Section: Obstetrics and Gynaecology

Original Research Article

A STUDY TO ASSESS OVARIAN RESERVE OF WOMEN ATTENDING INFERTILITY OPD AT GOVERNMENT INSTITUTE

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Received : *30/06/2024*
Received in revised form : *26/08/2024*
Accepted : *11/09/2024*

Keywords:
Antimullerian hormone, Antral follicular count, Ovarian Reserve, Artificial reproductive technology.

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DOI: 10.47009/jamp.2024.6.5.47

Source of Support: Nil,
Conflict of Interest: None declared

Int J Acad Med Pharm
2024; 6 (5); 256-261

Abstract

Background: It is known that ovarian factors contribute to about 30-40% of all cases of infertility. Also an idea of ovarian reserve status can help us in counseling and decide appropriate line of management so that women's chances of conception can be enhanced. Ovarian reserve is an estimate of primordial follicle pool in the ovaries. It is used to reflect women's reproductive age and her remaining reproductive life span. It can be determined by various ovarian reserve tests. By providing the appropriate plan of management and investigations targeted treatment options can be offered to the infertile couple. Our study was conducted at Government institute which further extends approach towards patients coming from rural area and those who are unaware of facilities available for infertility evaluation and treatment plan. This study aims; 1. To assess ovarian reserve using sr. AMH levels and ultrasound parameters like AFC, ovarian volume and stromal blood flow of infertile women. 2.To compare ovarian reserve between different age groups and classify them according to the Poseidon group classification. 3.To correlate various factors associated with poor ovarian reserve. **Materials and Methods:** It is a cross sectional study conducted in the study period from 1st March 2021 to 31st August 2022, the patients attending OPD in Infertility clinic, total 81 cases included and tests for ovarian reserve done. **Result:** Total 81 cases were studied. As per the results mean of age of the cases in study was 28.99,mean BMI was 25.37,mean of sr. AMH 2.61 and mean AFC was 5.73.It is observed that there is decreasing trend of mean value of AFC and sr. AMH with increasing age. There is significant inverse correlation between mean sr. AMH and age(P=0.001). As per the Poseidon classification of study group 52 cases were of group1, group 3 included 15 cases. **Conclusion:** Larger studies are needed to formulate AMH and AFC nomograms especially in relation to age and BMI, these can help in prognosticating and counseling patients with reference to treatment outcome. AMH and AFC are currently the most reliable and simplest marker of ovarian reserve. POSEIDON stratification of low prognosis cases provides more detail idea regarding planning and implementation of treatment options in patient undergoing ART.

INTRODUCTION

Infertility is the failure of a couple to conceive after 1 year of regular, unprotected intercourse. It is an established fact that ovulatory disorder is one of the most common reasons of female factor infertility 30% of all cases. Reproductive aging is considered to be the consequence of a decrease in the quantity and quality of the ovarian follicle pool. Between women of the same chronological age, the quantitative ovarian reserve may vary substantially. To assess the individual quantitative ovarian reserve, various ovarian reserve tests (ORTs) have been developed.[1] This study was done at government institute and it mainly aims to assess various factors affecting the ovarian reserve of reproductive age female and to provide them with appropriate plan for infertility workup who are attending Infertility clinic at Government institute.[2] There are around 1032 infertility patient registered annually at our institute and workup needed for the evaluation and treatment of infertility done. Among these cases this has been found that, women in their early reproductive life also have ovarian factors associated with their infertility and which should be evaluated and test for ovarian reserve should be advised to them.[3]

Ovarian reserve is an estimate of the primordial follicle pool in the ovaries. It reflects woman's reproductive age and her remaining reproductive life span. It can be determined by sonographic visualization and measurement of the ovaries and calculation of Ovarian Volume (OV), Antral Follicle Count (AFC), mean follicular volume, or by biochemical assessment of follicle stimulating hormone, estrogen, Anti-Mullerian hormone and inhibin-B.[4] As there is lack of infrastructure available and easy accessibility for performing these ovarian reserve test at Government institute, we could use reliable test which can give better information for predicting the functional ovarian reserve. In our study we assessed ovarian reserve by using serum marker i.e. sr. AMH levels and ultrasound marker i.e. Antral follicle count.[5] Women who begin their life with low antral follicle count or low ovarian reserve tend to experience low AMH levels relative to other women of similar age.It is mentioned that AMH has ovarian functions that are unrelated to primordial follicle activation, such as modulation of FSH sensitivity in granulosa cells.[6] The slow change in AMH levels suggest that it regulates long term processes, across the lifespan. Correlation studies suggest that the size of developing follicle pool is the primary determinant of AMH concentration in women.[7,8]

MATERIALS AND METHODS

This prospective study was conducted at the Infertility Outpatient Department (OPD) of Netaji Subhash Chandra Bose (NSCB) Medical College and Hospital, Jabalpur. The aim was to assess the ovarian reserve of women attending the infertility clinic.

Inclusion Criteria

- Women attending the infertility OPD.
- Women willing to participate in the study.

Exclusion Criteria

- Women not willing to participate in the study.

Sample Size

A total of 81 women were included in the study. The sample size was calculated using the following formula:

n=z2pqd2n = \frac{z^2pq}{d^2}n=d2z2pq

Where:

- n = sample size
- z = 1.96 (for 95% confidence level)
- p = 30% (as ovarian factors contribute to approximately 30% of all infertility cases, p=0.30p = 0.30p=0.30)
- q = 1 - 0.30 = 0.70
- d = 10% (absolute error, d=0.10d = 0.10d=0.10)

Substituting the values into the formula:

n=(1.96)2×0.30×0.70(0.10)2=80.67≈81n = \frac{(1.96)^2 \times 0.30 \times 0.70}{(0.10)^2} = 80.67 \approx 81n=(0.10)2(1.96)2×0.30×0.70=80.67≈81

Material and methods

Data collection involved registering patients with their personal details, including name, age, address, occupation, education, socioeconomic status, and contact number. Information regarding the type of infertility (primary or secondary) and its duration was documented. A detailed menstrual history was obtained, covering aspects such as age at menarche, the date of the last menstrual period, cycle regularity, and any associated complaints like dysmenorrhea, menorrhagia, or oligomenorrhea. Past medical history was also reviewed. Obstetric history was thoroughly assessed, including the duration of marriage, cohabitation history, previous pregnancies and their outcomes, contraceptive use, previous marriages, and any prior infertility treatments. Personal history included inquiries about diet, lifestyle, and any addictions. Sexual history covered the frequency of coitus, knowledge of the fertile period, and any issues such as dyspareunia, erectile dysfunction, or premature ejaculation. Examinations and investigations followed, beginning with a general physical examination, followed by systemic and local assessments. The key investigations for evaluating ovarian reserve included serum Anti-Müllerian Hormone (AMH) testing and transvaginal ultrasound (TVS) to measure the antral follicle count (AFC).

Statistical Analysis

Data were recorded in a pre-designed proforma and analyzed using IBM-SPSS version 23.0. The association between variables was interpreted using frequency and percentage. The ANOVA test was applied to assess the statistical significance of the findings.

RESULTS

[Table 1] Distribution of Cases According to Age of Cases in Study The age distribution of participants is shown in Table 1. The majority of the study participants (51.9%) were between the ages of 25-29, followed by 29.6% in the 30-34 age group. Only 11.1% of participants were between 20-24 years, and 7.4% were aged 35 or older. This distribution suggests that most cases fall within the reproductive age bracket of 25-34 years, with fewer participants at younger or older extremes.

[Table 2] Distribution of Cases According to Serum AMH Levels Table 2 details the distribution of serum AMH levels among the participants. A significant portion of the participants (38.3%) had serum AMH levels in the 4.0-6.8 ng/ml range, which indicates optimal fertility. Meanwhile, 34.6% had satisfactory fertility with AMH levels between 2.2-4.0 ng/ml. Lower fertility, represented by AMH levels between 0.3-2.2 ng/ml, was observed in 23.6% of cases, and very low fertility (AMH 0.0-0.3 ng/ml) was observed in only 3.7% of cases. This distribution reflects a wide range of ovarian reserves among the study population.

[Table 3] Distribution of Cases According to Antral Follicular Count (AFC) In Table 3, the distribution of antral follicular count (AFC) is presented. The majority of participants (66.6%) had an AFC between 5-10, which is considered within the normal range for reproductive-age women. A smaller proportion (29.6%) had an AFC of less than 5, indicating a reduced ovarian reserve, while only 3.7% of participants had an AFC greater than 10, reflecting a high ovarian reserve. The data suggest that most participants had an AFC that falls within the typical range, with a notable percentage showing a lower count.

[Table 4] Association Between Age of Cases and Serum AMH Levels Table 4 highlights the association between age and serum AMH levels. Among participants aged 20-24, 33.33% had optimal fertility, while 44.44% had satisfactory fertility. In the 25-29 age group, 45.23% had optimal fertility, but as age increased, fertility declined. In the 30-34 age group, 50% of participants had satisfactory fertility, but 20.83% had low fertility. For participants aged 35 and older, 33.33% had low fertility, with a higher percentage having very low fertility (16.66%). These results show a clear decline in AMH levels with increasing age, consistent with decreased ovarian reserve as women age.

[Table 5] Association Between Age of Study Subjects and Mean Serum AMH Table 5 reports the mean serum AMH levels across different age groups. The mean AMH level was highest in participants aged 20-24 (3.68 ng/ml) and gradually declined with increasing age, with those aged ≥35 years having a mean AMH level of 1.36 ng/ml. The observed decline in AMH with age is statistically significant (p=0.001), indicating that younger participants had significantly higher ovarian reserves compared to older participants. The standard deviation values highlight the variation within each age group, but the trend shows a consistent age-related decline in AMH levels.

[Table 6] Association Between Age of Study Cases and AFC Table 6 shows the relationship between age and antral follicle count (AFC). In the 20-24 age group, all participants (100%) had an AFC between 5-10, indicating good ovarian reserve. For participants aged 25-29, 59.1% had an AFC < 5, while 47.2% had an AFC between 5-10, and 66.7% had an AFC > 10, indicating a mixed ovarian reserve distribution. The 30-34 age group showed that 27.3% had an AFC < 5, while 32.1% had an AFC between 5-10, and 16.7% had an AFC > 10. Among those ≥ 35 years old, 50% had low AFC (either < 5 or 5-10), reflecting a decline in ovarian reserve with age.

[Table 7] Association Between Age of Study Subjects and Mean AFC Table 7 displays the mean AFC across different age groups. The mean AFC was highest in the 20-24 age group (6.11 ± 1.26), with a gradual decline as age increased, showing a mean AFC of 4.67 ± 0.81 in participants aged ≥ 35. Although there is a decreasing trend in AFC with age, the association was not statistically significant (p = 0.42). The standard deviation indicates variability within age groups, but overall, AFC values decreased with age, indicating reduced ovarian reserve.

[Table 8] Association Between AFC and Serum AMH Levels Table 8 examines the relationship between AFC and serum AMH levels. Among participants with an AFC > 10, 83.33% had optimal fertility (AMH 4.0-6.8), while 16.7% had low fertility (AMH 0.3-2.2). In participants with an AFC between 5-10, the majority (43.4%) had optimal fertility, 41.5% had satisfactory fertility, and 15.1% had low fertility. Among those with an AFC < 5, a significant portion (45.5%) had low fertility, and 13.6% had very low fertility (AMH 0-0.3). These results show that higher AFC is associated with higher AMH levels, suggesting better ovarian reserve.

[Table 9] Association Between BMI and Serum AMH Levels Table 9 shows the association between body mass index (BMI) and serum AMH levels. In participants with a normal BMI (18.5-24.9), 41.2% had optimal fertility, 41.3% had satisfactory fertility, and 14.7% had low fertility. Among overweight participants (BMI 25-29.9), 39.0% had optimal fertility, while a larger percentage (26.8%) had low fertility. Obese participants (BMI 30-34.9) had a much higher incidence of low fertility (50%) and very low fertility (16.7%), with only a small portion having optimal or satisfactory fertility. These results suggest that higher BMI is associated with lower AMH levels and decreased ovarian reserve.

[Table 10] POSEIDON Classification of the Study Group Table 10 classifies the participants based on the POSEIDON criteria. Group 1 (age < 35 years, AFC ≥ 5, AMH ≥ 1.2 ng/ml) had the highest representation with 52 participants, indicating a strong ovarian reserve in younger women. Group 2 (age ≥ 35, AFC ≥ 5, AMH ≥ 1.2 ng/ml) included only 3 participants, suggesting a decline in ovarian function in older participants. Group 3 (age < 35 years, AFC < 5, AMH < 1.2 ng/ml) had 15 participants, reflecting compromised ovarian reserve in younger women. Group 4 (age ≥ 35, AFC < 5, AMH < 1.2 ng/ml) also had 3 participants, indicating the poorest ovarian reserve in older women. These classifications highlight the variations in ovarian reserve across different age groups and reproductive health statuses.

Table 1: Distribution of Cases According to Age of Cases in Study.

Age (years)	Frequency	Percentage
20-24	9	11.1%
25-29	42	51.9%
30-34	24	29.6%

ISSN (O): 2687-5365; ISSN (P): 2753-6556

≥ 35	6	7.4%
Total	81	100%

Table 2: Distribution of Cases According to Serum AMH Levels

Serum AMH Levels (ng/ml)	Frequency	Percentage
4.0-6.8	31	38.3%
2.2-4.0	28	34.6%
0.3-2.2	19	23.6%
0.0-0.3	3	3.7%
Total	81	100%

Table 3: Distribution of Cases According to Antral Follicular Count

Antral Follicular Count	Frequency	Percentage
> 10	3	3.7%
5-10	54	66.6%
< 5	24	29.6%
Total	81	100%

Table 4: Association Between Age of Cases and Serum AMH Levels

Age (years)	4-6.8 (Optimal Fertility)	2.2-4 (Satisfactory Fertility)	0.3-2.2 (Low Fertility)	0-0.3 (Very Low Fertility)	Total
20-24	3 (33.33%)	4 (44.44%)	2 (22.22%)	0 (0%)	9 (11.1%)
25-29	19 (45.23%)	11 (26.19%)	10 (23.80%)	2 (4.76%)	42 (51.9%)
30-34	7 (29.16%)	12 (50%)	5 (20.83%)	0 (0%)	24 (29.6%)
≥ 35	2 (33.33%)	1 (16.66%)	2 (33.33%)	1 (16.66%)	6 (7.4%)
Total	31 (100%)	28 (100%)	19 (100%)	3 (100%)	81

Table 5: Association Between Age of Study Subjects and Mean Serum AMH

Age (years)	N	Mean Serum AMH	SD	F value	P value
20-24	9	3.68	1.63	6.24	0.001
25-29	42	3.09	1.92		
30-34	24	1.71	1.01		
≥ 35	6	1.36	0.79		
Total	81	2.62	1.76		

Table 6: Association Between Age of Study Cases and AFC

Age (Years)	< 5	5-10	> 10	Total
20-24	0 (0%)	9 (17%)	0 (0%)	9 (11.1%)
25-29	13 (59.1%)	25 (47.2%)	4 (66.7%)	42 (51.9%)
30-34	6 (27.3%)	17 (32.1%)	1 (16.7%)	24 (29.6%)
≥ 35	3 (13.6%)	2 (3.8%)	1 (16.7%)	6 (7.4%)
Total	22 (100%)	53 (100%)	6 (100%)	81

Table 7: Association Between Age of Study Subjects and Mean AFC

Age (Years)	N	Mean AFC	SD	F value	P value
20-24	9	6.11	1.26	0.93	0.42
25-29	42	5.93	2.26		
30-34	24	5.50	1.79		
≥ 35	6	4.67	0.81		
Total	81	5.73	1.97		

Table 8: Association Between AFC and Serum AMH Levels

AFC	4.0-6.8 (Optimal Fertility)	2.2-4.0 (Satisfactory Fertility)	0.3-2.2 (Low Fertility)	0-0.3 (Very Low Fertility)	Total
> 10	5 (83.33%)	0 (0%)	1 (16.7%)	0 (0%)	6 (100%)
5-10	23 (43.4%)	22 (41.5%)	8 (15.1%)	0 (0%)	53 (100%)
< 5	3 (13.6%)	6 (27.3%)	10 (45.5%)	3 (13.6%)	22 (100%)
Total	31 (38.3%)	28 (34.6%)	19 (23.5%)	3 (3.7%)	81

Table 9: Association Between BMI and Serum AMH Levels

BMI	4.0-6.8 (Optimal Fertility)	2.2-4.0 (Satisfactory Fertility)	0.3-2.2 (Low Fertility)	0-0.3 (Very Low Fertility)	Total
18.5-24.9	14 (41.2%)	14 (41.3%)	5 (14.7%)	1 (2.9%)	34 (41.97%)
25-29.9	16 (39.0%)	13 (31.7%)	11 (26.8%)	1 (2.4%)	41 (50.16%)
30-34.9	1 (16.7%)	1 (16.6%)	3 (50%)	1 (16.7%)	6 (7.40%)
Total	31 (38.3%)	28 (34.6%)	19 (23.5%)	3 (3.7%)	81

ISSN (O): 2687-5365; ISSN (P): 2753-6556

Table 10: POSEIDON Classification of the Study Group

Group	Criteria	N
Group 1	Age < 35 years, AFC ≥ 5, Sr. AMH ≥ 1.2 ng/ml	52
Group 2	Age ≥ 35 years, AFC ≥ 5, Sr. AMH ≥ 1.2 ng/ml	3
Group 3	Age < 35 years, AFC < 5, Sr. AMH < 1.2 ng/ml	15
Group 4	Age ≥ 35 years, AFC < 5, Sr. AMH < 1.2 ng/ml	3

DISCUSSION

Ovarian reserve assessment is known to be very useful for interpretations of various factors associated with infertility along with ovarian factor. Serum AMH and AFC are found to be better markers to assess ovarian reserve. Our study aims to assess individual AMH and AFC, to correlate it with other clinicals variables like age and BMI as many studies shows association between these variables.

The majority of participants in the study were in the 25-29 (51.9%) and 30-34 (29.6%) age groups, with smaller numbers in the younger (20-24) and older (≥ 35) groups. This distribution aligns with studies by Broekmans et al. (2009) and Seifer et al. (2011), who demonstrated that most reproductive-age women fall within this range, reflecting the natural decline in fertility with age. Both studies found that fertility significantly decreases after age 35, corroborating the small percentage (7.4%) of older participants in this study.[9,10]

In this study, 38.3% of participants had AMH levels indicating optimal fertility (4.0-6.8 ng/ml), 34.6% had satisfactory fertility (2.2-4.0 ng/ml), and 23.5% showed low fertility (0.3-2.2 ng/ml). A small percentage (3.7%) had very low fertility (AMH < 0.3 ng/ml). Similar patterns have been observed in a study by La Marca et al. (2010), where AMH levels were shown to decline with age and strongly correlated with ovarian reserve.[11] Additionally, Seifer et al. (2011) also reported a decrease in AMH levels as age increases, supporting the findings of this study.[10]

Most participants had an AFC between 5-10 (66.6%), while 29.6% had an AFC < 5, suggesting reduced ovarian reserve. Only 3.7% had an AFC > 10, indicative of a high ovarian reserve. These results are consistent with studies by Jayaprakasan et al. (2012) and Dewailly et al. (2014), both of which found that AFC tends to decrease with age, and AFC values between 5-10 are typical for reproductive-age women. Lower AFC values were associated with poorer fertility outcomes.[12,13]

AMH levels declined as age increased, with the highest levels observed in the 20-24 group and a significant decrease in those aged 35 and older. These results are supported by studies such as those by Nelson et al. (2011), which showed a strong inverse relationship between age and AMH levels.[14] The decline in AMH with age is a well-established phenomenon, indicating a reduction in ovarian reserve as women age.

The mean serum AMH levels also demonstrated a clear age-related decline, with younger participants (20-24 years) showing the highest levels (3.68 ng/ml) and those aged ≥35 years showing the lowest levels (1.36 ng/ml). This trend was statistically significant (p = 0.001). This finding aligns with studies by La Marca et al. (2010), which demonstrated a similar decline in AMH levels across age groups.[11] The statistical significance of the decline further supports the established understanding that AMH is a reliable marker of ovarian aging.

A similar age-related decline was observed in AFC, with younger participants having higher AFC counts. In the 20-24 group, all participants had an AFC between 5-10, indicating a good ovarian reserve, while older participants showed reduced AFC counts. These findings align with studies by Jayaprakasan et al. (2012), where AFC was shown to decrease with age, particularly after the age of 35, when ovarian reserve declines significantly.[12]

The mean AFC also decreased with age, though this relationship was not statistically significant (p = 0.42). Studies by Broekmans et al. (2009) and Dewailly et al. (2014) similarly found that while AFC tends to decrease with age, the variation within age groups can reduce statistical significance, reflecting individual differences in ovarian aging.[9,13]

Higher AFC was associated with higher AMH levels, as expected. Participants with an AFC > 10 were more likely to have optimal AMH levels, while those with an AFC < 5 had a higher likelihood of low fertility. These findings are consistent with a study by Dewailly et al. (2014), which also demonstrated a strong correlation between AFC and AMH, reinforcing the use of both markers to assess ovarian reserve.[13,14]

Higher BMI was associated with lower AMH levels, with obese participants showing a higher incidence of low or very low fertility. This trend aligns with studies by Freitas et al. (2016), who demonstrated that obesity is linked to decreased AMH levels and reduced ovarian reserve.[15] These findings suggest that BMI should be considered when assessing ovarian reserve and fertility potential.

The POSEIDON classification of participants highlights the variability in ovarian reserve across different age groups and reproductive statuses. Most participants fell into Group 1, which represents younger women with a good ovarian reserve. This distribution is consistent with studies by Esteves et al. (2017), who used the POSEIDON criteria to stratify patients based on their age, AMH levels, and AFC, showing that younger women tend to have better ovarian reserve outcomes compared to older women.16 Likewise by using POSEIDON criteria we can identify and classify the patients who have low prognosis in ART and can aim at designing an

ISSN (O): 2687-5365; ISSN (P): 2753-6556

indualised treatment plan to maximize the chances of achieving treatment outcome in each group.

CONCLUSION

Larger studies are needed to formulate AMH and AFC nomograms especially in relation to age and BMI, these can help in prognosticating and counseling patients with reference to treatment outcome. AMH and AFC are currently the most reliable and simplest marker of ovarian reserve. POSEIDON stratification of low prognosis cases provides more detail idea regarding planning and implementation of treatment options in patient undergoing ART.

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ISSN (O): 2687-5365; ISSN (P): 2753-6556