work as part of the therapies improving male semen quality. From a public health perspective, healthy dietary patterns may promote male reproductive health and thus improve semen quality in the population.

AUTHOR CONTRIBUTIONS

LLC and PYS designed the study. LLC and JJC wrote the manuscript and performed the statistical analysis. LLC, JJC, SJW, YHL, MYY, GFW, and PYS commented in detail on the drafts. LLC, SJW, and JJC participated in the quality assessment. LLC, JJC, SJW, YHL, MYY, GFW, and PYS performed the data analysis and explanation. LLC, JJC, and PYS reviewed the manuscript. All authors read and approved the final manuscript.

COMPETING INTERESTS

All authors declare no competing interests.

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Supplementary Table 1: Preferred reporting items for systematic reviews and meta-analyses guidelines

Section/topic	Number	Checklist item	Reported on page number
Title			
Title	1	Identify the report as a systematic review, meta-analysis, or both	1
Abstract			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number	2
Introduction			
Rationale	3	Describe the rationale for the review in the context of what is already known	2–5
Objectives	4	Provide an explicit statement of questions being addressed with reference to PICOS	5 Table 1
Methods			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number	No
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale	6–7
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched	6
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated	6
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis)	6–7
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators	7
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made	No
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis	7–8
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means)	8–9
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., <i>I</i> ² for each meta-analysis)	8–9
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies)	8–9
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified	No
Results			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram	9 Figure 1
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations	10–11 Table 2
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12)	12 Table 3
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot	12–15 Supplementary Table 1
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency	15–16 Figure 2 and 3
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15)	15–16
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16])	No
Discussion			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers)	17–20
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias)	20–21
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research	21
Funding			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review	22

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). PRISMA: The PRISMA Statement. *PLoS Med* 6 (6): e1000097. doi: 10.1371/journal.pmed1000097. PICOS: Participants, Interventions, Comparisons, Outcomes, and Study design; PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses

Supplementary Table 2: Participants, Interventions, Comparisons, Outcomes, and Study design criteria for inclusion of studies

Parameter	Criterion
Participants	Male with healthy dietary pattern
Intervention	Studies confirming diet assessed by dietary patterns, dietary scores, dietary indices, and food patterns
Comparison	Lower consumption of healthy dietary pattern
Outcomes	Six semen parameters
Study design	Cross-sectional studies

PICOS: Participants, Interventions, Comparisons, Outcomes, and Study design

Supplementary Table 3: The data of the included studies in semen parameters

Study	Treat			Control		
	Size	Mean	s.d.	Size	Mean	s.d.
Sperm concentration (millions ml ⁻¹)						
Gaskins <i>et al.</i> ³² 2012	47	51.20	68.63	47	44.30	58.92
Cutillas-Tolín <i>et al.</i> ¹⁷ 2015	52	44.20	46.51	53	35.70	36.46
Karayiannis <i>et al.</i> ³³ 2017	66	37.90	26.43	76	30.50	27.14
Salas-Huetos <i>et al.</i> ³⁵ 2019	27	25.63	15.26	30	30.13	34.72
Ricci <i>et al.</i> ³⁴ 2019	35	37.67	36.15	31	34.30	44.56
Danielewicz <i>et al.</i> ³¹ 2019	70	41.13	51.09	62	24.10	24.21
Total sperm count (millions)						
Cutillas-Tolín <i>et al.</i> ¹⁷ 2015	52	138.00	134.7	53	91.90	84.71
Karayiannis <i>et al.</i> ³³ 2017	66	82.10	70.75	76	63.80	72.67
Salas-Huetos <i>et al.</i> ³⁵ 2019	27	74.50	45.00	30	82.30	84.61
Ricci <i>et al.</i> ³⁴ 2019	35	85.77	75.62	31	81.43	107.29
Danielewicz <i>et al.</i> ³¹ 2019	70	154.27	188.78	62	90.53	102.02
Normal sperm morphology (%)						
Gaskins <i>et al.</i> ³² 2012	47	7.80	6.30	47	8.70	6.30
Cutillas-Tolín <i>et al.</i> ¹⁷ 2015	52	8.17	5.57	53	9.25	6.15
Karayiannis <i>et al.</i> ³³ 2017	66	4.60	2.44	76	4.30	2.63
Salas-Huetos <i>et al.</i> ³⁵ 2019	27	6.43	2.35	30	6.27	2.80
Danielewicz <i>et al.</i> ³¹ 2019	70	8.33	6.81	62	6.33	3.79
Total sperm motility (%)						
Cutillas-Tolín <i>et al.</i> ¹⁷ 2015	52	56.10	11.31	53	56.20	10.70
Karayiannis <i>et al.</i> ³³ 2017	66	42.60	20.33	76	32.10	20.57
Salas-Huetos <i>et al.</i> ³⁵ 2019	27	73.37	13.70	30	55.23	26.15
Danielewicz <i>et al.</i> ³¹ 2019	70	53.90	20.59	62	50.90	17.30
Progressive sperm motility (%)						
Gaskins <i>et al.</i> ³² 2012	47	63.70	20.26	47	57.10	19.58
Karayiannis <i>et al.</i> ³³ 2017	66	26.00	16.26	76	20.00	16.63
Salas-Huetos <i>et al.</i> ³⁵ 2019	27	52.60	14.87	30	40.17	20.50
Danielewicz <i>et al.</i> ³¹ 2019	70	33.80	17.49	62	30.80	14.88
Semen volume (ml)						
Cutillas-Tolín <i>et al.</i> ¹⁷ 2015	52	2.97	1.99	53	2.50	1.61
Karayiannis <i>et al.</i> ³³ 2017	66	2.10	0.81	76	2.00	1.31
Salas-Huetos <i>et al.</i> ³⁵ 2019	27	3.27	1.80	30	3.07	2.02
Ricci <i>et al.</i> ³⁴ 2019	35	2.73	1.88	31	2.77	1.58
Danielewicz <i>et al.</i> ³¹ 2019	70	3.77	1.89	62	4.17	1.59

s.d.: standard deviation