Original Article

Association of Dietary Protein on Level of Anti Mullerian Hormone (AMH) and Antral Follicle Count (AFC) in Patients of Infertility

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Background : "Infertility is defined as the inability to conceive within a year with normal frequency of sexual intercourse and no contraceptives." Relatively little is known about the effect of nutritional content on fertility.

Objective : To study the correlation of level of AMH and AFC with dietary habits especially protein intake in patients of infertility.

Material and Methods : It is hospital based study.

Design of study : Cross sectional study.

Place of study : Teerthankar Mahaveer Medical College & Research Centre, Moradabad, India .

Number of Patients : 95 patients of infertility included in the study .Patients were between 30 and 45 years.

Time Period : 18 months from January 2020-July 2021.

Method : All cases underwent full history taking; clinical examination and all completed a questionnaire consisting of demographic characteristics, FFQ (Food Frequency Questionnaire).

Main Outcome Measures : Moderate to high protein intake in diet corresponds to those having met their more than 20% calorie intake by protein had a higher mean AMH as well as had a higher mean AFC.

Result : Based on the results of the current study the effects of higher protein intake was found to be significant on the level of ovarian reserve .

Conclusion : This study suggests that good and healthy Nutrition, rich in proteins , in fertility treatment is required for better outcome and also helps in limiting the financial burden.

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Key words **: Antral Follicle Count, Anti Mullerian Hormone, In Vitro Fertilization, Body Mass Index**

On a global basis, roughly 15% of eligible couples experience infertility, which translates to about 60-80 million couples[2]. Infertility rates may differ by area[3]. According to the World Health Organization (WHO), prevalence of primary infertility in India ranges between 3.9 percent to16.8 percent. Lifestyle, diet, sexual health, substance abuse and psychosocial factors all have an impact not only on the outcome of fertility treatment but also on pregnancy health, which is essential in pre-conception care[4-6]. While it is well accepted that diet and modifiable lifestyle variables affect female[7-11] as well as male fertility[12-14], knowledge in this area is sparse, which make planning of pregnancy limited with few evidence-based resources to aid with pre-conceptional nutrition and lifestyle advice. Relatively little is known about the effect of nutritional content on fertility .

The Antral Follicle Count (AFC) and serum Anti Mullerian Hormone (AMH) are the most relevant indicators of ovarian reserve[16]. AMH and AFC have shown to be more accurate predictors of the number of ovarian primordial follicles

Editor's Comment :

- Females with poor health due to unhealthy and unbalanced diet have poor fertility as is seen in many infertile ladies. A good and healthy Nutrition, rich in proteins is required for better fertility and also helps in limiting the financial burden.

Although it is widely assumed that lifestyle variables have a detrimental influence on ovarian reserve and AMH levels[17-20], not all research agree[19] and the majority of studies do not examine the response of other contributing factors.

The purpose of this study is to examine the probable influence of dietary factors (proteins) in determining the level of both the mentioned markers of ovarian reserve in infertile patients.

As limited number of studies are available in India of regarding impact of dietary and lifestyle factors in patients of infertility. Therefore, this study would aim at studying the correlation of level of AMH and AMC with dietary habits.

Aims :

To Study the association of dietary proteins on level

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of Anti Mullerian Hormone (AMH) and Antral Follicle Count (AFC) in patients of infertility.

Review of Litrature :

(Rich-Edwards JW, *et al*)[28] Unbalanced meals high in carbs, fat, proteins constituents and other micronutrients negatively influence ovulation (Chavarro JE, *et al*)[29]. in their study "Diet and lifestyle in the prevention of ovulatory disorder infertility" concluded that in an otherwise healthy women, following a "fertility diet" plan may help them conceive.

(Chavarro JE, *et al*)[29], researchers at Harvard Medical School, found that carbohydrates influences ovulation and fertility in healthy women because it affects the metabolism of glucose and demand of insulin and its sensitivity (PCOD).

(Hohos NM, *et al*)[32] stated that diet with high fat has ill effect on female fertility, having possible impacts ovulation. Similar findings were seen in a research done by (KaboodMehri R, *et al*)[33] studied the correlation between Anti Müllerian Hormone (AMH) levels and food consumption in Iranian females. Additionally, some have discovered diet rich in fat exacerbates atresia of follicles[34,35].

Materials and Methods

Study Design : A cross sectional study

Study Setting : The Department of Obstetrics and Gynaecology, Teerthanker Mahaveer Medical College & Research Centre.

Study Population : Women attending the Department of Obstetrics and Gynaecology with Diagnosis of Primary or Secondary Infertility.

Study Duration : 18 Months

Sample Size : 95

To calculate sample size based on the prevalence we can use the following formula :

Sample Size = $(Z^2 PQ)/ E^2$

Z = Standard Normal Variate

P = Prevalence of infertility

E = absolute error (5-10%) acceptable

Q= (100-P)

Z =2.58 at 1% type 1 error

Here

Z^2 = 2.58 at 1% type 1 error

P=3.7%

E=5%

n = [(2.58)2 X 3.7X (100-3.7)]/ (5^2) =94.86 =95

Inclusion Criteria :

- Women attending the Department of Obstetrics and Gynaecology with diagnosis of primary or secondary infertility.
- Women between ages of 30-45 years, married for at least 1 year and sexually active.
- Patients with both ovaries intact
- Patients ready to give consent to participate in the study

Exclusion Criteria :

- Patients <30 years and >45 years
- Patients with immune infertility
- Patients with male factors of infertility
- Patients with sexually transmitted infection
- Traumatic or congenital malformation in the genital systems.
- Endometrial cause of infertility
- Patients of primary amenorrhea

23040 number of patients attended the Gynaecology OPD during the duration of 18 months, 691 number of patients were patients of primary or secondary infertility anxious to concieve out of which 389 belonged to the age group of 30-45 years. After applying inclusion and exclusion criteria, we enrolled 95 patients in our study. All the participants were assesed on the basis of the prevalidated questionnaire to assess their protein intake .

Calculation of Antral Follicle Counts Using Ultrasound Techniques :

Each participant in the study underwent a standard infertility evaluation, that included determination of the AFC using the Siemens ACCUSON S2000 ultrasound machine on third day of an unstimulated menstruation or on third day of bleeding after progesterone withdrawal. One examiner performed all transvaginal ultrasounds. AFC was done on 1st visit of each patient in OPD, before starting any treatment so as to avoid any bias.

AMH Assessment :

A sample for AMH obtained for all subjects under aseptic precaution on the 1st visit of patient to OPD. The sample sent to Biochemistry Department.

AMH values differentiated according the normal value depending upon age group[41].

Value of Normal Range of AMH According to Age Groups

Age	ng/ml	pmol/L
Under 33 years old	2.1-6.8	15.0-48
33-37 years old	1.7-3.5	12.14-32.13
38-40 years old	1.1-3.0	7.8-21.42
41 + years old	0.5-2.5	3.57-17.85

Statistical analysis : Data so collected was tabulated in an excel sheet, under the guidance of statistician. The means and standard deviations of the measurements per group were used for statistical

analysis (SPSS 22.00 for windows; SPSS inc, Chicago, USA). For each assessment point, data were statistically analyzed using one way ANOVA. Difference between two groups was determined using student t-test and the level of significance was set at $p \leq 0.05$.

Observation and Results

Table 1 — *Co-relation of Age to AMH*

Age	Group					
	Frequency	Percent	Mean	STD Deviation	F value	p value
Under 33	63	66.3	4.66	1.11	4.93	0.021*
37-40	2	2.1	1.53	1.40		
33-37	21	22.1	4.25	1.18		
>41	9	9.5	1.43	1.41		

Table 2 — *Co-relation of AFC to Age*

Age	Group					
	Frequency	Percent	Mean	STD Deviation	F value	p value
Under 33	63	66.3	4.44	1.75	3.08	0.042*
37-40	2	2.1	2.50	0.71		
33-37	21	22.1	4.33	1.76		
>40	9	9.5	4.78	1.59		

The subject were classified according to their age corrected AMH groups.We found that a total of 31 subjects that amounts to about 32.6% had low AMH values while 24.3% had high AMH values while the rest 41% which was the majority belonged to the nomal range of AMH (Table 3).

We found that a total of 27 subjects that amounts to about 28.4% had low AFC values while 13% had high AFC values ie >10 while the rest 55% which was the majority belonged to the normal range of AFC ie 3-8 in each ovary (Table 4).

The majority of the participants 57.9% preffered mixed diet while 42.1 % prefered vegetarian diet. It was found that the mean AMH was higher in the patients

Table 3 — Level of AMH

AMH	Frequency (n)	Percent (%)
Low	31	32.6
Normal	41	43.2
High	23	24.2

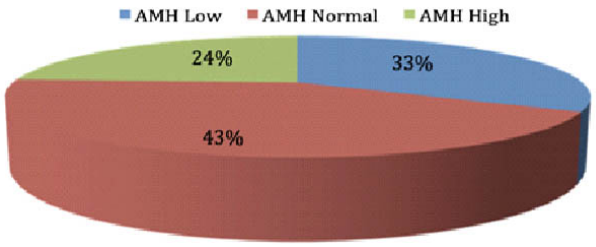
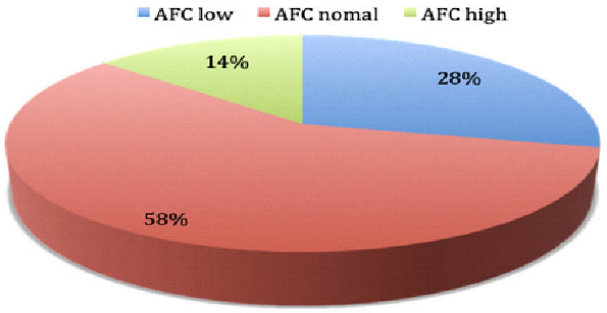


Table 4 — Level of AFC

AMH	Frequency (n)	Percent (%)
Low	27	28.4
Normal	55	57.9
High	13	13.7



that were vegetarian as compared to the ones who prefered mixed diet but the p value was 0.653 so the relationship was not significant (Table 5).

Majority of the patients 69.5% had moderate protein intake that is about 20-35% of their calorie intake was from the protein. The relationship of moderate to high protein intake in their diet that corresponds to those having met their more than 20% calorie intake by protein had a significant relation with the level of AMH and had a higher mean AMH (Table 6).

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Discussion

Our study found a strong association between a high ovarian reserve and AMH and AFC in patients on a moderate protein diet, defined as individuals consuming between 20% and 35% of calories from protein. Patients on this diet had a higher ovarian reserve in both AMH and AFC. The link between carbohydrate and fat consumption and ovarian reserve, on the other hand, was determined to be statistically negligible. There have been few studies examining the link between the calorie percentage from various macronutrients and the degree of ovarian research such as AMH and AFC. Souter I, *et al*[1] according to their findings, total protein intake (as a percentage of total calories) was not associated with AFC in 264 women. The researchers identified a negative connection between protein derived from dairy products consumption and Antral follicular count when they looked at protein from other dietary sources

independently of dairy protein consumption. There were no associations found between protein derived from non-dairy diet or protein derived to vegetable components and AFC in the research.

Conclusion

- Based on the results of the current study the effects higher protein intake was found to be significant on the level of ovarian reserve.
- This study suggests that inculcation of Nutrition and lifestyle modification Counseling into fertility treatment is required for better outcome and also help in limiting the financial burden.

Limitations of the study included :

Data acquired was self-reported via questionnaires administered to research participants for many lifestyle variables. For example, it is widely established that participants tend to underreport non-socially desirable activities such as alcohol consumption and smoking, which may result in some persons being misclassified.

Additionally, the very small sample size, in particular, may create some statistical power issues. This may be represented in the fact that some of the results are statistically insignificant, while others indicate substantial and steady trends.

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Contributions : PS contributed to the design and drafting of the manuscript. PS contributed to the formal analysis, statistical analysis and editing of the manuscript. RA contributed to the methodology and experimental test. The authors have read and approved the final manuscript.

Conflicts of Interest : There is no conflict of interests

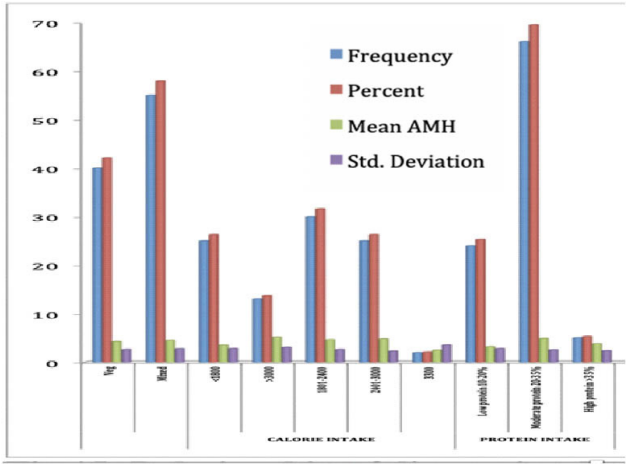
Table 5 — *Relationship of protein intake and level of AMH*

	Frequency(n)	Percent%	Mean	Std. Deviation(SD)	t value	p value
Veg	40	42.1	4.44	4.81	0.45	0.653
Mixed	55	57.9	4.02	3.83		
CALORIE INTAKE						
<1800	25	26.3	4.57	3.04	1.807	0.134
1801-2400	13	13.7	6.79	9.20		
2401-3000	30	31.6	3.48	2.45		
3000-3300	25	26.3	3.31	2.54		
>3300	2	2.1	4.50	2.12		
PROTEIN INTAKE						
Low protein 10-20%	24	25.3	1.92	1.03	8.11	0.002*
Moderate protein 20-35%	66	69.5	4.49	1.31		
High protein >35%	5	5.3	4.26	1.26		

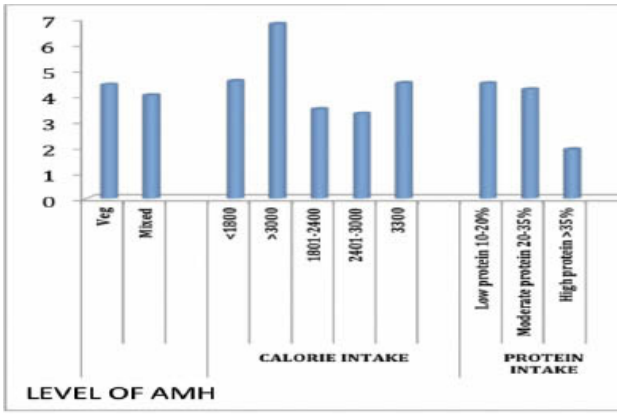
Table 6 — *Relationship of dietary intake and level of AFC*

	Frequency(n)	Percent%	Mean	Std. Deviation(SD)	t value	p value
Veg	40	42.1	4.28	2.63	-.419	0.676
Mixed	55	57.9	4.51	2.77		
CALORIE INTAKE						
<1800	25	26.3	3.52	2.82	1.380	.247
1801-2400	13	13.7	5.15	3.08		
2401-3000	30	31.6	4.63	2.63		
3000-3300	25	26.3	4.80	2.31		
>3300	2	2.1	2.50	3.54		
PROTEIN INTAKE						
Low protein 10-20%	24	25.3	3.21	2.81	3.77	0.02*
Moderate protein 20-35%	66	69.5	4.89	2.57		
High protein >35%	5	5.3	3.80	2.39		

Relationship of Protein Intake and Level of AMH



Relationship Protein Intake and Level of AMH



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