

Comparison of the effect high protein diet on indicators related to ovarian aging in subjects at risk of premature ovarian failure

Abstract

Introduction: we investigated the effects of high protein diet on the process of ovarian aging in subjects at risk of primary ovarian insufficiency.

Materials and Methods: The present study was conducted on 107 patients with early premature menopause. The patients were randomly assigned to three groups, including high-based animal protein diet group (Group A), high- based plant protein diet group (Group B) and normal protein diet(a mixture of animal and plant proteins diet). Blood sampling and ultrasound tests were performed before and after six month.

Results: In group A, decreasing in FSH and increasing in AMH and inhibin A were observed ($P < 0.05$). In the group B progesterone, AMH, and Inhibin A levels increased significantly, and FSH levels decreased significantly ($P < 0.05$). In the group C the levels of estradiol and AMH increased significantly, and the levels of CRP decreased significantly ($P < 0.05$). The statistical test results show that AMH increase in all tree group and FSH and estress decreased in all three groups. The intervention (six months) showed a significant difference among the three groups in terms of the presence of follicles. The group A showed a significant improvement in terms of the presence of follicles ($P = 0.03$).

Conclusion: Our findings reported a beneficial effect of a high-protein diet in improving the indicators affecting menopause in patients with premature menopause, also a remarkable improvement in hormones affecting ovarian function, however, high-base animal protein group showed better results than the other groups in terms of improving the number of follicles.

Keywords: *Diet, Ovary, Menopause, Ovarian Insufficiency, High-Protein Diet*

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Introduction

Primary ovarian insufficiency affects about 2% of women. Some women develop primary ovarian insufficiency in adolescence. These women will never have normal ovarian function [1]. In Iran, results have indicated that in genetic cases, many women are not aware of ovarian insufficiency, and this issue will cause psychological harm in their future [2, 3]. Women with primary ovarian insufficiency are prone to depression [4]. Only 10% of women with primary ovarian insufficiency become pregnant using their eggs with or without assisted reproductive techniques [5]. Menopause in healthy women occurs between the ages of 47 and 55, but premature menopause occurs at less than 40 years and affects about 4% of women [6]. Interruption or irregularity in menstrual cycles (shortening the interval between periods or increasing the interval between them) is a symptom of premature menopause. In the study conducted by Baghaei et al. in Azerbaijan, the rate of premature menopause was reported at 7.6% [7].

Premature menopause is associated with several factors, including race, genetic characteristics, socioeconomic status, fertility status, physical activity, sexual behaviors, and diseases [8]. Studies have investigated the effects of diet intervention, and supplement consumption like vitamins on premature menopause [7, 9, 10]. A study conducted in New Guinea

reported that women with malnutrition experience menopause 4 years earlier [7]. Other studies have reported that anti-Müllerian hormone levels are affected by diet [11, 12]. Another study conducted in Poland found that anti-Müllerian hormone levels in Polish women change about 2 to 3 years earlier than in women in industrialized countries due to nutritional status [12, 13]. The beneficial effects of consuming a DASH-containing diet on the Anti-Müllerian hormone have been reported [13]. Evaluation of levels of many reproductive hormones, including estradiol, FSH, anti-Müllerian (AMH), and inhibin, can help identify menopause [14]. Protein food sources affect menopause, infertility, ovarian aging rate, and quality.

In the field of fertility, there are conflicting conclusions about the priority of using an animal or plant diet. Studies showed that the consumption of plant proteins reduced the risk of premature menopause, while animal proteins did not show this effect [15]. Another study showed that the consumption of plant proteins such as soybean is associated with premature menopause [6, 7], but the positive effect of a protein diet and DASH in preventing inflammation has been proven [16]. Limited studies have examined the role of diet, especially protein diet, in premature menopause and no definitive and complete treatment has been identified for premature

menopause, also providing preventive and remedial strategies and gaining a better understanding of this disease is very effective. Therefore, the present study aimed to investigate and compare the effect of three protein-modified diets including a high-base animal protein diet, a high-base plant protein diet, and common proteins diet on indicators related to ovarian aging (premature menopause) in subjects at risk of premature ovarian failure.

Materials and Methods

Study design and participants

This double-blind randomized clinical trial was conducted on patients with premature menopause referred to medical clinics in 5 provinces of Tehran, Mazandaran, Gilan, Alborz, and Esfahan from July 2021 to January 2022. Eligible patients who met the inclusion criteria were randomly assigned to one of the three groups of high-base animal protein (A), high-base plant protein (b), and normal protein (mixture of animal protein and plant protein (C)). Written consent was obtained from patients before the study. This study was registered in RICT with the code IRCT20201124049481N1

Protocol

Menopausal women, aged < 35 years, who were willing to participate in the study were included. Exclusion criteria were hyperandrogenism, subjects with side effects of drugs such as glucocorticoids, anticonvulsants, liver or kidney, and spinal diseases, subjects with cancer, viral infections, autoimmune diseases because of the course of treatment and its effect on the menstrual cycle. The sample size was determined by using the 95% confidence level and 80% power and considering the insulin as the main outcome, taking into account the loss of 30 people in each group (13). A total of 107 eligible patients were assigned into three groups according to a random list generated using the relevant software using a randomized block method. Patients were randomly selected from a box with twenty sealed envelopes. One envelope containing codes A, B, and C (Method one: A, method two: B, method three: C) was selected for each patient. Except for the first researcher, the second researcher, and the statistician had access only to the contract codes of A and B, etc. They did not know any information about each group's treatment.

Groups and diets

Three groups of patients, including Group A (15% animal protein + 10 plant proteins), Group B (10% animal protein + 15% plant proteins), and Group C (15% protein: a mixture of animal and plant proteins (normal protein)) were examined for six months. In group A, 15% of the total protein was received from the animal proteins diet such as milk, yogurt, meat, chicken, fish, turkey, eggs and cheese, and meat of birds (partridge, quail), and 10% of the total protein was received from legumes, bread, and cereals. In group B, 15% of the total

protein was received from the plant proteins diet, such as legumes, bread, and cereals, and 10% of the total protein was received from the animal proteins diet such as milk, meat, chicken, fish, turkey, eggs and cheese and meat of all kinds of birds. In group C, 15% of total energy was received from a mix of animal and plant proteins such as milk, yogurt, cheese, meat, poultry, fish, eggs, turkey, and meat of a variety of birds, legumes, and bread and cereals.

Compliance

To assess whether individuals comply with the study diet, at the beginning, the third month, and the end of the study, a diet will be performed for three days a week during the intervention. In addition, they will be fed weekly counseling to ensure their diet. Since physical activity is a disruptive factor in our study, the IPAQ questionnaire will be completed by individuals at the beginning, the third month, and the end of the study. The stress and economic status questionnaire will be completed by individuals at the end of the intervention.

Also, the weight of these women in light clothes and without shoes was measured by a digital scale (710 Germany) with a measurement error of 100 grams, and their height without shoes and by a height gauge with an accuracy of ± 5 mm. BMI was calculated according to the formula.

Measurements

Five cc of blood was collected in standard tubes containing EDTA + NaF to analyze hormones and inflammatory markers levels. Blood samples were collected before and after the 6-month intervention in anticoagulant tubes and centrifuged at $1000 \times g$ for 15 minutes. Serum CRP was measured using an immunological turbidity instrument (Kit ... Finland). Hormone levels were measured using an immunosorbent assay kit connected to the human enzyme (CinnaGen Kit, Iran). In the case of menstruation, blood samples were taken in the days after menstruation.

Statistical Analysis

SPSS-22 statistical software was used to perform all analyses. Analysis of variance was used to compare quantitative variables with normal distribution between the two groups. The Chi-square test was used to evaluate qualitative variables. Spearman test was used for abnormal distribution. Multiple comparisons are also performed using the Tukey or Dante test. Regression models will also be fitted to investigate the relationship between variables and modulate the effect of confounding variables. The values will be shown as (Mean \pm SD). P values are calculated bilaterally and $P < 0.05$ is considered statistically significant. In order to investigate the raw and corrected relationship between the dependent variable and other variables, linear or logistic regression models appropriate to the response variable are used.

Results

Out of 107 female participants, 19 were excluded due to unwillingness to continue participating in the study, and three were excluded due to disease. One was excluded due to

underlying heart disease and two were excluded due to stomach problems (Fig 1).

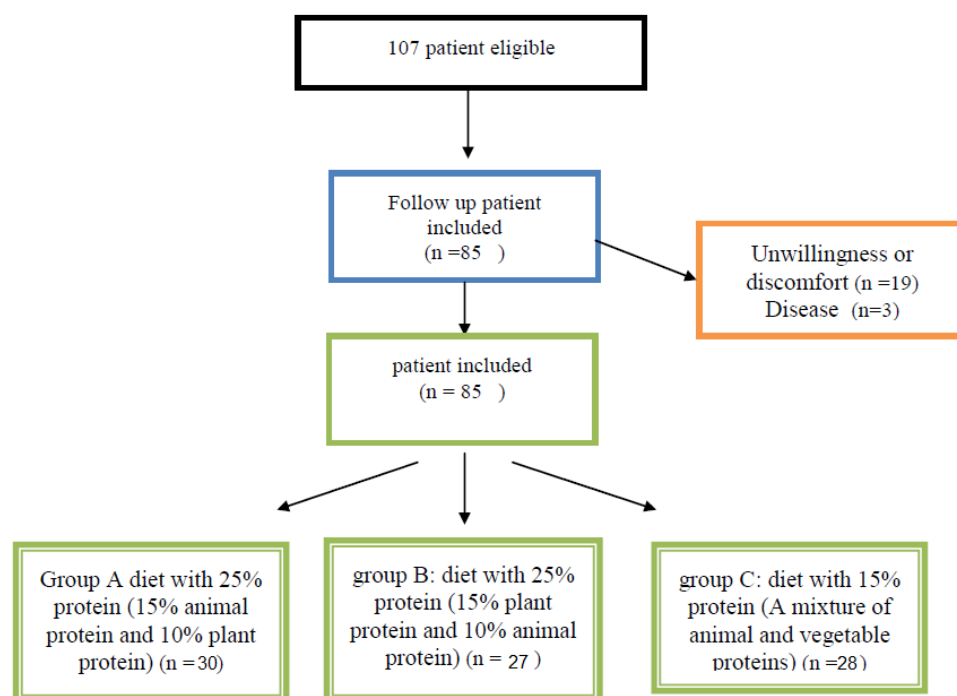


Fig1. Patient selection flow chart

A total of 85 patients with a mean age of 31.9 ±6.4 years participated in the study. Also, 53.8% had a diploma level education and 93.4% were married (Table 1).

Table 1: distribution of sociodemographic characteristics in groups.

Parameters		A (n=30)	B (n=27)	C (n=28)	p-value
Age (year)	(Mean ± SD)	31.9 ± 6. 21	29.86 ±5.32	30.86 ±6.03	0.12
Education	Less Than High School	6 (19.7)	5 (18.3)	3 (11.4)	0.23
	High School or GED	13(42.4)	14 (52.8)	14(50.6)	
	Some College	7(22.7)	4 (14.7)	4(14.7)	
	Less than Bachelor’s Degree	5 (15.2)	3 (9.8)	6 (19.3)	
Marital status	Married	28 (93.4)	26 (93.5)	27 (96.5)	0.14
	Single	2 (6.6)	2(6.5)	1 (3.5)	
Smoking		3 (8.5)	2 (6.2)	1 (3.5)	0.09

According to Table 2, the results showed that stress in all three groups was significantly reduced after the intervention. The mean body mass index was reduced in groups B and C (P =

0.03), but after 6 months of intervention, no significant change was seen in physical score variables (P> 0.05).

Table 2- Comparison of three groups in terms of levels of physical indicators ‘stress and BMI

Group Variables	A		B		C	
	(Mean ± SD)	sig	(Mean ± SD)	sig	(Mean ± SD)	Sig

stress	Before	1.847	*	1.92	*	1.67	*
	after	1.470	0.007	1.60	0.007	1.40	0.04
Physical score	Before	1.580	0.76	1.60	0.12	1.59	0.534
	after	1.600		1.75		1.63	
Body mass index (BMI)	Before	28.320	0.44	27.56	*	27.32	*
	after	28.173		26.14	0.01	26.02	0.004

Group A diet with 25% protein (15% animal protein and 10% plant protein), group B: diet with 25% protein (15% plant protein and 10% animal protein), group C: diet with 15% protein (A mixture of animal and vegetable proteins). A significant level less than one hundredth ** Significant level less than five-hundredths, *

According to Table 3, the levels of FSH, AMH, and Inhibin-A variables in the animal protein group changed significantly after eight months. Furthermore, the results showed that the test was significant for FSH, AMH, and Inhibin-A variables (P <0.05). In other words, dietary intervention (15% animal protein + 10% plant protein) reduced FSH and stress and

increased AMH and inhibin-A, and no significant effect was observed in other variables. In the second group (15% plant protein and 10% animal protein), the levels of progesterone, AMH, and Inhibin-A increased significantly, and FSH level was reduced significantly (P <0.05). In Group C (mixture of plant and animal proteins), the level of estradiol, AMH, increased significantly, and the CRP level was reduced significantly (P <0.05).

The statistical test results show that progesterone, estradiol, estrogen, AMH, Inhibin-A, and Inhibin-B were associated with an increase, and FSH, LH, and CRP decreased in all three groups (Table 3).

Table 3- Comparison of three groups in terms of hormone levels in pre intervention and post intervention

Group Variables	A (Mean ± SD)			B (Mean ± SD)		C (Mean ± SD)	
	sig			sig		Sig	
Progesterone	Before	9.673 ±1.19	0.12	7.48±1.19	0.006	8.57±1.22	0.50
	after	10.390 ±1.43		9.24±1.19		8.22±1.11	
Estradiol	Before	121.93±11.7	0.14	86.29±17.82	0.20	30.96±14.33	* 0.003
	after	130.00±11.7		84.44±17.71		46.21±12.14	
Estrogen	Before	102.23±19.52	0.78	77.14±20.32	0.20	74.14±10.16	
	after	101.33±14.81		83.55±17.41		79.35±16.15	0.05
FSH	Before	86.15±16.15	0.04 *	71.07±17.42	0.03 *	51.70±21.14	0.09
	after	82.08±16.79		64.09±19.18		47.84±17.93	
LH	Before	15.03±3.41	0.24	18.90±6.31	0.06	14.48±4.65	0.08
	after	14.27±3.72		16.90±5.17		14.08±4.83	
AMH	Before	2.41±0.98	* 0.007	2.75±1.12	0.01*	2.49±0.81	* 0.03
	after	2.76±0.74		2.91±0.62		3.17±0.79	
CRP	Before	5.04±1.98	0.05	3.21±1.46	0.18	8.02±3.05	
	after	4.46±1.19		2.91±0.82		7.00±2.69	0.018
Inhibin-A	Before	47.71±14.12	* 0.001	15.16±6.44	* 0.001	39.97±12.11	0.57
	after	102.23±23.29		86.29±18.91		30.96±15.87	
Inhibin-B	Before	71.88±29.17	0.63	84.87±28.62	0.47	101.48±34.64	0.84
	after	73.41±21.43		90.05±31.75		101.92±26.56	

Group A diet with 25% protein (15% animal protein and 10% plant protein), group B: diet with 25% protein (15% plant protein and 10% animal protein), group C: diet with 15%

protein (A mixture of animal and vegetable proteins). A significant level less than one hundredth ** Significant level less than five hundredth, * AMH; anti-mullerian hormone,

CRP; C-reactive protein; FSH; follicle-stimulating hormone, LH; luteinizing hormone

A comparison of the three groups in terms of frequencies of follicles before and after the intervention showed a significant difference among the three groups in terms of the presence of
 Table4: Number of follicles in the pre-test and post-test groups

follicles. The group of high-base animal protein (B group) showed a significant improvement in terms of the presence of follicles ($P = 0.03$). However, the number of follicles in the pre-test and post-test groups was not significantly different among the three groups ($P = 0.07$). Table 4

groups		Absence of follicle	presence of follicle	of total	Sig
A	n	22	8	30	0.03
	experimental group	73.3%	26.7%	100.0%	
	follicle after intervention	30.6%	61.5%	35.3%	
B	n	24	3	27	
	experimental group	88.9%	11.1%	100.0%	
	follicle after intervention	33.3%	23.1%	31.8%	
C	n	26	2	28	
	experimental group	92.9%	7.1%	100.0%	
	presence of follicle after intervention	36.1%	15.4%	32.9%	
Sum	n	72	13	85	0.07
	Percentage in the experimental group	84.7%	15.3%	100.0%	
	presence of follicle after intervention	100.0%	100.0%	100.0%	

Discussion

The present randomized clinical trial investigated the role of animal high-protein, plant high-protein, and a normal protein in individuals at risk of primary ovarian insufficiency for the first time in Iran. The results suggest that a diet containing an appropriate level of protein can affect and improve hormones affecting fertility. The levels of progesterone, estradiol, estrogen, AMH, InhibinA, and InhibinB increased in all three groups after six months weeks intervention. FSH, LH, and CRP were reduced in all three groups, but the increase in InhibinA level was significant only in the plant high-protein and animal high-protein diets. Also, the CRP level in Group C (a mixture of 15% plant protein and animal protein) showed a significant reduction.

Froozanfar et al. conducted studies to evaluate the effect of diet methods to stop hypertension (DASH diet) on weight loss, anti-Müllerian hormone (AMH), and metabolic profiles in women with polycystic ovary syndrome (PCOS). The results showed that consumption of a DASH-containing diet had a beneficial effect on Anti-Müllerian hormone and insulin metabolism [13]. Levels of the Anti-Müllerian hormone, which plays a major role in fertility. Nybaka et al. showed that dietary management is effective in improving anti-Müllerian hormone levels in obese women with polycystic ovary syndrome [17]. in another study, no significant relationship

was observed between low-fat and high-carbohydrate diets and the age of premature menopause in women (15).

Our results revealed that the CRP levels in the groups receiving the diet were significantly reduced after the intervention. This reduction was especially significant in the group that received a mixture of animal and plant proteins. In this regard, Smidowicz et al. reported that diet patterns rich in fruits and vegetables had a beneficial effect on endothelial function, as estimated by CRP concentrations [16]. Other researchers have reported that diet, as one of the most important environmental factors, is associated with the concentration of inflammatory markers [18]. The animal protein group (15% animal protein and 10% plant protein) in terms of the number of follicles showed the greatest effect compared to other groups. So that 26% of women in this group had follicles after the intervention. In a similar study conducted by Boutot et al., it was found that the animal protein diet did not affect premature menopause, but a plant high-protein diet was associated with premature menopause among American nursing women [15]. In contrast, Souter et al. reported that receiving a protein diet reduced the number of follicles. In addition, there was no difference between protein intake levels in animal or plant diets [19].

Findings show that animal protein intake is associated with a direct effect of hormones and metabolism with a reduced risk of metabolic syndrome and abdominal obesity (20). In the

present study, milk and dairy foods were used as the main animal protein diet. Another study has shown that dairy foods, carbohydrates, fats, proteins, and calcium from dairy sources, lactose, and galactose are inversely related to the rate of reduction of AMH and the risk of its rapid decline (21).

The present study showed that diets (animal high-protein, plant high-protein, and a normal protein) did not differ from each other in terms of therapeutic function in many cases, but all diets had a positive effect on the levels of hormones affecting fertility. In other words, the effect of diet used in the present study was likely effective in reducing the weight of the studied women. One of the limitations was that many menopausal patients by affecting the weight index of the population. Also, it played a role in reducing obesity-related inflammation in women, so CRP was reduced in all groups. Receiving a healthy diet in the studied women was the main reason for this result. In our study, many women stated that they avoided eating large amounts of food, and this issue may have affected women's physiological function. Previous studies have proven the role of a healthy diet and weight loss in reducing inflammation (22, 23).

had chronic diseases such as uterine cancer or were over 40 years old, making it difficult to identify and select the target population. To solve this problem, a list of patients was prepared, and then a selection was made among them, given the exclusion criteria. Due to a shortage of samples during the present study, a list of 521 patients with premature menopause was prepared from 5 different cities in Iran. The list of patients was prepared from Milad Tehran, Ghaem Karaj, Imam Reza Chalus, and several obstetric and ultrasound centers. Another limitation of the study was the high cost of the project, so it was prepared step-by-step with the necessary coordination. Among the strengths of the present study was accurate randomization.

Conclusion

The effective role of a high-protein diet in improving the indicators affecting premature menopause was identified. The animal high-protein group showed stronger results in improving the number of follicles than the other groups. The plant high-protein group also showed significant results in improving hormone levels affecting fertility. Paying attention to a diet containing animal and plant proteins is one of the main points in the diet of women with premature menopause. Finally, it is recommended to use a high-protein diet as a solution to reduce premature menopause in medical centers.

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Conflict of interest

The authors declare that there are no conflicts of interest.

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Ethical statements

All the information studied in this project is kept confidential and entering the study is accepted by fully reading and obtaining satisfaction from them. Consumables were procured from research credits of the Institute of Nutrition of Shahid Beheshti University of Medical Sciences, and no additional costs were imposed on the patients. The study was sent to the ethics committee of the university to receive the code of ethics and approval, and after receiving the code of ethics, it was registered in the Iranian clinical trial system with the code IRCT20201124049481N1 (irct.ir).

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