American Journal of Men's Health
2018, Vol. 12(3) 575–583

DOI: 10.1177/1557988315627139
journals.sagepub.com/home/jmh
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Dietary Patterns and Their Relationship With Semen Quality

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Abstract

Diet is a complex exposure variable, which calls for multiple approaches to examine the relationship between diet and disease risk. To address these issues, several authors have recently proposed studying overall dietary patterns by considering how foods and nutrients are consumed in combinations. The aim of the study was to investigate the associations between dietary patterns, semen quality parameters, and the level of reproductive hormones. The study population consisted of 336 men who attended the infertility clinic for diagnostic purposes and who had normal semen concentration of 20 to 300 mln/ml or slight oligozoospermia (semen concentration of 15-20 mln/ml). Participants were interviewed, and a semen sample was provided by them. Diet was assessed via food frequency questionnaire, and dietary patterns were identified by factor analysis. Men were classified into three groups according to scores of each dietary pattern: Western, Mixed, or Prudent. A positive association was observed between sperm concentration and Prudent dietary pattern, and level of testosterone and Prudent dietary pattern (p = .05, p = .03, respectively). Additionally, Prudent dietary pattern was identified to decrease the DNA fragmentation index (p = .05). The results were adjusted for sexual abstinence, age, smoking, past diseases, and alcohol consumption. Higher consumption of a Prudent dietary pattern was associated with higher sperm concentration and higher level of testosterone. Sperm chromatin structure was inversely related to higher consumption of a Prudent dietary pattern. Further research is needed to confirm these findings and extend these results to other populations.

Keywords

semen, dietary pattern, sperm chromatin structure

Introduction

While genetic, endocrine, congenital, intrauterine (e.g., maternal smoking), and demographic factors—such as age, smoking, and heavy alcohol use—are risk factors for decreased sperm quality, there is increasing evidence that nutrition could also play an important role (Attaman et al., 2012; Wong, Thomas, Merkus, Zielhuis, & Steegers-Theunissen, 2000). As a reflection in global changes in dietary behavior, 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 women and men within the reproductive age range (Vujkovic et al., 2007). To date, nutritional studies on semen quality in men have mostly focused on isolated micronutrients such as folate, zinc, and various antioxidants. Very few studies have focused on food groups or dietary patterns.

Diet is a complex exposure variable, which calls for multiple approaches to examine the relationship between diet and disease risk. Traditional analyses in nutritional epidemiology typically examine diseases in relation to single or few nutrients or foods. However, people do not eat isolated nutrients but eat meals consisting of a variety of foods with complex combinations of nutrients (Hu et al., 1999). Dietary pattern analysis is one approach that can examine the relationship between diet and disease. In contrast, while the conventional approach focuses on a single nutrient or a few nutrients or foods, this approach considers overall eating patterns. Several authors recently

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proposed studying overall dietary patterns by considering how foods and nutrients are consumed in combination (Huijbregts et al., 1997; Kant, 1996; Millen et al., 1996; Slattery, Boucher, Caan, Potter, & Khe-Ni, 1998).

Little is known of how diet may influence male reproductive potential. Emerging literature supports the hypothesis that specific nutritional factors (saturated fats, omega-3 fats, soy, soy isoflavones, beef consumption, folate, zinc, and antioxidants) can affect semen quality (sperm concentration, morphology, motility, sperm aneuploidy; Chavarro et al., 2011; Chavarro, Toth, Sadio, & Hauser, 2008; Eskenazi et al., 2005; Mendiola et al., 2010; Swan, Liu, Overstreet, Brazil, & Skakkebaek, 2007; Wong et al., 2000; Young, Eskenazi, Marchetti, Block, & Wyrobek, 2008). Men who consume a "health-conscious" diet (high intakes of fruits, vegetables, fish, and whole grains) have lower sperm DNA damage (Vujkovic et al., 2009). A Spanish study revealed positive associations between the intake of folate-rich food sources, such as fruit and vegetables, and semen quality (Mendiola et al., 2009). Gaskins, Colaci, Mendiola, Swan, and Chavarro (2012), who studied dietary patterns, reported that a diet rich in fruits, vegetables, chicken, fish, and whole grains may be positively associated with the percentage of progressively motile sperm.

Unlike some risk factors for subfertility that cannot be reversed, diet poses an opportunity for intervention, thus making it important to consider in the counseling of subfertile men. The present study adds more statistical power to the previous human sperm quality studies of dietary patterns by expanding outcome measures to include semen parameters, sperm chromatin structure, and the level of reproductive hormones, and by assessment of semen quality among a large number of subjects. The objective of the present analysis was to investigate the associations between dietary patterns and semen quality parameters. This is the first study to assess the dietary pattern and many different semen parameters (sperm concentration, motility, morphology, computer-aided semen analysis [CASA] parameters), sperm chromatin structure (DNA fragmentation index [DFI], high DNA stainability [HDS]), and the level of reproductive hormones (FSH [*follicle-stimulating hormone]*, estradiol, testosterone) in one study.

Materials and Method

Study Population

The study population initially consisted of 344 men who were attending an infertility clinic for diagnostic purposes and who had a normal semen concentration of 20 to 300 mln/ml or slight oligozoospermia (semen concentration of 15-20 mln/ml; World Health Organization [WHO], 1999), from the study titled "Environmental Factors and Male Infertility," which is a part of the project "Epidemiology of Reproductive Hazards in Poland—Multicentre Study in Poland." The Nofer Institute of Occupational Medicine Bioethical Committee Board approved the study (Resolution No. 9/2007 [04.06.2007]), and written informed consent was obtained from all subjects before their participation. Approximately 59% of eligible men agreed to participate. Those declining participation primarily cited lack of time during their visit as the reason for nonparticipation. All participants completed a questionnaire, which collected demographic information, lifestyle factors, and medical history. A physical examination of each participant was performed on the same day as semen sampling in which weight and height were assessed to determine body mass index and the presence of varicocele or other abnormalities was noted. Full details of the parent study have been described elsewhere (Jurewicz et al., 2014). The present analysis included only men (n = 336) who completed a food frequency questionnaire (FFQ). Smoking status was verified by measuring cotinine levels in saliva using high-performance liquid chromatography coupled with tandem mass spectrometry/positive electrospray ionisation (LC-ESI+MS/MS) and the isotope dilution method (ISO 17025 criteria; accredited by the Polish Center of Accreditation [Certificate AB215]). Men were identified as smokers when their saliva cotinine levels were greater than 10 ng/ml (Jurewicz et al., 2014).

Dietary Pattern Assessment

Diet was assessed using a previously validated FFQ (Rimm et al., 1992). Men were asked to report how often, on average, they consumed specified amounts of each food, beverage, and supplement included in the questionnaire during the previous year. Options for frequency of food intake included six categories ranging from *never* to *six or more times* per day. The selected frequency category for each food item was converted to a daily intake. For example, a response of "2 to 4/week" was converted to 0.43 servings/day. The individual food items were collapsed into 40 predefined food groups based on the similarity of nutrient profiles or culinary usage. These 40 food groups were similar to those used in other studies of Western men (Hu et al., 2000). Two dietary patterns were identified using factor analysis. The Prudent pattern was characterized by high intakes of fish, chicken, fruit, cruciferous vegetables, tomatoes, leafy green vegetables, legumes, and whole grains, while the Western pattern was characterized by high intakes of red and processed meat, butter, high-fat dairy, refined grains, pizza, snacks, high-energy drinks, mayonnaise, and sweets. For every subject, factor scores were calculated on each of the two

retained factors by summing the frequency of consumption multiplied by factor loadings across all food items. Thus, each participant was given a score for the "Prudent" and "Western" patterns according to his consumption of items from each.

As it was difficult to assign participants exclusively to either a Prudent or a Western group, the Mixed group was created. Men were classified into three groups according to scores of each dietary pattern for the main analysis: Western, Mixed, or Prudent.

Semen Collection and Analysis

Semen samples were collected by masturbation at the clinic where on arrival men were asked to report the time of their previous ejaculation. Sample processing was initiated within 30 minutes of collection.

Sperm morphology was quantified using strict Kruger criteria; the semen smears were air-dried, fixed, and stained according to Papanicolaou. Results were reported as the percent of abnormal forms among the spermatozoa examined. Technicians were trained to analyze the semen samples using standardized protocols based on the WHO (1999) Guidelines.

Sperm counts and percentage motility were determined with the use of CASA (Hamilton-Thorne Version 10HTM-IVOS) using two-chamber Leja slides (Leja, The Netherlands). A manual count of the same sample was also performed for the sperm concentration. For the manual count, the sperm concentration was obtained by averaging the total number of sperm in both chambers on the Leja slide. In the present study, straight-line velocity, curvilinear velocity, average path velocity, and linearity, were used as measures of sperm progression, sperm vigor, sperm velocity, and swimming pattern, respectively (Duty et al., 2004).

The assessment of the sperm chromatin structure assay (SCSA) was performed using flow cytometry (ASRM Practice Committee, 2006). After partial denaturation of the DNA (pH = 1.5), the samples were stained with metachromatic fluorochrome: acridine orange (Ex/Em = 488/525 and 615 nm). Fluorescence in green (515-530 nm) and red (>630 nm) bands was measured using a flow cytometer (DAKO Galaxym DAKO, Denmark). The fluorescence bands corresponded to intact, double-stranded DNA (green fluorescence) and fragmented, single-stranded (red fluorescence) sperm DNA. Cells with an abnormal chromatin structure (i.e., fragmented DNA) showed a distinct shift of the alpha t parameter value (alpha t = red/(green+red). The DFI was calculated according to the formula: DFI = (cells with a shift of the alpha t parameter/all cells) × 100 each analyzed sperm cell and was shown on a histogram. Cells with an abnormal chromatin structure showed a distinct shift of the alpha t parameter value (Evenson et al., 1999). HDS% was calculated on the basis of the percentage of sperm with high levels of green fluorescence, which are thought to represent immature spermatozoa with incomplete chromatin condensation (Evenson, Larson, & Jost, 2002).

Reproductive Hormones Analysis

Human plasma was collected in lithium heparin EDTA (Ethylenediaminetetraacetic acid) to determine levels of testosterone, FSH, and estradiol among the study participants. Measurement of FSH, testosterone, and estradiol was performed using a Chemiluminescent Microparticle Immunoassay (ARCHITECT System; Abbott, Longford, Ireland). The results are expressed as IU/L for FSH, pg/ml for estradiol, and ng/ml for testosterone. The lower limits of detection for FSH, estradiol, and testosterone were 0.05 IU/l, 17.9 pg/ml, and 0.08 ng/ml, respectively (Messinis et al., 2005). Male reference values for these assays were 2.49 to 8.36 ng/ml for testosterone, 1.7 to 12 IU/l for FSH, and <62 pg/ml for estradiol (Raven, de Jong, Kaufman, & de Ronde, 2006).

Statistical Analysis

Descriptive statistics on subject demographics were calculated, along with the type of diet and semen quality and reproductive hormone levels. Bivariate analysis was conducted between all sperm measures, reproductive hormones measure, type of diet, and demographic variables to investigate differences between distributions or categories and the potential for confounding. Differences were tested statistically, using nonparametric methods where appropriate. The following covariates were evaluated as potential confounders: sexual abstinence (days), age (years), smoking (yes/no), past diseases (yes/no), and alcohol consumption (none or < 1 drink/week, 1-3 drinks/week, 4-7 drinks/week). Multiple linear regression was used to assess associations between type of diet and sperm quality measures and the level of reproductive hormones. Some dependent variables were log transformed to obtain approximate normal distribution. Qqnorm plot was used to visually assess the normality of distribution. Sperm concentration (mln/ml) and DFI (%) were log transformed. R 2.15.1 statistical program was used to analyze data (R Core Team, 2013).

For every subject factor scores were calculated on each of the two retained factors by summing the frequency of consumption multiplied by factor loadings across all food items. Thus, each participant was given a score for the "Prudent" and "Western" patterns according to his consumption of items from each. Men were classified into three groups according to scores of each dietary pattern for the main analysis.

Table 1. Demographic and Dietary Characteristics of Participants.

Characteristics	Dietary patterns			
	Western (*N* = 150), *n* (%)	Mixed (*N* = 90), *n* (%)	Prudent (*N* = 96), *n* (%)	*p*
Age, years				.11
22-30	50 (33.33)	29 (32.22)	26 (27.08)	
31-40	92 (61.33)	60 (66.67)	64 (66.67)	
41-45	8 (5.33)	1 (1.11)	6 (6.25)	
Body mass index				.77
18.5-25	50 (33.33)	27 (30.00)	36 (37.50)	
26-30	66 (44.00)	45 (50.00)	40 (41.67)	
≥30	34 (22.67)	18 (20.00)	20 (20.83)	
Education				.71
Primary	35 (23.33)	18 (20.00)	18 (18.75)	
Secondary	57 (38.00)	32 (35.56)	38 (39.58)	
Higher	58 (38.67)	40 (44.44)	40 (41.67)	
Time of sexual abstinence, days				.33
<3	11 (7.33)	8 (8.89)	5 (5.21)	
3-7	131 (87.33)	76 (84.44)	85 (88.54)	
>7	8 (5.33)	6 (6.67)	6 (6.25)	
Smoking				.81
No	104 (69.33)	64 (71.11)	67 (69.79)	
Yes	46 (30.67)	26 (28.89)	29 (30.21)	
Past diseases				.83
No	128 (85.33)	77 (85.56)	83 (86.46)	
Yes	22 (14.67)	13 (14.44)	13 (13.54)	
Alcohol use				.55
None or <1 drink/week	48 (32.00)	31 (34.44)	32 (33.33)	
1-3 drinks/week	75 (50.00)	47 (52.22)	49 (51.04)	
Every day	27 (18.00)	12 (13.33)	15 (15.63)	

Results

The study population consisted of 336 men who attended infertility clinics for diagnostic purposes. Of the study subjects, 45% (n = 150) had a Western dietary pattern, 27% (n = 90) a Prudent, and 29% (n = 96) a Mixed diet. There were no differences in the demographic characteristics among men with different dietary patterns. The mean age of men participating in this study was 32 years.

Most of the participants had secondary and higher education and were nonsmokers (about 70%; p = .81); verification was based on the level of cotinine in saliva. Past diseases that may have an impact on semen quality were reported by about 14% (p = .83) of the participants. Sexual abstinence before the semen analysis lasted mostly 3 to 7 days, mean 5 days (p = .33; Table 1). About 62% to 70% of examined men had a body mass index ≥25 kg/m^2 (p = .77; Table 1). As summarized in Table 1, most of the participants drank 1 to 3 drinks per week (p = .55).

The semen quality among the study subjects is presented in Table 2. There was a statistically significant difference in sperm concentration, DFI, and level of testosterone between the three groups of men with different dietary patterns (p < .05). There were no differences in sperm motility, percentage of sperm with abnormal morphology, CASA parameters (average path velocity, straight-line velocity, curvilinear velocity, linearity), HDS, and level of reproductive hormones (FSH, estradiol) between the three groups of men with different dietary patterns (p > .05; Table 2).

A positive association was observed between sperm concentration (p = .04) and the level of testosterone (p = .01) with the Prudent dietary pattern as compared to participants with the Western dietary pattern. Additionally, the Prudent dietary pattern was found to increase the DFI (p = .05). The Mixed dietary pattern was not statistically significantly associated with any of the examined semen parameters, sperm chromatin structure, or the level of reproductive hormones (Table 3).

In multivariate analysis, the associations from univariate models remained significant. The Prudent dietary pattern was associated with increased sperm concentration, increased level of testosterone, and decreased percentage of sperm with DNA damage (p = .05, p = .03, and p = .05, respectively) compared to the Western dietary pattern. The

Table 2. Semen Parameters, Sperm Chromatin Structure, and the Level of Reproductive Hormones Among Men With Different Dietary Patterns.

	Dietary Patterns																	
	Prudent						Mixed						Western					
	Statistical variables						Statistical variables						Statistical variables					
Semen quality	*N*	*M*	*SD*	*Mdn*	Minimum	Maximum	*N*	*M*	*SD*	*Mdn*	Minimum	Maximum	*N*	*M*	*SD*	*Mdn*	Minimum	Maximum
Sperm concentration (mln/ml)[a]	96	53.18	64.19	33.20	1.70	230.70	90	47.19	44.48	31.70	1.90	360	150	38.12	38.00	37.00	1.00	215.30
% motility	96	55.95	19.98	54.50	6.00	96.00	90	54.12	17.21	52.50	4.00	96.00	150	56.68	21.42	56.00	6.00	99.00
% of sperm with abnormal morphology	96	54.19	24.33	50.00	13.00	98.00	90	51.88	22.70	47.50	15.00	97.00	150	54.53	24.38	49.50	11.00	97.00
CASA parameters																		
VAP	96	51.80	12.06	50.60	15.10	86.60	90	52.16	10.37	51.15	26.80	74.80	150	53.68	10.95	53.85	24.90	81.70
VSL	96	43.50	10.08	43.00	15.50	77.1	90	42.11	9.89	42.11	14.20	75.13	150	42.89	10.13	42.80	14.70	76.13
VCL	96	78.10	16.80	79.00	32.00	146.00	90	77.11	15.20	78.00	30.00	139.00	150	77.89	15.98	78.52	29.00	140.00
LIN	96	56.84	6.33	56.50	38.00	71.00	90	55.56	6.05	56.00	41.00	67.00	150	55.91	6.65	56.00	40.00	74.00
Sperm chromatin structure																		
DFI[a]	96	15.20	10.45	13.29	3.06	68.72	90	16.04	9.05	14.60	2.72	42.23	150	17.98	8.12	12.42	2.93	71.23
HDS	96	8.57	4.01	7.78	0.73	20.46	90	8.79	4.60	7.98	2.80	30.54	150	9.10	4.43	8.78	1.56	30.65
Level of reproductive hormones																		
FSH	96	4.75	3.59	3.50	0.10	25.20	90	4.34	2.42	3.53	1.10	12.60	150	4.14	2.82	3.42	0.10	16.80
Estradiol	96	27.64	8.37	27.80	2.60	55.10	90	29.26	12.22	27.90	2.80	72.40	150	28.19	9.48	27.10	9.00	75.20
Testosterone[a]	96	4.57	1.84	4.48	1.47	10.90	90	5.10	3.49	4.50	1.68	28.30	150	4.12	1.72	4.80	1.35	9.35

Note. CASA = computer-aided semen analysis; VSL = straight-line velocity; VCL = curvilinear velocity; VAP = average path velocity; LIN = linearity; DFI = DNA fragmentation index; HDS = high DNA stainability; FSH = *follicle-stimulating hormone*.

[a]Statistically significant differences $p<0.05$.

Table 3. Univariate and Multivariate Analysis of Semen Quality and the Level of Reproductive Hormones Among Men With Different Dietary Patterns.

	Dietary patterns					
	Mixed[a]			Prudent[a]		
Semen quality parameters and level of reproductive hormones	Estimate	95% Confidence interval	*p*	Estimate	95% Confidence interval	*p*
Sperm concentration (mln/ml) crude	−5.97	[−16.10, 4.15]	.25	0.08	[0.02, 57, 0.78]	**.04**
Sperm concentration (mln/ml) adjusted	0.05	[−0.26, 0.36]	.76	0.05	[0.02, 0.99]	**.05**
% Motility crude	−3.02	[−8.43, 2.39]	.28	1.18	[−6.51, 4.15]	.67
% Motility	−2.72	[−8.11, 2.68]	.32	0.76	[−6.18, 4.67]	.79
% of sperm with abnormal morphology crude	−2.65	[−9.50, 4.21]	.45	−0.37	[−7.04, 6.30]	.91
% of sperm with abnormal morphology	−2.13	[−8.96, 4.69]	.54	0.06	[−6.75, 6.68]	.99
VAP	−1.62	[−4.54, 1.29]	.28	−2.04	[−4.90, 0.82]	.16
VAP	−1.06	[−4.00, 1.88]	.48	−1.18	[−4.13, 1.78]	.44
VSL crude	−0.61	[−1.55, 0.33]	.20	−0.31	[−1.23, 0.61]	.51
VSL	−0.39	[−1.32, 0.55]	.42	−0.02	[−0.96, 0.92]	.97
VCL crude	−0.01	[−0.20, 0.18]	.89	−0.12	[−0.31, 0.06]	.19
VCL	−0.01	[−0.20, 0.18]	.90	−0.11	[−0.30, 0.08]	.26
LIN crude	−0.26	[−2.01, 1.50]	.77	0.92	[−0.80, 2.63]	.30
LIN	0.00	[−1.76, 1.76]	1.00	1.06	[−0.70, 2.82]	.24
DFI crude	0.05	[−0.13, 0.24]	.56	−0.03	[−0.04, 0.02]	**.05**
DFI	0.05	[−0.14, 0.24]	.58	−0.03	[−0.04, 0.01]	**.05**
HDS crude	−0.06	[−0.20, 0.07]	.35	−0.05	[−0.19, 0.08]	.42
HDS	−0.05	[−0.18, 0.09]	.50	0.00	[−0.13, 0.14]	.96
FSH crude	0.35	[−0.16, 0.86]	.17	0.22	[−0.29, 0.74]	.39
FSH	0.07	[−0.08, 0.22]	.35	0.06	[−0.09, 0.21]	.43
Estradiol crude	0.44	[−1.94, 2.82]	.72	−0.09	[−2.39, 2.20]	.94
Estradiol	−0.07	[−2.48, 2.34]	.95	−0.83	[−3.23, 1.56]	.50
Testosterone crude	−0.35	[−0.83, 0.13]	.16	0.36	[0.36, 0.84]	**.01**
Testosterone	−0.35	[−0.83, 0.13]	.15	0.36	[0.37, 0.13]	**.03**

Note. VAP = average path velocity; VSL = straight-line velocity; VCL = curvilinear velocity; LIN = linearity; DFI = DNA fragmentation index; HDS = high DNA stainability; FSH = *follicle-stimulating hormone*.

[a]Reference group: Western dietary pattern. Boldface-statistically significant values

results were adjusted for sexual abstinence, age, smoking, past diseases, and alcohol consumption (Table 3).

Discussion

The current study demonstrates that human nutrition affects semen quality and the level of reproductive hormones. Men who consume fish, chicken, fruits, cruciferous vegetables, tomatoes, leafy green vegetables, legumes, and whole grains had a lower DFI and higher sperm concentration and testosterone level. These findings also represent important information on the relationship between unhealthy diets and decline of semen quality in industrialized countries.

Studies on the association between diet and semen quality are in line with the results of the present study findings. The study of dietary fat and semen quality conducted among men attending a fertility clinic reported that high intake of saturated fats was negatively related to sperm concentration whereas higher intake of omega-3 fats was positively related to sperm morphology (Attaman et al., 2012). Chavarro et al. (2011) measured the sperm fatty acid composition in semen samples of 33 men undergoing infertility evaluation at an academic medical center. They identified that trans-fatty acids were present in human sperm and were inversely related to sperm concentration.

A study carried out by Swan et al. (2007) suggests that maternal beef consumption, and possibly xenobiotics (anabolic steroids) in beef, may alter a male fetus' testicular development in utero and adversely affect his reproductive capacity. Sperm concentration was inversely related to the mother's beef intake per week. In sons of "high beef consumers" (>7 beef meals/week), sperm concentration was 24.3% lower than in the men whose mothers ate less beef (Swan et al., 2007; vom Saal, 2007).

The intake of 15 soy-based foods in the previous 3 months was assessed for 99 male partners of subfertile couples (Chavarro et al., 2008). The data suggested that higher intake of soy foods and soy isoflavones was associated with lower sperm concentration.

Low intake of antioxidant nutrients was associated with poor semen quality in a case control study in Spain (Mendiola et al., 2010). Young et al. (2008) observed inverse relationships between folate, zinc, and antioxidant intake in healthy nonsmoking men in the United States and sperm aneuploidy. Eskenazi et al. (2005) identified positive associations between antioxidant intake (vitamin C, vitamin E, and beta-carotene) and semen quality, especially for motility but no association between antioxidant intake and DNA fragmentation as measured by the sperm chromatin structure assay (Silver et al., 2005).

Mendiola et al. (2009) compared dietary habits in normospermic (control group) and oligoasthenoteratospermic patients (cases group) from an infertility clinic. Participants had a lower intake of lettuce and tomatoes and fruits (apricots and peaches) and a significantly higher intake of dairy and meat processed products. Frequent intake of lipophilic foods like meat products or milk may negatively affect semen quality in humans, whereas some fruits or vegetables may maintain or improve semen quality (Mendiola et al., 2009).

Vujkovic et al. (2009) demonstrated that human nutrition affects semen quality in men undergoing in vitro fertilization/intracytoplasmic sperm injection procedures. Men who consumed the "health-conscious" (high intakes of fruits, vegetables, fish, and whole grains) diet had lower sperm DNA damage. Sperm concentrations were much higher in men who strongly adhered to the "traditional Dutch" dietary pattern (high intakes of meat, potatoes, and whole grains and low intakes of beverages and sweets).

The high content of folates and vitamin B6–rich foods may stimulate tHcy to be remethylated into methionine and trans-sulfurated into cystathionine and cysteine. In males, the regulation of homocysteine is mediated partially through the testosterone-dependent cystathionine-b-synthase pathway (Vitvitsky et al., 2007).

Additionally, Gaskins et al. (2012), who studied dietary patterns, reported that diet rich in fruits, vegetables, chicken, fish, and whole grains may be positively associated with the percentage of progressively motile sperm.

This is the largest study to date examining the influence of specific dietary patterns on male fertility: semen quality, sperm chromatin structure, and level of reproductive hormones. Strengths of the study include the ability to account for multiple potential confounders, which had not been the case in previous studies. This is the largest study in humans so far examining the relationship between dietary patterns and semen quality. The use of dietary pattern analysis as opposed to nutrient or whole food analysis more closely reflects the real world and allows for easier translation of these results to the public (Hu, 2002). To determine the typical daily intake, the FFQ for the 3-month window prior to sample collection sensitive period for semen quality was used.

The current study was not able to examine a representative sample of general male population. To overcome this disadvantage, only men with normal semen parameters or with oligozoospermia according to WHO (1999) classification were included in the selection. Although they may differ from men in the general population, there is currently no evidence showing that they would differ in ways that would alter their response to diet. Participants were heterogeneous in their semen profiles and had normal semen parameters. The relative homogeneity of study participants (educated, White) helped reduce the chance that the findings resulted from unmeasured health or behavioral factors. This homogeneity increases the internal validity of the study but limits the generalization of study findings to clinical groups and more diverse populations (Jha, Flather, Lonn, Farkouh, & Yusuf, 1995). A potential limitation of this study is the use of an FFQ to assess habitual dietary intake. Although the FFQ has been reported to have adequate validity and reproducibility for use in epidemiological studies (Willett & Lenart, 1998), it is nonetheless prone to measurement error usually leading to attenuation of the associations of interest. As in all observational studies, misclassification of dietary intake is possible.

Only one semen sample from each study participant was obtained. While several semen samples collected over 1 to 2 weeks may have been preferable, there are limited advantages to using more than one semen sample per participant in the setting of an epidemiologic study (Stokes-Riner et al., 2007). Eating patterns are likely to vary with different socioeconomic statuses, ethnic groups, and cultures. It is necessary to replicate the current study in other populations. In addition, because of changes in food preferences and food availability, the definition of a dietary pattern could change over time. While the homogeneity of the study population increased the internal validity of the study, it limits the generalizability of the finding to clinical groups and more diverse populations.

Higher consumption of a Prudent dietary pattern was associated with higher sperm concentration and higher level of testosterone. Sperm chromatin structure was inversely related to higher consumption of a Prudent dietary pattern. Further research is needed to confirm these findings and extend these results to other populations.

Acknowledgments

This study was performed under the project "The Association Between Environmental Exposure to Widespread Man Made Endocrine Disrupting Chemicals and Level of Hormones

Associated With the Activity of Hypothalamic Pituitary Testicular Axis Among Young Men" supported by National Science Centre in Poland from Grant no. UMO-2014/13/B/NZ7/02223 and the project financed with a grant for statutory activity IMP 10.23/2015.

Authors' Note

The study was performed in the Nofer Institute of Occupational Medicine.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: National Science Centre in Poland from Grant no. UMO-2014/13/B/NZ7/02223 and the project financed with a grant for statutory activity IMP 10.23/2015.

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