Original Article

Correlation Between Follicle Stimulating Hormone, Anti-Müllerian Hormone and Antral Follicle Count with Different Age Groups in Infertile Women

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Abstract

Background: *Reduced ovarian reserve predicts poor ovarian response and poor suc--cess rates in infertile women who undergo Assisted Reproductive Technology (ART). Ovarian reserve decreases with age but the rate of decline varies from one woman to another. Follicle Stimulating Hormone (FSH) Anti-Müllerian Hormone (AMH) and antral follicle count (AFC) represent the three most frequently utilized laboratory tests in determining Ovarian Reserve (OR). To determine correlation between FSH, AMH and AFC in infertile female.*

Materials and methods: *It was an observational (Cross sectional) study. This study was done in the Department of Reproductive Endocrinology and Infertility, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, between July 2018 to June 2019. The study population consisted of all the diagnosed female infertility patients of reproductive age. The women attending the study center during study period having primary or secondary infertility was considered as study population. They were divided in 4 age groups 21-25, 26-30, 31-35 yrs and 36-40 yrs years. Data was collected using a structured questionnaire following physical & lab examination. For D_2 FSH level fasting blood was collected on D2/3 of menstrual cycle, serum FSH level was measured by ADVIA Centraur(R) XP immunoassay system. For S. AMH level blood sample was collected on 2nd day of cycle and measured by BECKMAN COULTER machine using Chemiluminescent Immunoassay method. For AFC count TVS was done on D_{2-5} of cycle using KONTRON medical USG machine. Collected data were classified, edited, coded and entered into the computer for statistical analysis by using SPSS version 23.*

Results: *Out of 74 patients the mean age was found 32.6±5.5 years. Serum FSH, AMH and AFC were significantly associated with different age group. A negative correlation was found between serum FSH and serum AMH in all age group. But strong correlation found in age group 31-35 yrs and in 36-40 years age group. A negative correlation was found between serum FSH and total AFC in age group 26-30 years, 31-35 years and 36-40 years respectively. A positive correlation was found between serum AMH and total AFC in all age group but most strong in age group 31-35 years. In multivariate logistic regression analysis serum AMH (<1.0 ng/ml) and total AFC (<5 number) were found to be significantly associated with age group >35 years patients.*

Conclusion: *In all age grqoup, FSH, AMH and AFC correlates but it is more pronounced in advanced age that means >35 years age group.*

Key words: Follicle stimulating hormone; AntiMullerian Hormone; Antral follicle count.

INTRODUCTION

The term "ovarian reserve" has traditionally been used to describe a woman's reproductive potential, specifically the number and quality of oocytes she possesses[1].

A woman is born with about 2 million primordial follicles, yet by the onset of menarche only about 400,000 follicles are left due to natural follicular atresia. As a woman reaches her mid-30s, the pace of oocyte depletion begins to increase and by the time she reaches her late 30s, the number of follicles declines to approximately 25,000, concomitant with a significant increase in miscarriage rate[2].

Ovarian reserve is a complex clinical phenomenon influenced by age, genetics, and environmental variables.[2] The decline in a woman's ovarian reserve with time is irreversible and the rate at which women lose primordial follicles varies considerably, with wide variation regarding the onset of sterility and timing of the menopausal transition[2].

Ovarian reserve tests started to emerge during the rise of ART in the late 1980s to predict both responsiveness to super ovulation drugs and the odds of pregnancy with treatment. They include both biochemical basal and provocative tests and ultrasound imaging of the ovaries. The first test to be introduced - was day-3 follicle-stimulating hormone (FSH) (1988), followed by Clomiphene Citrate Challenge Test (CCCT) (1989) Gonadotropin Releasing-Hormone (GnRH) agonist (1989) inhibin B (1997) Antral Follicular Count (AFC) (1997) and Anti Müllerian Hormone (AMH)[2].

Early follicular phase (Basal) FSH as a marker of ovarian reserve was proposed almost 30 years ago, as a tool to predict ovarian response to In Vitro Fertilization (IVF)[3]. This test is an indirect assessment of ovarian reserve and is based on the feedback inhibition of FSH pituitary secretion by ovarian factors.

Women with normal ovarian reserve have sufficient production of ovarian hormones at this early stage of the menstrual cycle to maintain FSH levels within normal range[2].

However, basal FSH testing has several major limitations including significant intercycle and intracycle variability that limits its reliability[4]. it requires a functional hypothalamus-pituitary-ovarian axis, and it is not adequately sensitive for clinical utility–only elevations carrying significance[5].

A single abnormal FSH value in a woman <40 years of age may not predict a poor response to stimulation or failure to achieve pregnancy and should prompt repeat testing[6].

The ovary begins producing AMH in utero at about 36 weeks of gestation[7]. Its levels rise in young women beginning in adolescence and peak at about 25 years of age, then gradually decline until reaching undetectable levels a few years prior to menopause. Since AMH is expressed during normal early folliculogenesis (Secreted by early follicles up to 6 mm), it is relatively independent of gonadotropins circulating at physiologic levels and allows for testing anytime throughout the cycle[2].

AFC is the sum of follicles in both ovaries as observed on ultrasound in the early follicular phase (Day 2-4) of the menstrual cycle. Antral follicles are defined as those measuring 2-10 mm in largest mean diameter on 2-dimensional plane. AFC is easy to carry out, provides an immediate result and has good intercycle reliability and good interobserver reliability when measured in experienced centers using a minimal number of sonographers. Its precision is compromised with overweight and obese individuals or when using multiple sonographers[8].

MATERIALS AND METHODS

It was an observational (Cross sectional) study. This study was done in the Department of Reproductive Endocrinology and Infertility, Bangabandhu Sheikh Mujib Medical University (BSMMU) Dhaka, between July 2018 to June 2019. The study population consisted of all the diagnosed female infertility patients of reproductive age. The women attending the study center during study period having primary or secondary infertility was considered as study population. They were divided in 4 groups, 21-25, 26-30, 31-35, 36-40 years. Data was collected using a structured questionnaire following physical & lab examination. For D_2 FSH level fasting blood was collected on D2/3 of menstrual cycle, serum FSH level was measured by ADVIA Centraur[(R)] XP immunoassay system. For S. AMH level blood sample was collected on any day of cycle and measured by BECKMAN COULTER machine using Chemiluminescent Immunoassay method. Statistical analysis was carried out by using the Statistical Package for Social Sciences version 16.0 for Windows (SPSS Inc Chicago, Illinois, USA). The mean values were calculated by frequencies and percentages. The quantitative observations were indicated by frequencies and percentages. Chi square test was used for categorical variables. Unpaired t-test was used for continuous variables. Pearson's correlation coefficient was used to test the relationship between the groups. Multivariate logistic regression analysis was used for risk factors of infertile women. P values <0.05 was considered as statistically significant.

RESULTS

A total of 74 infertile women's were included in this study with maintaining inclusion & exclusion criteria. They were divided in 4 age groups- 21-25 years,26-30 years, 31-35 years and 36-40 years.

Table I : Distribution of the study patients by age (n=74).

Age (Years)	Number of patients	Percentage
21-25	6	8.1
26-30	23	31.1
31-35	18	24.3
36-40	27	36.5
Mean±SD	32.6 ±5.5	
Range (Min-max)	22.0 -40.0	

Table II : Distribution of the study patients according to serum FSH (n=74).

Serum FSH (IU/L)	Age 21-25 years (n=6)		Age 26-30 years (n=23)		Age 31-35 years (n=18)		Age 36-40 years (n=27)		p value
	n	%	n	%	n	%	n	%	
10.0 (Normal)	6	100.0	22	95.7	9	50.0	12	44.4	
>10.0 (Abnormal)	0	0.0	1	4.3	9	50.0	15	55.6	
Mean±SD	5.2	±1.1	6.4	±2.5	10.7	±6.1	10.2	±3.1	0.001[s]
Range (min-max)	4.0	-7.2	3.02	-15.9	4.0	-27.0	5.0	-16.0	

s= significant, p value reached from ANOVA test.

Table III : Distribution of the study patients according to serum AMH (n=74).

Serum AMH (ng/ml)	Age 21-25 years (n=6)		Age 26-30 years (n=23)		Age 31-35 years (n=18)		Age 36-40 years (n=27)		p value
	n	%	n	%	n	%	n	%	
<1.0 (Low)	0	0.0	1	4.3	5	27.8	13	48.1	
1.0-3.5 (Normal)	6	100.0	22	95.7	13	72.2	14	51.9	
Mean±SD	2.87	±0.80	2.44	±0.77	1.77	±1.10	1.37	±1.06	0.001[s]
Range (min-max)	1.50	-3.50	0.46	-3.50	0.18	-3.30	0.02	-3.48	

s= significant, p value reached from ANOVA test.

Table IV : Distribution of the study patients according to total AFC (n=74).

Total AFC (Number)	Age 21-25 years (n=6)		Age 26-30 years (n=23)		Age 31-35 years (n=18)		Age 36-40 years (n=27)		p value
	n	%	n	%	n	%	n	%	
<5 (Low)	0	0.0	1	4.3	1	5.6	6	22.2	
5-15 (Normal)	6	100.0	19	82.6	17	94.4	20	74.1	
>15 (High)	0	0.0	3	13.0	0	0.0	1	3.7	
Mean±SD	13.5	±1.7	13.3	±3.2	9.8	±3.1	8.5	±3.3	0.001[s]
Range (Min-max)	11.0	-15.0	4.0	-18.0	4.0	-14.0	4.0	-16.0	

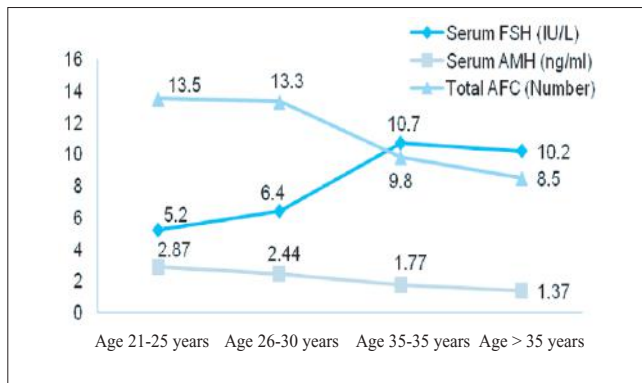s= significant, p value reached from ANOVA test.

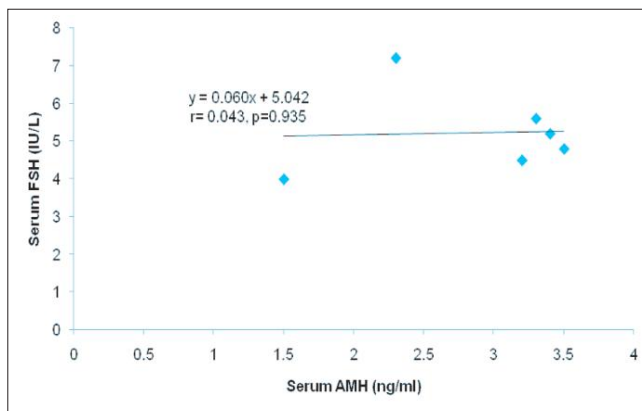**Figure 1 :** Line diagram showing mean serum FSH, serum AMH and total AFC in different age years.

Figure 2 : The scatter diagram showing no correlation (r= 0.043, p=0.935) between serum FSH and serum AMH in age group 21-25 years.
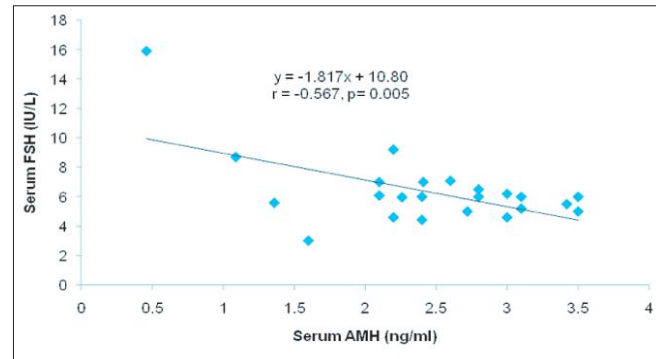

Figure 3 : The scatter diagram showing negative correlation (r= -0.567, p=0.005) between serum FSH and serum AMH in age group 26-30 years.
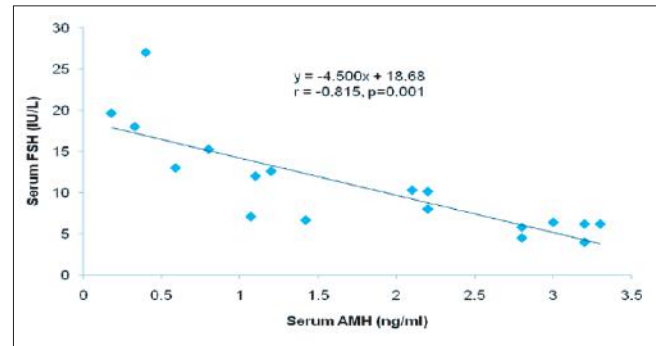

Figure 4 : The scatter diagram showing negative correlation (r= -0.815, p=0.001) between serum FSH and serum AMH in age group 31-35 years.
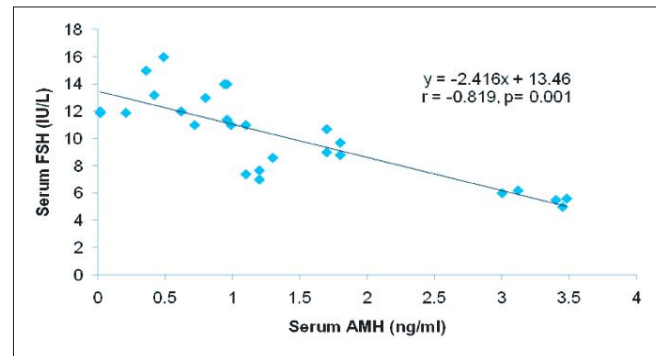

Figure 5 : The scatter diagram showing negative correlation (r= -0.819, p=0.001) between serum FSH and serum AMH in age group 36-40 years.
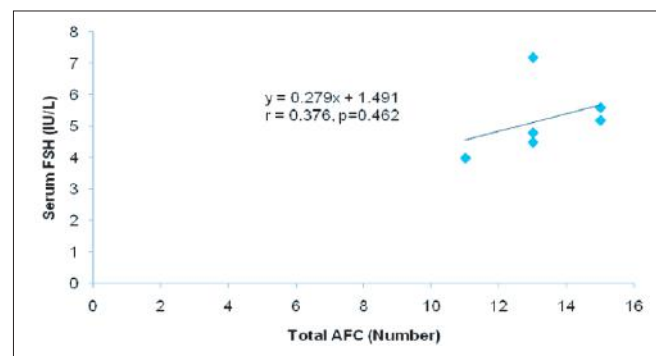

Figure 6 : The scatter diagram showing positive correlation (r= 0.376, p=0.462) between serum FSH and total AFC in age group 21-25 years.
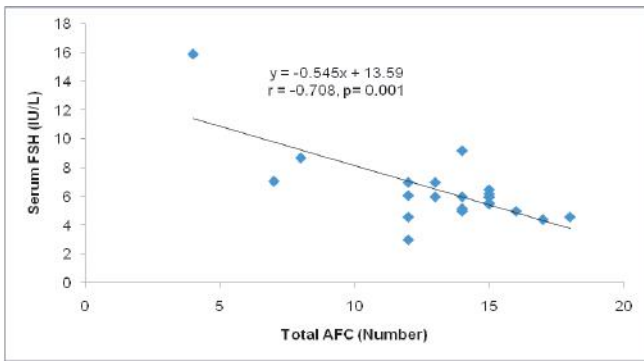
Figure 7 : The scatter diagram showing negative correlation (r= -0.708, p=0.001) between serum FSH and total AFC in age group 26-30 years.
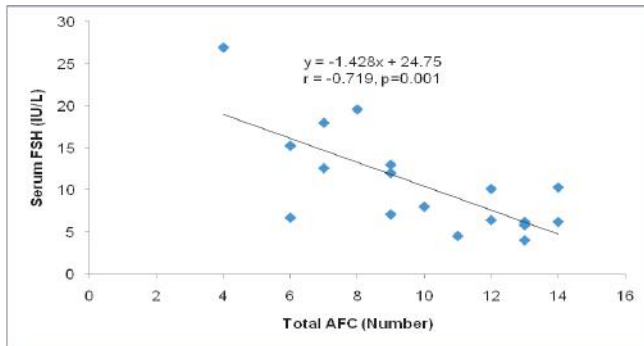

Figure 8 : The scatter diagram showing negative correlation (r= -0.719, p=0.001) between serum FSH and total AFC in age group 31-35 years.

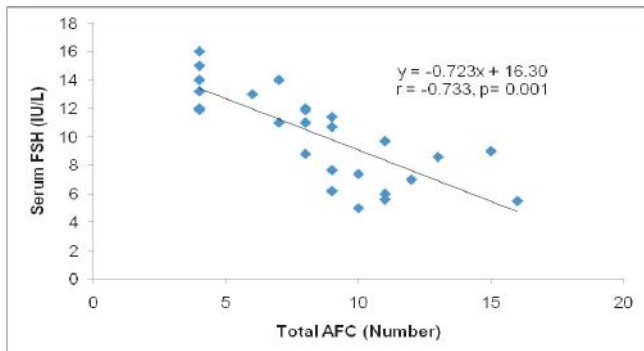**Figure 9 :** The scatter diagram showing negative correlation (r= -0.733, p=0.001) between serum FSH and total AFC in age group 36-40 years.
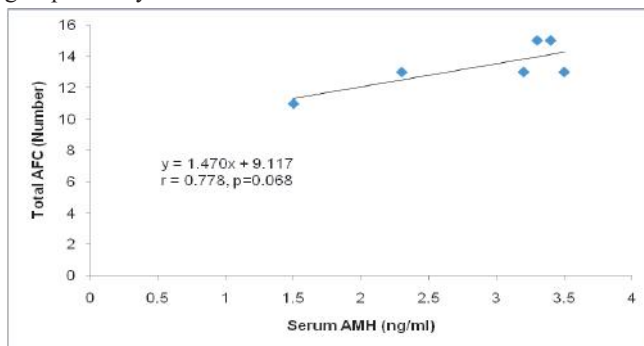

Figure 10 : The scatter diagram showing positive correlation (r= 0.778, p=0.068) between serum AMH and total AFC in age group 21-25 years.
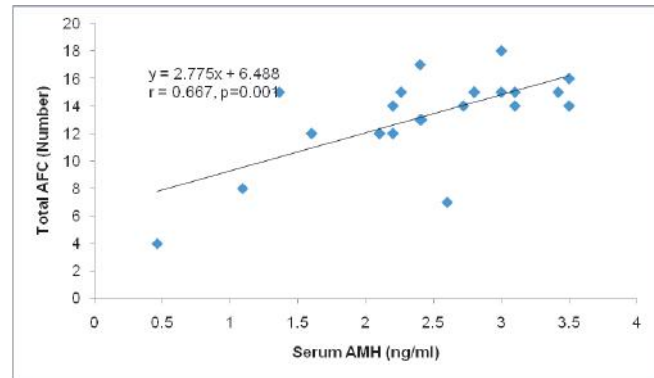

Figure 11 : The scatter diagram showing positive correlation (r= 0.667, p=0.001) between serum AMH and total AFC in age group 26-30 years.
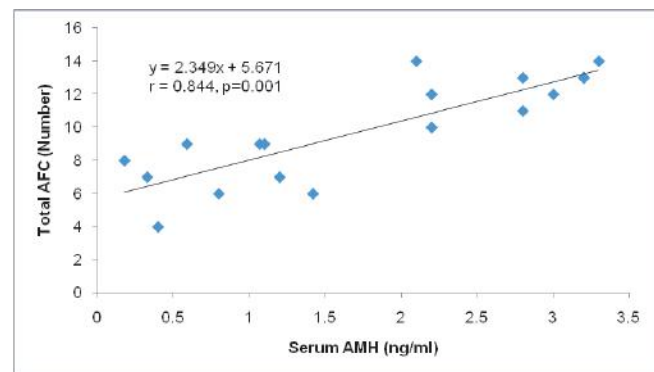

Figure 12 : The scatter diagram showing positive correlation (r= 0.844, p=0.001) between serum AMH and total AFC in age group 31-35 years.
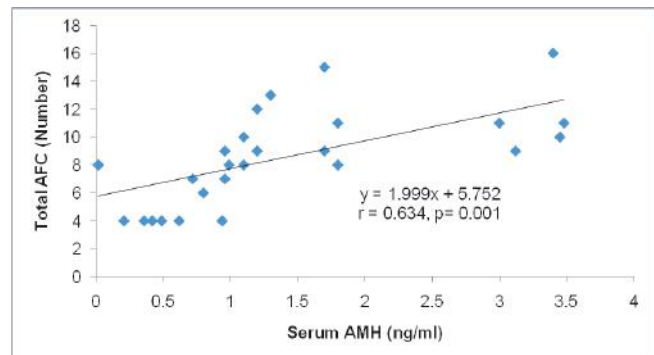

Figure 13 : The scatter diagram showing positive correlation (r= 0.634, p=0.001) between serum AMH and total AFC in age group 36-40 years.

Table V : Multi variable logistic regression analysis for age >35 years.

Risk factors	Regression coefficient (β)	Odds Ratio (OR)	95% CI for OR	P value
Serum FSH (>10.0 IU/L)	0.934	2.544	0.901-7.182	0.078[ns]
Serum AMH (<1.0 ng/ml)	1.531	4.626	1.649-12.976	0.004[s]
Total AFC (<5 number)	2.242	9.412	2.543-34.838	0.001[s]

s=significant, ns=significant, p-value reached from multivariate analysis by binary logistic regression analysis

DISCUSSION

In present study it was observed that all (100%) patients have normal (10 IU/L) serum FSH in age group 21-25 years, 95.7% in 26-30 yrs, 50% in 31-35 yrs and 44.4% in age group 36-40 yrs. The mean difference was statistically significant (p<.05) among four group. Barbakadze et al found significant association between serum FSH with different age group. They divided their subject into 3 age groups, <35 yr, 35-40 yrs and 41-46 yrs. In their study serum FSH level showed a significantly higher result only in age group 41-46 yrs compared to age group <35 yrs[9].

Ozcan et al revealed that the AMH concentration declined significantly with increasing age. This decline began at the age of 30, and it became dramatically evident from the age of 35. This suggests that some women may be candidates of poor response due to the unexpected risk of a diminishing ovarian reserve after age 30[10]. In this study 100% patient were found normal (1.0-3.5 ng/ml) serum AMH in age group 21-25 yrs, 95.7% in 26-30 yrs, 72.2% in 31-35 yrs and 51.9% in age group 36-40 yrs. The mean serum AMH was found 2.87±.8 ng/ml in age group 21-25 yrs, 2.44±.77 ng/ml in age group 26-30 yrs, 1.77±1.1 ng/ml in age group 31-35 yrs and 1.37±1.06 ng/ml in 36-40 yrs. The mean difference was statistically significant (p<0.05) among 4 groups.

In the largest study analyzing age-specific medians for serum AMH by Seifer et al reported that both median and mean AMH values were inversely associated with age[11]. The average yearly decrease in the median serum AMH value was 0.2 ng/ml/year upto age 35 then diminished to 0.1 ng/mL/year after the age of 35. The most striking study on means of AMH in general population is the study of Tremellen and Kolo[12]. They evaluated a total of 1032 women aged between 18 and 43 years and found that the mean serum AMH level is relatively stable at approximately (4.1 ng/ml) (1 ng AMH is 7.143 pmol) in the under 30-year-old range, however, from 30 years of age onwards the serum AMH levels decline rapidly, became half in concentration to an average of only (1.95 ng/ml) in the 35-39 year old age group.

Barbakadze et al found significant negative correlation of serum AMH with advancing age group[9]. A study by Nelson et al with 9601 infertile women showed that serum AMH will decrease with age and found that serum AMH in all percentiles were lower compared to the study. This difference might be caused by several factors, including different populations with different genetic and environmental backgrounds, which could lead to a different ovarian biological age compared to chronological age.

In current study 100% patients were found normal (5-15 numbers) total AFC in age group 21-25 yrs, 82.6% in 26-30 yrs, 94.4% in 31-35 yrs and 74.1% in age group 36-40 yrs. The mean AFC was found 13.5±1.7 in 21-25 yrs, 13.3±2 in 26-30 yrs, 9.8±3.1 in 31-35 yrs and 8.5±3.3 in 36-40 yrs group. The mean difference was significant among 4 groups (p<0.05).

In this study it was observed that there was a moderate negative correlation (r=-0.567, p= .005) between serum FSH and serum AMH in age group 26-30 yrs. But strong negative correlation

(r= -0.815, p= 0.001) in 31-35 yrs and (r= -0.819, p= 0.001) in 36-40 yrs age group. Barbakadze et al consisted that AMH showed a negative correlation with FSH (rs=-0.48, p<0.0001)[9]. Gada et al found that there was a negative correlation between AMH and FSH (R= -0.41)[13]. Okunola et al showed in their study the Pearson's coefficient for the correlation between FSH and AMH after controlling for age was -0.311 (p=0.012)[14]. Scheffer et al documented that AMH was significantly correlated with FSH (r=-0.32, p<.01)[15].

Gleicher et al reported that women with normal AMH and FSH produced high number of oocytes, whereas women with normal FSH but decreasing AMH produced a significantly lower number of oocytes[16]. This also indicates that serum AMH levels are more important predictors of ovarian aging than FSH levels. This is similar with previous studies by Barad et al that shows that AMH levels are better predictors of response to ovarian stimulation and clinical pregnancy than baseline FSH[17]. The relatively lower slopes of increasing FSH in older age have made FSH a late predictor of ovarian reserves[18].

In this study there was a negative correlation between FSH and AFC in age group 26-30 yrs (r= -0.708, p= 0.001) 31-35 yrs (r= -0.719, p= 0.001) and 36-40 yrs (r= -0.733, p= 0.001). Barbakadze et al showed significant negative correlation between FSH and AFC only in age group > 40 yrs.(r= -0.42, p= 0.001)[9]. In this current study there was a positive correlation between serum AMH and AFC in all age group. But it is most strong in age group 31-35 yrs group (r= 0.844, p= 0.001). Barbakadze et al showed in their study AMH and AFC level had positive association for group I (r= 0.57, P<0.001) group II (r= 0.69, p< 0.001) and group III (r= 0.47, p<0.002) which were significant[9].

Scheffer et al reported that AMH was significantly correlated with AFC (r=0.81, p<.00001)[15]. Gada et al showed that there was a strong correlation between AMH and AFC (Correlation coefficient, R= 0.72)[13].

Barbakadze et al reported that according to regression analysis, age only explained the variation of AMH in 22%, the variation of FSH in 14% and the variation of AFC in 27% of changes[9]. Tehraninezhad et al showed that among AFC and age, AFC was the independent predictor (beta=0.6, p=0.001)[19]. Among FSH and age, age was the only independent predicting variable (beta=-0.4, p=0.001). In this study it was found that in multivariate logistic regression analysis, patients having serum AMH (<1.0 ng/ml) was 4.626 (95% CI 1.649 to 12.976) times in age group >35 years. Patients having total AFC (<5 number) was 9.412 (95% CI 2.543 to 34.838) times in age group >35 years. Serum AMH and total AFC were found to be significantly (p<0.05) associated with age group >35 years patients.

CONCLUSION

In all age group, FSH, AMH and AFC correlates but it is more pronounced in advanced age that means >35 years age group. Further studies can be undertaken by including large number of patients.

DISCLOSURE

All the authors declared no competing interest.

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