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ASSOCIATIONS BETWEEN DIETARY PATTERNS AND PARAMETERS OF OVARIAN RESERVE IN POLISH WOMEN OF REPRODUCTIVE AGE

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Abstract
Objectives: Research investigating the association between reproductive health and diet has predominantly focused on the antenatal and prenatal periods, as well as childbirth in women, and semen quality in men. There is a limited amount of research addressing female fertility assessed as ovarian reserve in relation to diet. The main aim of this study was to evaluate the association between dietary patterns and the parameters of ovarian reserve, such as antral follicle count (AFC), anti-Müllerian hormone (AMH), follicle-stimulating hormone (FSH), and estradiol (E2) – predictors of reproductive health in women of childbearing age. **Material and Methods:** Women aged 24–39 years (N = 511) were enrolled from fertility clinic in central Poland. The count of antral follicles was determined using ultrasonography (USG), FSH and E2 levels were measured using a chemiluminescence method and for determination of AMH level, an enzyme-linked immunosorbent assay method was employed. Diet was assessed according to food frequency questionnaire and dietary patterns were identified by factor analysis. Women were classified into 3 groups according to scores of each dietary pattern: Western, mixed, prudent. **Results:** Higher adherence to the prudent dietary pattern in obese women was associated with significantly higher AFC (p = 0.03) and AMH (p = 0.05) as compared to participants with the Western dietary pattern. The results were adjusted for

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age, BMI, smoking and duration of fertility. Increased consumption of mixed dietary pattern was not statistically significant associated with any of examined ovarian reserve parameters. **Conclusions:** The prudent dietary pattern is positively associated with ovarian reserve in a cohort of women seeking fertility care. Continued research in this area will provide nutritional guidance for clinicians and their patients and provide novel insight on potential modifiable lifestyle factors which can be associated with ovarian reserve. Int J Occup Med Environ Health. 2024;37(4):411–20

Key words:
ovarian reserve, urine, dietary patterns, mixed, prudent, Western

INTRODUCTION

Our diet and nutrition habits are significant not only for well-being but also have influence on human health. Increasing evidence indicates that following specific dietary patterns is associated with risk of cardiovascular diseases, incidence of cancers, developing of diabetes, infertility, neurodegenerative diseases and infertility [1,2]. Previous studies describing association between reproductive health and dietary in women mainly were focused on time to pregnancy [3], preterm birth [4], birth weight [5], pregnancy loss [6] or pregnancy rate [7,8]. Till now only few epidemiological studies aimed at exploring the potential impact of different dietary patterns on ovarian aging and fertility, which now represent an important and expanding field of research. Studies examining the associations between individual nutrients and foods and biomarkers of ovarian reserve yield mixed results [9,10]. Souter et al. [9] found that higher dairy protein intake (≥5.24% of energy) is associated with a decreased antral follicle count (AFC) but the study has some important limitations, such as the lack of anti-Müllerian hormone (AMH) levels to correlate with the AFC findings, and the intake of 1 nutrient may reflect other unknown dietary or lifestyle factors. While Anderson et al. [10] reported that dietary fat consuming may be inversely linked to AMH concentrations. To better understand the role of overall diet in ovarian reserve, many studies have evaluated dietary patterns instead of focusing on single nutrients, since human eating habits involve the consumption of a whole diet rather than isolated nutrients. Insufficient number of studies have described association between dietary pattern and ovarian reserve. Diet has influence on ovarian reserve in women with or without diminished ovarian reserve (DOR) [11]. Moreover profertility dietary pattern (PFD) which includes in diet wholewheat, soy products and seafood, low-residue products and supplementation with folic acid, B_{12} and vitamin D can increases the levels of AMH and AFC [12]. Moreover women who follow a healthy diet aligned with the Mediterranean and Dietary Approaches to Stop Hypertension (DASH) eating patterns exhibit improved ovarian morphology, which indicates enhanced ovarian function [13]. On the other hand Maldonado-Carceles et al. [14,15] have analyzed association between dietary patterns and AFC level in 2 different studies and found that none of following parameters of low-to-moderate intakes of caffeinated, alcoholic, sugar-sweetened, and artificially sweetened beverages, adherence to Mediterranean diet, fertility diet, and profertility diet, associate with ovarian reserve. The results of the mentioned studies are inconclusive, as they were conducted in different examined groups, sometimes without the use of confounding factors. The study represents the first prospective cohort study among a large group of participants, with a proper assessment of various parameters of ovarian reserve. The purpose of the present analysis was to determine whether prudent, Western and mixed dietary patterns are associated with the parameters of ovarian reserve. The prudent diet is characterized by a high intake of vegetables, fruit, legumes, whole grains, fish, and other seafood. The Western diet is characterized by a high intake of processed and convenience foods, refined grains, red and processed meats, high-fat dairy products, and sugary beverages and snacks. It typically includes lower amounts of fruits, vegetables, and whole grains. In comparison, the Mediterranean diet is widely considered one of the healthiest eating patterns due to its balance and emphasis on natural, minimally processed foods. Similar to the prudent diet, it also promotes health but has a different cultural and culinary context. In contrast, the Western diet, with its higher levels of processed foods and unhealthy fats, is associated with poorer health outcomes. According to the authors' best knowledge, this is the first epidemiological study which assesses Western and pudent dietary patterns and 4 parameters of ovarian reserve like AFC, AMH, follicle-stimulating hormone (FSH) and estradiol (E2) including relative high number of participants.

MATERIAL AND METHODS

Characteristics of study population and data gathering

The study population initially comprised 700 women within the reproductive age range of 25–39 years, who were attending a fertility hospital. Finally 511 (73%) participants agreed to take part in the study. Inclusion criteria concerned women with menstrual and ovulatory cycles, devoid of concurrent chronic illnesses such as adrenocortical insufficiency, fragile X syndrome, or abnormal karyotype. Exclusion criteria encompassed women presenting with symptoms or chronic conditions potentially compromising ovarian reserve, including a history of ≥3 spontaneous miscarriages, extensive in vitro fertilization procedures, pelvic chemotherapy or radiotherapy, prior ovarian surgical interventions, premature ovarian failure, polycystic ovary syndrome, ovarian cysts with endometrial involvement, hypogonadotropic, hyperprolactinemia, and hypogonadism. Detailed socio-demographic characteristics, lifestyle behaviors (e.g., smoking, alcohol consumption, physical activity), health status (e.g., coexisting medical conditions), and food consumption frequency were captured through participant-completed questionnaires. Ethical consent was purchased from the Bioethical Committee Board of the Nofer Institute of Occupational Medicine (Ethical Approval No. 10/2018, dated May 30, 2018), guaranteeing compliance with relevant protocols and regulations. Informed consent was acquired from all recruited subjects prior to study commencement.

Assessment of ovarian reserve parameters

In this investigation, parameters relating to ovarian reserve were examined, including the antral follicular count and levels of reproductive hormones such as FSH, AMH and E2. The count of antral follicles was determined using ultrasonography (USG) following the criteria outlined by Broekmans et al. [16], with antral follicles ranging in size 2–10 mm being considered [16]. All assessments were conducted by a trained gynecologist, and the total AFC across both ovaries was utilized for analysis.

Blood samples were collected during the early follicular phase of the menstrual cycle (days 2–4). Serum concentrations of AMH, FSH, and E2 were then measured. After centrifugation, blood samples underwent serum separation and were transferred to polypropylene test tubes. Subsequently, they were stored at a temperature of –80°C until the time of analysis. For determination of AMH level, an enzyme-linked immunosorbent assay method was employed, utilizing Gen-II ELISA kits from Beckman Coulter, Inc. (Brea, CA, USA) following the manufacturer's instructions. The FSH and E2 levels were measured using a chemiluminescence method on the Vitros® ECiQ System (Immunodiagnostic System with MicroWell technology, QuidelOrtho Corporation, San Diego, CA, USA). Commercially available High Wycombe, Vitros Reagent Packs and Vitros Calibrators were used in accordance with the manufacturer's instructions (Ortho-Clinical Diagnostics Johnson & Johnson, High Wycombe, UK).

Dietary pattern assessment

Diet was assessed using a previously validated *Food Frequency Questionnaire* (FFQ) [17]. Participants reported the average frequency of their consumption of specified amounts of each food, beverage, and supplement over the past year. The frequency options included 6 categories ranging from never to ≥6 times per day. Each selected frequency category was converted to a daily intake. For example, a response of "2 to 4 times per week" was converted to 0.43 servings per day. The individual food items were then grouped into 40 predefined categories based on similar nutrient profiles or culinary usage, consistent with groupings used in other studies of Western populations.

Two distinct 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. In contrast, the Western pattern was characterized by high intakes of red and processed meats, butter, high-fat dairy products, refined grains, pizza, snacks, high-energy drinks, mayonnaise, and sweets [18]. Factor scores for each dietary pattern were calculated for each participant by summing the frequency of consumption multiplied by the factor loadings across all food items. This calculation resulted in each participant receiving a score for both the prudent and Western dietary patterns. To account for participants whose diets did not clearly fit into either the prudent or Western patterns, a mixed dietary group was created. Women classified into 3 groups according to dietary pattern were involved for the main analysis.

Statistical analysis

Descriptive statistics were computed for the demographic characteristics of subjects, their diet types, semen quality, and reproductive hormone levels. Bivariate analysis examined relationships among all parameter of ovarian reserve, diet types, and demographic variables to identify differences in distributions or categories and potential confounding factors. Statistical differences were assessed using nonparametric methods when necessary. The following covariates were considered as potential confounders: age, BMI, smoking, duration of infertility. Multiple linear regression analyzed the associations between diet type and ovarian reserve measures. Data analysis was performed using the R 2.15.1 statistical program (R Core Team, 2013).

RESULTS

The study population consisted of 511 women who attended to infertility clinics for diagnostic purposes. General participant background information are depicted in Table 1.

Of the study subjects 42.9% had Western dietary pattern, 35% mixed, and 22.1% prudent diet. There were no differences in the demographic characteristics among women with different dietary patterns. The mean (M) age of volunteers participating in this study was 33.3 years. Most of them had higher education (75.34%, secondary 21.14% and 3.52% for vocational) and most of them were nonsmokers (92.17%); verification was based on the level of cotinine in saliva. Regarding alcohol consumption, the majority of participants reported either no alcohol intake or <1 drink/week (55%). The duration of infertility for most couples exceeded 5 years (35.23%), with couples notable proportion experiencing infertility for 3–5 years (29.55%). The primary diagnosis for infertility was predominantly male factor (37.8%), followed by idiopathic infertility (31.1%), endometriosis (13.7%), tubal factor (10.2%), and ovarian factor (4.7%). Of the surveyed women 5.28% were obese with BMI ≥30 kg/m^2, 30.14% overweight with BMI 25–29 kg/m^2 and 58.9% had normal weight with BMI <25 kg/m^2. Five point sixty eight percent of participants were in underweight range of BMI <18.5 kg/m^2 (Table 1).

Table 2 depicts the ovarian reserve parameters observed within the study cohort. The arithmetic M ± standard deviation (SD) for AFC was 12.73±8.94. Levels of reproductive hormones were M±SD 1.17±1.46 ng/ml for AMH, 6.38±2.18 IU/l for FSH, and 93.74±16.63 pg/ml for E2 (Table 2).

Univariate and multivariate analysis of parameters of ovarian reserve with different dietary patterns are presented in Table 3. There were no differences in AFC, AMH, FSH and E2 between the 3 groups of women with different dietary patterns ($p > 0.05$) (Table 3). Model was adjusted for: age, BMI, smoking, and duration of infertility.

Multivariate analysis of parameters of ovarian reserve with different dietary patterns and 3 ranges of BMI is presented in Table 3. Subjects were stratified by BMI in relation to markers of ovarian reserve [19]. The prudent dietary pattern was associated with increased AFC and AMH compared to the Western dietary pattern in obese women with BMI ≥30 kg/m^2. There were not association between AFC, AMH, FSH and E2 and prudent dietary patterns in case of overweight women (BMI 25–29 kg/m^2) and normal weight women (BMI <25 kg/m^2). The mixed dietary pattern compared to the Western dietary pattern was not associated with any parameters of ovarian reserve in different BMI range groups (Table 3).

DISCUSSION

Among cohort of women of reproductive age from fertility clinic, a positive relationship between higher adherence to the prudent dietary pattern and improved 2 markers of ovarian reserve is observed in obese women (BMI ≥30 kg/m^2). Markers of ovarian reserve were not associated with a mixed dietary pattern for all analyzed ranges of BMI and for women with BMI 25–29 kg/m^2 (overweight) and BMI <25 kg/m^2 (normal) in case of prudent dietary pattern.

In this study prudent dietary pattern is characterized by high intakes of fish, chicken, fruit, cruciferous vegetables, tomatoes, leafy green vegetables, legumes, and whole grains. Concerning the intake of specific components of diet it is well-established that consumption of red meat negatively impact the implantation rate and the probability of preg-

Table 1. Characteristics of the study population – women in reproductive age from central Poland, 2017–2019

Variable	Participants (N = 511) [n (%)]	M±SD
Education		
vocational	18 (3.52)	
secondary	108 (21.14)	
higher	385 (75.34)	
Age		33.30±3.69
24–30 years	121 (23.68)	
31–39 years	390 (76.32)	
BMI		23.18±3.80
<18.5 kg/m^2	29 (5.68)	
18.5–24.9 kg/m^2	301 (58.90)	
25–29.9 kg/m^2	154 (30.14)	
30–40 kg/m^2	27 (5.28)	
Current smoking		
no	471 (92.17)	
yes	40 (7.83)	
Initial infertility diagnosis		
male factor	193 (37.8)	
idiopathic	159 (31.1)	
endometriosis	70 (13.7)	
ovarian factor	24 (4.7)	
tubal factor	52 (10.2)	
missing data	13 (2.5)	
Duration of couple's infertility		
1–2 years	39 (7.63)	
2–3 years	141 (27.59)	
3–5 years	151 (29.55)	
>5 years	180 (35.23)	
Alcohol use		
none or <1 drink/week	281 (55.0)	
1–3 drinks/week	224 (44.0)	
everyday	6 (1)	
Dietary pattern		
prudent	113 (22.1)	
mixed	179 (35.0)	
Western	219 (42.9)	

Table 2. Ovarian reserve parameters among study population – 511 women in reproductive age from central Poland, 2017–2019

Parameter	AM±SD	GM±SD	Min.	Q25	Me	Q75	Q95	Max
Antral follicle count (AFC) [n]	12.73±8.94	12.25±1.73	1	8	11	20	30	40
Anti-Müllerian hormone (AMH) [ng/ml]	1.17±1.46	1.21±1.4	0.02	0.9	1.3	2.9	9.36	18
Follicle-stimulating hormone (FSH) [IU/l]	6.38±2.18	6.00±1.43	0.9	4.86	6.14	7.51	10.48	13.5
Estradiol (E2) [pg/ml]	93.74±16.63	91.33±12.89	75	83	95	120	180	200

AM – arithmetic mean; GM – geometric mean; Q25 – 25th percentile; Q75 – 75th percentile; Q95 – 95th percentile.

Table 3. Univariate and multivariate analysis of parameters of ovarian reserve with different dietary patterns and by body mass index (BMI) – 511 women in reproductive age from central Poland, 2017–2019

Parameter	Dietary pattern					
	mixed[a]			prudent[a]		
	estimate	95% CI	p	estimate	95% CI	p
Ovarian reserve						
AFC						
univariate	0.11	–0.11–0.32	0.37	0.20	–0.03–0.45	0.095
multivariate	0.06	–0.18–0.34	0.43	0.04	–0.10–0.18	0.46
AMH						
univariate	0.07	–0.11–0.42	0.61	–0.03	–0.18–0.14	0.70
multivariate	–0.06	–0.42–0.17	0.68	–0.15	–0.31–0.04	0.16
FSH						
univariate	–0.04	–0.32–0.24	0.65	–0.04	–0.188–0.118	0.78
multivariate	–0.04	–0.20–0.31	0.77	0.03	–0.12–0.15	0.75
E2						
univariate	–0.05	–0.18–0.15	0.54	–0.07	–0.23–0.07	0.29
multivariate	0.13	–0.12–0.62	0.24	0.04	–0.14–0.17	0.83
BMI[b]						
<25 kg/m^2						
AFC	0.29	–0.06–0.44	0.06	–0.12	–0.39–0.05	0.19
AMH	–0.40	–0.70–0.09	0.22	–0.08	–0.25–0.28	0.92
FSH	–0.13	–0.31–0.17	0.34	–0.60	–0.91–0.17	0.74
E2	–0.04	–0.50–0.11	0.41	–0.12	–0.32–0.02	0.16
25–29 kg/m^2						
AFC	0.05	–0.15–0.26	0.65	0.05	–0.14–0.25	0.64
AMH	–0.16	–0.37–0.06	0.29	–0.09	–0.26–0.14	0.64
FSH	–0.05	–0.29–0.70	0.64	–0.02	–0.25–0.12	0.99
E2	0.08	–0.18–0.24	0.50	0.09	–0.28–0.26	0.74

Table 3. Univariate and multivariate analysis of parameters of ovarian reserve with different dietary patterns and by body mass index (BMI) – 511 women in reproductive age from central Poland, 2017–2019 – cont.

Parameter	Dietary pattern					
	mixed[a]			prudent[a]		
	estimate	95% CI	p	estimate	95% CI	p
BMI[b] – cont.						
≥30 kg/m^2						
AFC	–0.05	–0.13–0.18	0.28	0.04	0.02–0.67	**0.03**
AMH	0.03	–0.32–0.55	0.43	0.05	0.03–0.99	**0.05**
FSH	0.07	–0.22–0.26	0.81	–0.12	–0.34–0.14	0.25
E2	0.08	–0.21–0.38	0.71	0.08	–0.18–0.26	0.88

AFC – antral follicle count; AMH – anti-Müllerian hormone; E2 – estradiol; FSH – follicle-stimulating hormone.
Model adjusted for: age, BMI, smoking, duration of infertility.
Bolded are statistically significant value.
[a] Reference group: Western dietary pattern.
[b] Multivariate analysis.

nancy [20] farther substituting chicken and red meats with vegetable protein sources could potentially decrease the risk of infertility resulting from anovulation [21]. Soy and soy products can potentially affect reproductive health and fertility [22] but fruit and vegetables in some studies are high pesticide residue products which are associated with lower probability of clinical pregnancy and lower probability of live birth [23]. To the authors' best knowledge, only few studies assess the impact of dietary patterns on ovarian reserve. Their results are mostly in line with the findings. Eskew et al. [12] reported that higher serum AMH concentration and increased AFC are associated with consuming whole grains, soy and seafood, low pesticide residue products and supplementation with folic acid, B_{12} and vitamin D (PFD) in women with BMI ≥25 kg/m^2. There are growing evidence over the past decades which has shown that vitamin D has regulatory function during production AMH [24,25] and can regulates ovarian reserve. Moreover women who follow a healthy diet aligned with the Mediterranean (9 types of products: fruits, vegetables [excluding potato], nuts, legumes, whole grains, fish, ratio of monounsaturated to saturated fat, red and processed meats and alcohol) and DASH (8 food items: fruits, vegetables, nuts and legumes, low-fat dairy, whole grains, sodium, sugar-sweetened beverages, and red and processed meats) eating patterns may exhibit improved ovarian morphology, which likely indicates enhanced ovarian function [13].

There are important differences between food composition in Mediterranean and DASH diet pattern, but both can improve obesity and metabolic control in female with ovarian dysfunction, including polycystic ovary syndrome (PCOS) [26,27]. The positive association between Diet Quality Index – International (DQI-I) and AFC as well as AMH was also described at group of 120 women with DOR and 250 women with normal ovarian reserve as controls. The authors concluded that following the DQI-I characterized by 4 main categories of the index: dietary variety, adequacy, moderation, overall balance and quality index, is based on food and nutrients, more closely may reduce the risk of DOR and enhance the ovarian reserve in women already diagnosed with this condition [11]. In turn, 2 other studies conducted by Maldonado-Carceles [14,15] did not find relation between ovarian reserve and various dietary patterns. The Mediterranean diet, the fertility diet (developed according to dietary risk factors related to ovulatory infertility), the profertility diet and self-reported caffeinated (coffee, tea, soda), alcoholic (wine, beer, liquor), sugar sweetened, and artificially sweetened beverages intakes were not statistically significant associated with the level of AFC. The lack of associations observed in these studies could be explained by the relatively short exposure window assessed. Moreover, utilization of more readily available markers of reserve, such as serum AMH measurements, might be more practical in such studies.

The strengths of the study include analyzing novel parameters of ovarian reserve which were not described so far (FSH and E2) in reproductive age, healthy women without history of chronic diseases. Moreover, the sample size is large which may be crucial since the size of the effect to obtain significant differences may depend on the size of the study group. Relatively high number of participants takes into account much greater inter-individual variability and minimize the effect of large dispersion of results. The next strength is the ability to account for multiple potential confounders, which had not been the case in previous studies. Furthermore, the authors employed principal components analysis to uncover dietary patterns within study group and scrutinized predefined dietary profiles. This enabled authors of the study to explore whether dietary patterns not previously recognized could elucidate variations in markers of ovarian reserve. Utilizing dietary patterns, rather than solely focusing on dietary intake or serum levels of specific nutrients, provides enhanced means for advising women regarding lifestyle factors that can be modified to promote reproductive health. Another limitation is that the group of participants consisted of reproductive-age women from central Poland, which may limit the relevance of the results to women in other regions. As ovarian reserve decreases during a woman's reproductive years, it is difficult to determine the most relevant biologic window for assessing dietary patterns and ovarian reserve.

CONCLUSIONS

In this study, increased adherence to the prudent dietary pattern was associated with higher AFC and AMH in women with BMI ≥30 kg/m^2 recruited from fertility clinic. Higher intakes of fish, poultry, fruit, cabbage vegetables, tomatoes, leafy green vegetables, legumes, and whole-wheat can improve ovarian reserve. This finding needs to be verified by further cohort research and extend these results to other populations.

Author contributions

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