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The Adherence Mediterranean Diet and the Quality of Sperm Parameters in Men with Infertility: A Cross-Sectional Study

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ABSTRACT

Background: It is well known that dietary factors can affect male fertility. The aim of this study was investigating the effect of Mediterranean diet on sperm quality. **Methods:** This cross-sectional study was conducted on 218 men with infertility aged 20-50. A 168-item food frequency questionnaire (FFQ) was used to evaluate dietary intakes. Mediterranean diet scores were calculated based on the participant's dietary intake. The evaluation of anthropometric and biochemical variables was performed through standard methods. To evaluate the relationship between Mediterranean dietary patterns and sperm parameters, the multivariate logistic regression controlled for potential confounders was used. **Results:** The prevalence of sperm's low concentration in people with low, moderate, and high adherence to the Mediterranean diet was 43.4%, 34% and 25.9%, respectively. Participants in the highest tertile of adherence to the Mediterranean diet had lower odds of sperm low concentration (odds ratio: 0.37; 95% confidence interval: 0.15–0.91 P=0.02) compared with those in the lowest tertile after adjusting for potential confounders including energy intake, body mass index (BMI), smoking, age, level of education and physical activity. This association was observed between increasing trend adherence to the Mediterranean diet and decreasing the odds of sperm low concentration after full adjustments (odds ratio: 0.60; 95%, confidence interval: 0.98–0.94, P_{trend}=0.02). **Conclusion:** In the present study, the authors observed a protective relation between Mediterranean diet and sperm's low concentration. However, the association between adherence to this diet and other sperm parameters is unexplored. In future, more investigations are needed in this era.

Introduction

Infertility has been identified as a probably severe and costly problem for affected families (Vayena *et al.*, 2002). Males are found to be entirely responsible for 20–30% of infertility cases overall (Vander Borght and Wyns, 2018). 90% of the couples with fertility problems have low quality and/or count sperm (Leaver, 2016). Lifestyle factors such as smoking, stress, and diet

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can play a role in infertility (Hart, 2016).

It is well acknowledged that diet and metabolic factors can affect male fertility (Nätt *et al.*, 2019). Interestingly, some studies have shown that obesity may decline the quality of sperm (Ibañez-Perez *et al.*, 2019, Liu and Ding, 2017, Salas-Huetos *et al.*, 2017). High calorie diets, foods rich in trans-fatty acids, saturated fats, and cholesterol have been related to testicular interruption, involving damage in spermatogenesis, likely affecting male fertility (Morgan *et al.*, 2014, Ng *et al.*, 2010, Rato *et al.*, 2014). Excessive energy intake may also cause mitochondrial dysfunction, which is closely related to reactive oxygen species (ROS). It easily targets spermatozoa DNA and lipids, which diminishes sperm quality (Rato *et al.*, 2014).

However, epidemiological studies have shown that the use of dietary pattern can help better understand the correlation between dietary intake and chronic diseases such as infertility (Karayiannis *et al.*, 2017, Salas-Huetos *et al.*, 2017). The Mediterranean dietary pattern was proposed for the first time in 1995 to decrease cardiovascular risk factors in Mediterranean countries (Esposito and Giugliano, 2002).Vegetables, fruit, and olive oil are main components of Mediterranean diet. These compounds are rich in vitamins C and E, carotenoids, and polyphenols because they had antioxidant capacity (Lopes *et al.*, 2003, Visioli, 2000). Antioxidants can play the role of scavenger ROS before they lead to damage to various biomolecules, or prohibit oxidative damage from spreading out by breaking down the radical chain reaction of lipid peroxidation (Frei, 1994).

Although some projects reported protective association of the Mediterranean diet on health (Guasch-Ferré and Willett, 2021, Schröder, 2007), the results of a review study reported that Mediterranean diet is associated with lower rates of incident diabetes, and has better glycemic control in diabetic patients compared to control diets (Guasch-Ferré and Willett, 2021). Some studies reported that high adherence to Mediterranean diet has a protective effect on sperm quality (Cutillas-Tolín *et al.*, 2015a, Karayiannis *et al.*, 2017). Considering the effects of Mediterranean diet on health, the limited number of studies on the effect of Mediterranean diet on infertility, the fact that most of the research has been done in western countries where the dietary habits and the prevalence and features of infertility are different, the aim of this study is to investigate the effect of following the Mediterranean diet on sperm quality.

Material and Methods

Study population

This cross-sectional study was conducted on aged 20-50 year infertile men attending the Yazd Research and Clinical Center for infertility. Eligible participants were infertile men (with any disorder in morphology, motility, and concentration of sperm). Exclusion criteria were chronic disease, azoospermia, genetic disorders, history of vasectomy, varicocele, macroorchidism, and cryptorchidism. In addition, the participants who did not answer more than 35 food items of the food frequency questionnaire items, and the participants that received calorie, 800-4200 kcal were excluded. 250 men were eligible after applying all inclusion and exclusion criteria, and finally, the study was conducted on 218 men.

Dietary intake and physical activity

Dietary intake data was collected by trained dietitians with face to face interviews and a validated semi-quantitative food frequency questionnaire (FFQ) including 168 food items (Mirmiran *et al.*, 2010). The participants were asked about consumption frequency of each item in the questionnaire over the period of one year. The size of standard units and items reported based on household measures were converted to grams using instructions from nutritionist 4 scales (Afeiche *et al.*, 2013). Then, the amount of food intake of each subject was reported based on their daily intake in gram. The responses of participants were transformed into grams per day. The authors used the information included in the

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food ingredients table adapted for Iranian food items to calculate energy, macronutrients, and micronutrients. Daily physical activity (MET h/week) was measured by a short International Physical Activity Questionnaire (IPAQ) (Chiu *et al.*, 2014).

Mediterranean diet scale

The score of adherence to Mediterranean diet was calculated using the proposed method by Trichouplou *et al*. (Trichopoulou *et al.*, 2003) . The scoring was based on 9 food items: vegetables, legumes, fruits and nuts, dairy products, cereals, red meat and meat products, fish, poultry, and the ratio of monounsaturated fatty aside (MUFA) to Poly Unsaturated Fatty Acids (PUFA). The median consumption of these food items was calculated for each of the 9 components. For red and processed meat consumption, poultry and dairy products of less than median were given a value of 1, and for their consumption at or higher than the median was a value of 0 was given. For beneficial components (vegetables, legumes, fruits and nuts, cereal, and fish) consumption of less than the median, a value of 0 was considered, and for their consumption at or higher than the median, a value of 1 was dedicated. Also, for fat intake, the ratio of MUFA to saturated fatty aside (SFA) was used. The total Mediterranean-diet score ranged from 0 to 9.

Semen collection and analysis

Semen samples were taken by masturbation in the room near the laboratory and kept at 37 °C. Patients were asked not to ejaculate for at least 48 h before sampling. Semen analysis was conducted based on the WHO guidelines (Word Health Organization, 2010). Sperm parameters like sperm count (10^6/ml), motility, viability, and normal morphology were assessed on behalf of 200 spermatozoa for each sample. Sperm count and motility were evaluated by means of Makler chamber using light microscopy (Olympus Co., Tokyo, Japan). The viability and morphology were assessed by Eosin and Papanicolaou staining tests, respectively(Jörundsson *et al.*, 1999).

General, medical, and anthropometric measurements

General information including education, economic, and employment status were collected by personal interview. Morphology, motility, concentration of sperm, and sperm volume examinations were recorded according to the individual tests (World Health Organisation, 1999). Anthropometric measurements were performed using the standard methods. Body weight was determined with 100 g accuracy using a Seca scale. Body height was measured with a tape meter in a standing position without shoes, while the shoulders were in normal position using a Seca scale. Body mass index (BMI) (kg/m^2) was calculated by dividing weight in kilograms by the square of height in meters. Waist circumference (cm) was measured with the accuracy of 0.1 cm using a tape measure without any pressure on the body surface at the midpoint between the last rib and the upper part of the pelvis at the end of a normal exhalation. When it was hard to measure the narrowest area of the waist in individuals, waist circumstance was measured exactly under the last rib since in most subjects waist would be the narrowest area between the iliac crests and the lower ribs (Holm *et al.*, 1991). Hip circumstance (cm) was measured by a tape measure with the accuracy of 0.1 cm in a standing position and without any extra cover in the head area of the biggest hip bone that is equal to the biggest circumstance of thigh bone.

Ethical considerations

The protocol of this study was approved by the ethics committee of Shahid Sadoughi University of Medical Sciences, Yazd, Iran (approval code: IR.SSU.SPH.HER.1399.193). Informed consent was obtained from each participant before enrollment.

Data analysis

To compare general characteristics across tertiles, one-way ANOVA test was used for continuous variables and chi-square test was conducted for categorical variables. Dietary intakes across tertiles were compared using

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ANOVA test. To evaluate the relation between Mediterranean dietary pattern and sperm parameters, the authors used multivariate logistic regression in crude and adjusted models; they fit a model adjusted for energy intake, BMI, and physical activity.

Results

Characteristics of the study population

Characteristics of the participants across tertiles score of Mediterranean diet participants are presented in **Table1**. The mean age of the participants was 33.7±5-79 year. There was no significant difference in age, BMI, fat mass, visceral fat, waist circumference, hip circumference, smoking, education level and physical activity between tertiles Mediterranean dietary pattern scores.

Table 1. Characteristics of the study participants across tertiles of Mediterranean diet scores.

Variables	Mediterranean diet tertile			P-value[b]
	T1	T2	T3	
Quantitative variables				
Age (year)	34.44±5.67[a]	33.60±5.91	33.40±5.76	0.61
Body mass index (kg/m^2)	25.81±4.84	25.73±5.00	25.37±4.38	0.88
Fat mass (%)	23.25±7.84	23.21±12.65	22.31±8.61	0.81
Waist circumference (cm)	94.15±13.43	94.00±12.65	92.60±10.40	0.79
Hip circumference (cm)	99.94±8.04	99.65±8.56	95.04±21.85	0.10
Visceral fat (%)	8.76±4.56)	8.56±4.37)	7.91±4.07)	0.63
Qualitative variables				
Low sperm concentration	23(43.6)	33(34.0)	14(25.9)	0.16
Low sperm volume	7(13.7)	5(5.7)	5(9.6)	0.27
Low sperm progressive motility	22(41.5)	33(34.4)	14(25.9)	0.68
Low morphology	43(86)	79(83.2)	73(81.5)	0.82
Smoking	22(38.6)	39(38.6)	21(35.0)	0.88
Education				
High school	16(29.6)	39(40.6)	22(37.3)	0.40
Diploma	23(42.6)	36(37.5)	28(47.5)	
Bachelor's degree	7(13.0)	6(6.3)	2(3.4)	
Master's degree and higher	8(14.8)	15(15.6)	7(11.9)	
Physical activity				
Low	19(34.5)	25(26.6)	17(32.1)	0.86
Moderate	30(54.5)	56(59.6)	30(6.6)	
High	6(10.9)	13(13.8)	6(11.3)	

[a]: Data are presented in quantitative variables as mean ±standard deviation (SD) and for qualitative variables as number (%); [b]: For qualitative and quantitative variables, Chi-square test and One-way ANOVA, were used respectively.

Dietary intakes of participants

Components of the Mediterranean diet across tertiles of Mediterranean diet scores are presented in **Table2.** Consumption of whole grain and dairy was not significantly different among tertiles of Mediterranean diet scores. However, intake of refined grain and poultry, fish, fruit, vegetable, legumes, nut, MUFA to SFA ratio, and meat significantly increased with increasing tertiles.

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Table 2. Characteristics of the study participants based on tertiles of Mediterranean diet scores.

Variables	Mediterranean diet tertile			P-value[b]
	T1	T2	T3	
Energy intake (kcal)	3113.08±653.15[a]	3106.08 ±653.15	3262.72±522.91	0.27
Whole grain (g)	91.44±112.40	106.08±104.04	110.63±89.31	0.56
Refine grain (g)	386.36±184.74	385.57±160.83	324.49±116.55	0.03
Fish (g)	14.45±20.17	25.61±33.20	39.23±35.49	<0.001
Meat (g)	57.88±56.67	41.05±37.66	35.25±38.27	0.01
Poultry (g)	100.48±77.89	72.27±76.37	69.19±59.02	0.03
Fruit (g)	417.42±290.93	440.76±284.94	767.99±591.88	<0.001
Vegetable (g)	87.57±45.81	111.83±125.53	187.14±132.85	<0.001
Legumes (g)	35.69±26.75	50.89±33.75	62.46±42.00	<0.001
Nut (g)	8.76±8.09	17.66±21.16	21.88±22.90	<0.01
Dairy (g)	359.17±355.71	313.76±231.48	434.46±667.30	0.22
MUFA/SFA	0.92±0.29	0.09±0.43	1.15±0.35	<0.01

***MUFA**: Monounsaturated fatty acid; **SFA**: Saturated fatty acid; [a]: Data reported on mean± SD; [b]: One-way ANOVA.*

Association between Mediterranean diet and sperm quality

Crude and multivariate-adjusted odds ratios for decreased sperm quality based on tertiles of Mediterranean diet adherence are listed in **Table 3**. There was no significant association between adherence to Mediterranean diet and odds of low volume, low morphology, and low progressive motility in crude and adjusted models. Moreover, there was no significant association between odds of low concentration and score of Mediterranean diet in crude model (Odds Ratio (OR): 0.45; 95% confidence interval (CI): 0.20–1.03, *P*=0.06).

After adjusting for energy intake, BMI, age and physical activity, and smoking, significant protective association was observed between higher adherence to Mediterranean diet and odds of low concentration sperm. Men in the higher tertile of Mediterranean diet had 64% lower odds of sperm's low concentration than the first tertile after full adjustment (OR=0.37, 95% CI: 0.15 to 0.91; P= 0.03).

Table 3. Odds ratio of the considered sperm disorder across tertiles of Mediterranean diet score (Multivariable-adjusted odds ratios and 95% CI).

Variables	Mediterranean diet tertile			P-value[a]	P_{trend}
	T1	T2	T3		
Low concentration					
Crude	1.00	0.67(0.23-1.33)	0.45 (0.20-1.03)	0.06	0.05
Adjusted model[a]	1.00	0.58 (0.28-1.20)	0.37 (0.15-0.91)	0.03	0.02
Low progressive motility					
Crude	1.00	0.73(0.37-1.47)	0.87 (0.40-1.88)	0.72	0.72
Adjusted model	1.00	0.75(0.36-1.56)	0.94 (0.40-2.17)	0.89	0.87
Low volume					
Crude	1.00	0.37(0.11-1.26)	0.66 (0.19-2.26)	0.51	0.47
Adjusted model	1.00	0.42 (0.12-1.50)	0.67 (0.17-2.64)	0.56	0.50
Low morphology					
Crude	1.00	0.80 (0.30-2.10)	0.71 (0.25-2.05)	0.53	0.53
Adjusted model	1.00	0.87 (0.31-2.44)	0.62 (0.20-0.18)	0.40	0.39

[a]: Adjusted model: Adjusted for energy intake, BMI, physical activity, age, and smoking.

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Discussion

This study was conducted to explore the association between adherence to Mediterranean diet and quality of sperm in Iranian adults as a Middle East country. The results showed that higher adherence to Mediterranean diet can reduce the odds of sperm's low concentration but not sperm's low volume and low progressive motility after full adjustment. Previous observational studies stated a positive relationship between the intake of individual components of a Mediterranean dietary pattern such as vegetables (Eslamian *et al.*, 2012), fruits (Braga *et al.*, 2012), fish (Afeiche *et al.*, 2014b), poultry (Eslamian *et al.*, 2012), whole grains (Braga *et al.*, 2012), low-fat dairies (Afeiche *et al.*, 2014a) and the improvement of some sperm quality parameters.

The findings regard a protective association between adherence to a Mediterranean dietary pattern and concentration of sperm, which was consistent with some studies. In a cross-sectional study of 225 men, Karayiannis *et al*. (Karayiannis *et al.*, 2017) reported that men in the lowest tertile of the Mediterranean diet adherence compared to the highest one, had a higher abnormal sperm concentration, total sperm count, and motility. Furthermore, in a study by Cutillas-Tolın *et al.* (Cutillas-Tolín *et al.*, 2015b), the total sperm count of men in the higher quartile of Mediterranean diet consumption was 51.5% higher than those in the lower quartile adherence. In a study population of healthy men from the USA, higher adherence to a "prudent" dietary pattern, that was similar to the Mediterranean dietary pattern, was related to higher progressive motility (Gaskins *et al.*, 2012). Hence, the findings confirmed some of the results but only in relation to the concentration of sperm as a key fertility-related parameter.

Regarding other dietary patterns assessing sperm parameters, in a study by Oostingh *et al.* (Oostingh *et al.*, 2017) a positive relationship between higher adherence to a healthy dietary pattern and semen parameters in men with poor semen quality was found. On the other hand, Cutillas-Tolın *et al* Observed an inverse relationship between adherence to Western diet and semen concentration among overweight or obese men (Cutillas-Tolín *et al.*, 2015a). In addition, the findings of main components of western dietary patterns, including processed meat (Afeiche *et al.*, 2014c), potatoes (Mendiola *et al.*, 2009), full-fat dairy (Afeiche *et al.*, 2013), sweets (Eslamian *et al.*, 2012), sugar-sweetened beverages (Chiu *et al.*, 2014), and alcohol (Anifandis *et al.*, 2014) were adversely related to several quality parameters of semen.

Vegetables and fruits were two main food groups of the Mediterranean diet with high contents of micronutrients and antioxidants. The combination of losing cytoplasm, generating ROS by mitochondria, and accumulation of an oxidizable substrate in its cell membrane led to the susceptibility of sperm to oxidative damage (Nassan *et al.*, 2018). Evidence from randomized trials demonstrated that antioxidant supplementation among men caused improving semen quality, particularly motility (Agarwal *et al.*, 2004, Nassan *et al.*, 2018).

This was while the intake of folic acid as affluent vitamins of fruit and vegetables in Mediterranean diet can affect sperm production. Folates may play a key role in spermatogenesis by acting as substrates or cofactors in the metabolism of one-carbon (Schröder, 2007). The metabolism of one-carbon occurs in testes (Chen *et al.*, 2001, Holm *et al.*, 1991, Holm *et al.*, 1999). Generally, this pathway contains a series of related metabolic pathways that one-carbon moieties are transferred from donors to intermediate carriers and used in reactions of methylation or the synthesis of purines and thymidine; then, they are used in DNA synthesis (Eslamian *et al.*, 2012, Lucock, 2000). In a randomized trial, the supplementation of folic acid led to an increase in sperm concentration (Bentivoglio *et al.*, 1993). Additionally, in another randomized trial, sub fertile men who assigned to the folate and zinc arm showed a remarkable increase in total sperm count (Wong *et al.*, 2002). The composition of fatty acids in sperm cell membrane is very important for proper function of sperms, and the sperm cell membrane have a main role in fertilization events (Flesch and Gadella, 2000). The PUFA content of sperm cell membrane,

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especially docosahexaenoic acid (DHA) increases as the sperm matures (Lenzi *et al.*, 2000). Moreover, 20% of the fatty acid content in mature sperm is comprising of DHA (Lenzi *et al.*, 2000). PUFAs must be obtained from consuming seafood, fish, nuts, seeds, and vegetable oils consumed highly in Mediterranean diet. It was demonstrated that intake of these food sources could alter the fatty acid composition of sperm quality (Nassan *et al.*, 2018). In addition, evidence showed that supplementation with fish oil, as a rich source of DHA and eicosapentaenoic acid (EPA), could elevate the concentration of DHA in animals testicular and humans sperm membrane, resulting in higher sperm concentration, motility, and normal morphology (Conquer *et al.*, 1999, Frei, 1994, Safarinejad, 2011, Schröder, 2007, Tavilani *et al.*, 2006, Tavilani *et al.*, 2007).

Low-fat dairy products as components of Mediterranean diet have been established with association between dairy products, and specifically, sperm parameters reference. In a longitudinal cohort study among men in the USA, the consumption of low-fat dairy products was related to a higher sperm concentration and progressive sperm motility (Afeiche *et al.*, 2014a). Inversely, a cross-sectional investigation of a cohort of men found that the intake of full-fat dairy products was adversely associated with normal sperm morphology and better motility (Vujkovic *et al.*, 2009). In another cross-sectional study of men in the Netherlands, dairy consumption was not associated with semen quality (Afeiche *et al.*, 2013).

The strengths of the present study were the large study population, and using priori dietary patterns method due to the major advantage of method as a reference where data was achieved regarding adherence to healthy dietary habits. In addition, infertility clinic is referral in Middle East countries. Because of unique diet cultures and backgrounds, the findings of this study may identify novel insight into Mediterranean diet and infertility. One of the strengths of the study was that FFQ answering was not self-reported, and each item was explained for the participants, and then, marked the chosen option in the answer sheet by trained interviewer. Also, the other was controlled for a wide range of confounders such as energy intake, BMI and physical activity.

This study had several limitations as well. The cross-sectional nature of present study led to causal inference. Moreover, using one-year FFQ might have caused errors in measuring dietary intakes and patterns causing measurement error and misclassification of the study subjects and reporting bias. Therefore, generalizing the results should be done with caution.

Conclusion

Generally, the Mediterranean diet has consistently been related to improving sperm concentration. However, in the present study, the association between adherence to the Mediterranean diet and other sperm quality parameters is unexplored. In future, analytic studies such as clinical trials and cohorts are needed in this era for approve these results.

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Authors' contributions

Hosseinzadeh M and Babaie S conceived the idea and supervised the study. Beigrezaei S and Darabi Z wrote the first draft. Darabi Z and Hosseinzadeh M were involved in study analysis. Hosseinzadeh M revised the manuscript. All the authors read and approved the final version of the manuscript.

Conflict of interest

The authors declared no conflict of interests.

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