A Study of Correlation between Serum Level of Antimullerian Hormone (AMH) and Follicle Stimulating Hormone (FSH) in Infertile Women at a Tertiary Care Hospital of Eastern India

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ABSTRACT

BACKGROUND

Worldwide infertility affects 8 % - 10 % of couples. In recent years, assessment of ovarian reserve has become one of the important tests for diagnosis of infertility. Biochemical tests for follicle stimulating hormone (FSH), anti-mullerian hormone (AMH), luteinizing hormone (LH) and estradiol (E2) are important in assessment of ovarian reserve.

METHODS

This is a hospital based prospective observational study conducted in the Department of Obstetrics and Gynaecology, VIMSAR, Burla from November 2018 to October 2020. The study was done on 70 infertile women with regular menstrual cycle. AMH estimation was done using the Beckman Coulter Access® 2 Automated Immunoassay System. Serum FSH and LH were estimated by ADVIA Centaur CP auto analyzer. Correlation between serum AMH, FSH, LH, LH / FSH ratio with body mass index (BMI) and different age groups were performed with appropriate statistical tests.

RESULTS

In our study, we observed that serum AMH levels and LH/FSH ratio were significantly decreased in patients with advanced age, but basal FSH and LH levels were significantly increased according to age. Serum AMH and serum FSH showed a significantly negative correlation between each other. Serum AMH and LH / FSH showed a positive correlation but not statistically significant. Serum FSH and LH/FSH showed a significantly negative correlation.

CONCLUSIONS

Serum AMH levels and the LH / FSH ratio were decreased with increase in age. Serum FSH and LH levels were significantly increased according to age. Serum AMH is a better predictor of ovarian reserve as it is relatively stable throughout the cycle and can be assayed at different times during the cycle.

KEYWORDS

Infertility, Anti-Mullerian Hormone, Follicle Stimulating Hormone, Luteinizing Hormone

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BACKGROUND

The global health community had great success in improving maternal and child health in the past decade, partly through a focus on reproductive health.[1,2] Infertility is a critical component of reproductive health, and has often been neglected in these efforts.[3] Worldwide infertility affects approximately 8 % – 10 % of couples.[4] Out of 60 - 80 million couples suffering from infertility globally, about 15 - 20 million (25 %) are from India alone.[5] The fertility rate in India has declined from 3.346 in 2000 to 2.636 in 2010 to 2.220 in 2019.[6] The birth rate in India has declined from 26.635 in 2000 to 21.508 to 2010 to 17.806 in 2019.[6] The long-term decline in birth and fertility rate has been attributed to greater focus on education and careers, improvement in contraception and easy access to family planning services, delayed childbearing, decreased family size and late marriage and more frequent divorce.[7] In recent years, assessment of ovarian reserve for treatment of female infertility has become very essential.[8,9] Traditionally, for the evaluation of ovarian reserve age, follicle stimulating hormone , estradiol (E2) levels and antral follicle count (AFC) by ultrasound investigation at the early follicular phase have been used. For years, biochemical markers like the levels of FSH and E2 were considered for assessment of low ovarian reserve.[10] However, it has been found that the FSH level is raised above the normal, only in cases when the ovary function is largely compromised. Recently identification of anti-mullerian hormone levels became important in assessment of ovarian reserve.[11]

Aims and Objectives

Primary Objective

The aim of present study is to investigate the age-dependent changes in circulating anti-mullerian hormone and follicle stimulating hormone.

Secondary Objective

To correlate changes in serum AMH levels with serum FSH and LH values and LH / FSH ratio.

METHODS

The present study was conducted in the Department of Obstetrics and Gynaecology, VIMSAR, Burla, Sambalpur. The study period was from November 2018 to October 2020. It is a cross sectional type of study which included a total of 70 women with regular menstrual cycles attending Obstetrics and Gynaecology out-patient department (OPD) of VIMSAR, Burla. Written and informed consents were obtained from all participants before study recruitment. The research was approved and received ethical clearance from Institutional Ethics Committee, bearing a reference numberRegn. No. ECR / 861 / Inst / OR / 2016 VIMSAR, Burla.

Inclusion Criteria

Infertile women in the age group of 30 - 40 years.

Exclusion Criteria

1. Women with other factors of infertility i.e. Uterine, tubal or male factor
2. History of sterilization.
3. Diagnosed case of poly-cystic ovarian syndrome (PCOS).
4. Surgically diagnosed endometriosis.
5. Infertile male partner.
6. Body mass index (BMI) ≥ 30 kg / m^2
7. Serum prolactin levels > 50 ng / ml
8. Patients with endocrine diseases like hypothyroid, diabetes mellitus, Cushing's syndrome

Serological Hormones Estimation

Blood samples were collected for AMH, LH and FSH by venipuncture. Serum levels of Day 3 AMH, FSH, LH and LH/FSH ratio of all patients were measured. Serum AMH estimation was done using the Beckman Coulter Access®2 Automated Immunoassay System under the principle of the chemiluminescent immunoassay (CLIA). The clinically reportable range for AMH in our laboratory was set at 0.3 - 10 ng / mL. Bench quality controls from Bio-Rad were used for internal quality assessment. Serum FSH and LH is estimated by ADVIA Centaur CP auto analyzer by chemi-luminescence immunoassay. Serum AMH level is an age-specific marker for the ovarian reserve, hence the patients were classified into two groups, group I – 30 - 35 years and group II – 36 - 40 years. Patients were also classified on the basis of aetiology of infertility into primary and secondary infertility.

Statistical Analysis

All data analysis was performed using SPSS (Statistical Package for the Social Sciences) software version 20.0. Comparisons of AMH, FSH, LH, LH / FSH ratio and BMI, between different age groups were performed using one-way analysis of variance (ANOVA). Variables were presented as mean ± S.D. The correlation between AMH, FSH and LH / FSH ratio were analyzed and expressed with the Pearson's correlation coefficient. A p value < 0.05 was considered statistically significant.

RESULTS

The majority of cases were present in the 30 - 35 years age group (60 %). Overall the mean age was 34.7 ± 2.6 years. There was no significant difference observed in the distribution of cases between the two age groups (p = 0.094). According to BMI, 55 (78.6 %) cases were normal weight and 15 cases (21.4 %) were overweight. None of the patients were underweight or obese. There was significantly higher proportion of cases in the normal

weight category of BMI. Out of 70 cases, 58 (82.9 %) cases had primary infertility and 12 (17.1 %) cases had secondary infertility. In the study group, primary infertility was significantly higher than secondary infertility. In our study, 59 (84.3 %) cases were nulliparous and 11 (15.7 %) cases had one child. Treatment and drug history of cases reveals out of 70 cases, 62 (88.6 %) cases had received no treatment and 8 (11.4 %) cases had undergone ovulation induction with clomiphene citrate. Serum levels of AMH, FSH, LH, LH/FSH ratio are shown in Table - 1.

Descriptive Statistics	S.AMH (ng / ml)	S.FSH (mlU / ml)	S.LH (mlu / ml)	LH / FSH
Total number of cases (n)	70	70	70	70
Mean	2.21	7.57	5.45	0.75
SD	1.26	2.10	1.15	0.16

Table 1. Descriptive Statistics of S.AMH, S.FSH, S.LH, LH/FSH Ratio

Comparisons of serum levels of AMH, FSH, LH, LH/FSH ratio between types of infertility are shown in table 2.

Variables	Type of Infertility: Primary Infertility (n = 58) Mean	SD	Median (IQR)	Secondary Infertility (n = 12) Mean	SD	Median (IQR)	P' Value*
S.AMH (ng / ml)	2.3	1.2	2.2 (1.4,2.8)	1.7	1.4	1.5 (0.9,2.0)	0.072
S.FSH (mlU / ml)	7.5	2.1	7.4 (6.1,8.6)	7.9	2.1	7.2 (6.3,9.8)	0.469
S.LH (mlu / ml)	5.4	1.1	5.3 (4.5,6.3)	5.9	1.2	6.2 (4.6,6.8)	0.113
LH/FSH	0.7	0.2	0.8 (0.6,0.9)	0.8	0.1	0.7 (0.6,0.8)	0.935

Table 2. Comparison of S.AMH, S.FSH, S.LH, LH/FSH Ratio between Type of Infertility

* Mann-Whitney U 'p' value

There was no significant difference in serum level AMH (p = 0.072), FSH (p = 0.469), LH (p = 0.113), LH/FSH ratio (p = 0.925) between two types of infertility.

Table 3 showed comparison of serum parameters between two age groups 30 - 35 years and 36 - 40 years age groups.

Variables	Age Group: 30 - 35 (n = 42) Mean	SD	Median (IQR)	36 - 40 (n = 28) Mean	SD	Median (IQR)	P - Value
S.AMH (ng / ml)	2.7	1.1	2.5 (2.0,2.9)	1.4	1.1	1.2 (0.9,1.7)	0.000*
S.FSH (mlU / ml)	6.9	1.6	6.8 (5.7,8.5)	8.6	2.3	7.8 (6.7,10.5)	0.002*
S.LH (mlu / ml)	5.1	1.0	5.0 (4.2,5.8)	6.0	1.2	5.9 (4.8,6.8)	0.001*
LH / FSH	0.8	0.2	0.8 (0.7,0.9)	0.7	0.2	0.7 (0.6,0.8)	0.080

Table 3. Comparison of S.AMH, S.FSH, S.LH, LH/FSH Ratio between Age Group

* indicates statistically significant difference at $P<0.05$

The mean and median of serum AMH are significantly lower in group II (36 - 40 years age) as compared to group I (30 - 35 years age). The mean and median for LH / FSH ratio was lower in group II (older age group) but did not vary significantly in both group with 'p' value of 0.080. Serum FSH and LH values were found to be higher in age group of 35 - 40 as compared to 30 - 35 year age group.

Serum AMH depicted a significant negative correlation of -0.625 with age ($p < 0.01$) (R^2 Linear = 0.391). Serum FSH depicted a significant correlation of 0.520 with age ($p < 0.01$) (R^2 Linear = 0.270). Serum LH show a significant positive correlation of 0.456 with age ($p < 0.01$) (R^2 Linear = 0.208). LH / FSH ratio showed a negative correlation of -0.237 with age ($p < 0.01$) (R^2 Linear = 0.056).

Correlation between serum AMH, FSH, LH and LH / FSH ratio with age within the age group 30 - 35 and 36 - 40 years are shown in table 4.

Variables	Age Group: 30 - 35 (n = 42)	36 - 40 (n = 28)
	Karl Pearson Correlation with Age	
S.AMH (ng / ml)	-.495**	-0.319
S.FSH (mlU / ml)	0.054	.725**
S.LH (mlu / ml)	-0.052	.628**
LH / FSH	-.138**	-0.335

Table 4. Correlations between Serum AMH, FSH, LH, LH/FSH Ratio with Age Group

** Correlation is significant at the 0.01 level (2-tailed), * Correlation is significant at the 0.05 level (2-tailed)

Serum AMH showed significant stronger negative correlation of (r = -0.495) with age in the younger age group 30 - 35 years as compared to older age 35 – 40 (r=-0.319). Serum FSH showed an insignificant correlation with age in 30 - 35 years age group while it showed a significant positive correlation in 36 - 40 years age group with correlation co-efficient 0.725. Serum LH depicted an insignificant correlation of -0.052 with age in 30 - 35 years age group while in 36 - 40 years group the correlation of LH with age was significantly positive with correlation of co-efficient 0.628. LH / FSH ratio showed a weak negative correlation of - 0.159 with age in 30 - 35 years age group as compared to 36 - 40 years group the correlation of LH / FSH with age was negative with correlation of co-efficient - 0.335. This indicated that in the 36 - 40 years of age group, inverse relationship with age is more pronounced than the younger age group of 30 - 35 years.

Correlation between serum AMH, FSH, LH and LH / FSH ratio with age with type of primary and secondary infertility are shown in Figure - 1.

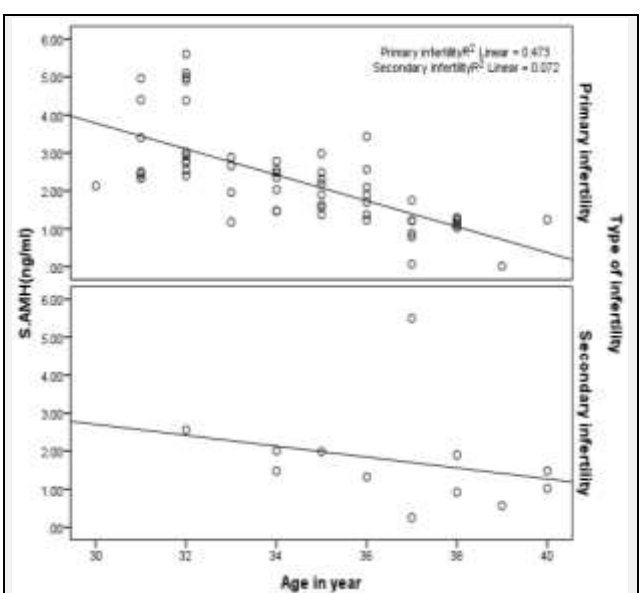

Figure 1A. Correlation with S.AMH and Age with Type of Infertility

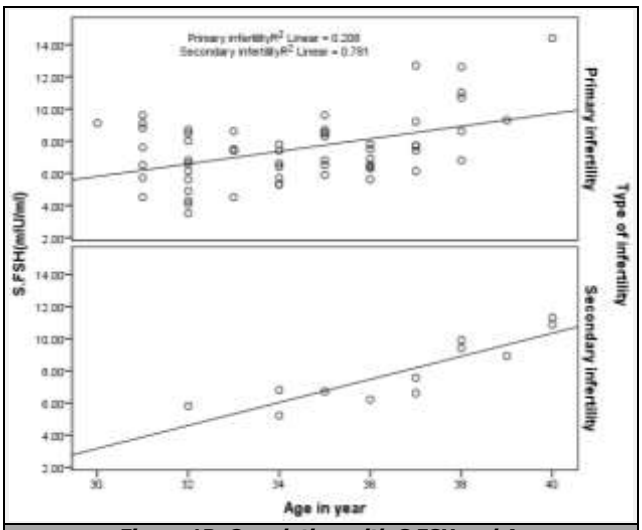

Figure 1B. Correlation with S.FSH and Age with Type of Infertility

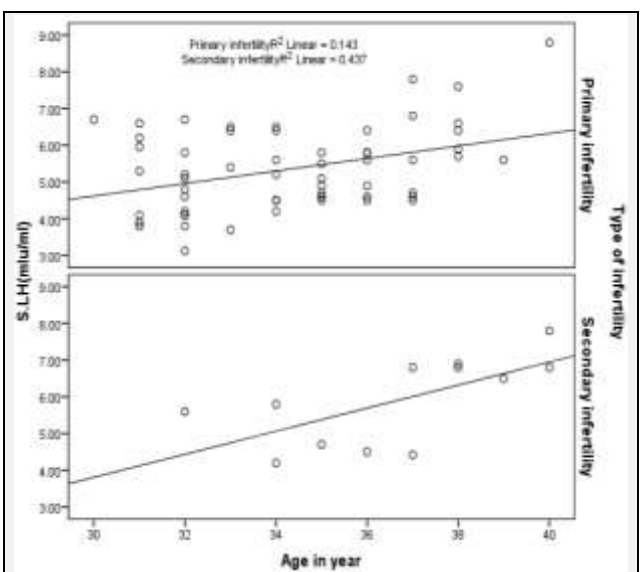

Figure 1C. Correlation with S.LH and Age with Type of Infertility

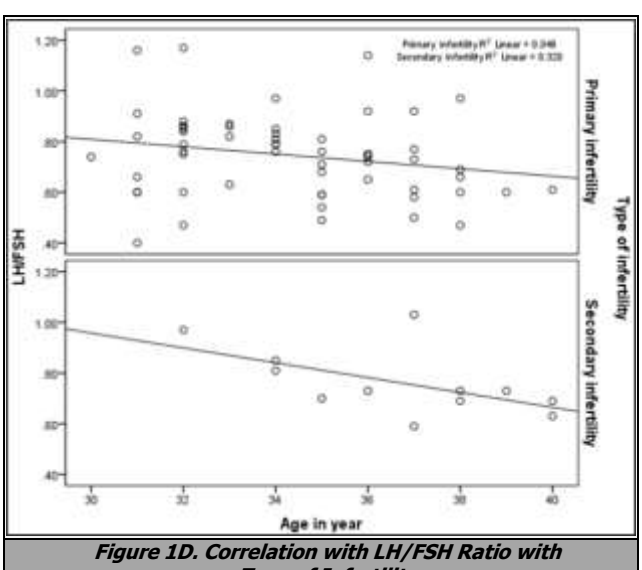

Figure 1D. Correlation with LH/FSH Ratio with Type of Infertility

Serum AMH depicted a significant negative correlation of - 0.688 ($p < 0.01$) with primary infertility and negative correlation of -0.269 with secondary. Serum FSH showed a significant positive correlation of 0.456 with primary infertility and positive correlation of 0.884 with secondary infertility.

Relationship between serum AMH, FSH and LH/FSH ratio are presented in Table 5.

Variable	S.AMH (ng / ml)	S.FSH (mIU / ml)	LH / FSH
S.AMH (ng / ml)	1	-.270*	.220**
S.FSH (mIU / ml)		1	-.642*
LH / FSH			1

Table 5. Correlation between Serum AMH, FSH and LH/FSH Ratio

** Correlation is significant at the 0.01 level (2 - tailed).

Serum level of AMH and FSH showed a significantly negative correlation between each other with correlation coefficient of - 0.270 ($p < 0.05$). Serum AMH level and LH / FSH ratio showed a positive correlation with each other (correlation coefficient 0.220) but not statically significant ($p < 0.067$). Serum FSH and LH / FSH ratio showed a significantly negative correlation with each other (correlation coefficient - 0.642, $p < 0.01$).

DISCUSSION

In the present study, majority of cases (60 %, 42) were present in Group I – 30 - 35 years of age. Twenty eight cases (40 %) were in group II – 36 - 40 years of age. Overall the mean age was 34.7 ± 2.6 years. There was no significant difference in the distribution of cases between the two age groups ($p = 0.094$). Ludmila Barbakadze et al. (2015)[12] studied 112 infertile women and divided subjects into three age groups: group I < 35 years (n = 39, 35 %), group II 35 - 40 years (n = 31, 28 %), and group III 41 - 46 years (n = 42, 37 %). The finding of the previous study was close to our study. The mean BMI in our study was 23.5 ± 1.7. The mean BMI of study done by Jyoti Bala et al. (2018)[13] was 23.26 ± 1.47 which is accordance with this study. In the present study, majority of cases were (58) present with primary infertility. Ludmila Barbakadze et al. (2015)[12] studied 112 cases out which primary infertility was present in 59.3 % (n = 67) of patients. Out of 70 cases, 84.3 % (n = 59) cases were nulliparous and 15.7 % (n = 11) cases had one living child. Out of 70 cases, 88.6 % (n = 62) cases had never undergone any treatment and 11.4 % (n = 8) had undergone ovulation induction with clomiphene citrate.

In our study the mean serum AMH was 2.21 ± 1.26 ng/ml. The mean FSH was 7.57 ± 2.10 mIU / ml, mean LH was 5.45 ± 1.15 mIU / ml and mean LH / FSH ratio was 0.75 ± 0.16. Eddie Racoubian et al. (2020)[14] observed mean serum AMH was 2.47 ± 2.29, FSH was 7.96 (0.09 – 177.80), LH was 7.02 (0.10 – 113.60) and LH / FSH was 0.72 (0.02 – 7.59) in their study. Jong Eun Lee et al. (2015)[15] observed the mean level for AMH 3.53 ± 3.02 ng / mL, serum FSH was 8.87 ± 5.95 mIU / mL and for LH / FSH ratio 0.79 ± 0.51. There was no significant difference in serum AMH ($p = 0.072$), FSH ($p = 0.469$), LH ($p = 0.113$), LH / FSH ratio ($p = 0.925$) between type of infertility. It is found that mean and median of serum AMH

level, are significantly lower in group II (36 - 40 years age) as compared to group I (30 - 35 years age) with 'p' value of 0.000. The mean and median for LH / FSH ratio was lower in group II (older age group) but did not vary significantly in both group with 'p' value of 0.080. FSH and LH values were found to be higher in age group of 35 - 40 as compared to 30 - 35 year age group with 'p' value of 0.002. Study by Jong Eun Lee et al. (2015)[15] observed serum AMH levels and the LH / FSH ratio were significantly decreased in patients with advanced age and basal FSH and LH levels were significantly increased according to age.

Eddie Racoubian et al. (2020)[14] concluded that the mean (± SD) and median AMH were 2.47 ± 2.29 and 1.80, respectively. There was an inverse proportion of AMH and subject's age ($p < 0.001$), which declined from 5.14 ± 3.21 ng/ml in the 20 – 25 year age group, to 0.68 ± 0.45 ng/ml in women older than 25 years. FSH values progressively increased from [median (range)] 5.89 (0.11 – 62.10) ng/ml in the 20 – 25 year category females to 9.17 (0.18 – 167.00) ng / ml in 40 – 45 year-old women. Age-dependent changes in LH / FSH ratio paralleled those of AMH, they dropped from 1.16 (0.11 – 7.59) in 20 – 25 year-old females to 0.56 (0.27 – 2.78) in 45 – 50 year-old females. No clear trend for age-related changes in LH levels were seen ($p = 0.299$).

Serum AMH depicted a significant negative correlation of - 0.625 with age ($p < 0.01$). This indicated that increase in age is associated with decrease in the S. AMH and vice-versa. FSH depicted a significant correlation of 0.520 with age ($p < 0.01$), which indicated that increase in age with increase in serum FSH. Serum LH shows a significant positive correlation of 0.456 with age ($p < 0.01$) which showed that increase in age is likely to result in increase in LH. LH/FSH showed a negative correlation of - 0.237 with age ($p < 0.01$). This indicated that increase in age is likely to result in decrease in the LH/FSH. Jong Eun Lee et al. (2015)[15] and Ludmila Barbakadze et al. (2015)[12] also had similar results corresponding to our study.

AMH level showed significant stronger negative correlation of ($r = - 0.495$) with age in the younger age group 30 - 35 years while showed a negative correlation of ($r = - 0.319$) in older age 35 - 40. This indicates both age groups are negatively correlated with age. Serum FSH level showed an insignificant correlation with age in 30 - 35 years age group while it showed a significant positive correlation in 36 - 40 years age group with correlation co-efficient 0.725. Serum LH depicted an insignificant correlation with age in 30 - 35 years age group while in 36 - 40 years group the correlation of S. LH with age was significantly positive with correlation of co-efficient 0.628. This indicated that in the 30 - 35 years group with correlation of S.LH and age was not significant while the said correlation is very high in 36 - 40 years age group. LH / FSH ratio showed a weak negative correlation with coefficient of - 0.159 in 30 - 35 years age group as compared to 36 - 40 years group, which showed negative correlation with correlation of co-efficient - 0.335. Serum AMH depicted a significant negative correlation of - 0.688 ($p < 0.01$) with primary infertility and negative correlation of - 0.269 with secondary infertility. This indicated in the primary infertility inversely relationship with S. AMH is more pronounced than the secondary infertility. FSH showed a significantly positive correlation of 0.456 with primary infertility and positive correlation of 0.884 with secondary infertility. This indicated that increase in the both in primary and secondary infertility is likely to result in increase in the serum FSH level.

S. LH showed a significantly positive correlation of 0.378 with primary infertility with ($p < 0.01$) level while in secondary infertility the correlation of S. LH with age was significantly positive of 0.661 ($p < 0.05$).This indicated that both the primary and secondary infertility results in increase in S. LH.

LH / FSH depicted a weak negative correlation of - 0.220 with primary infertility ($p < 0.01$) level while in secondary infertility a significantly negative correlation of - 0.566 ($p < 0.01$) level is seen. This indicated that there is an inverse relationship between LH/FSH with primary and secondary infertility.

Serum AMH and FSH showed a significantly negative correlation between each other. However, serum AMH and LH / FSH showed a positive correlation with each other but not statistically significant. FSH and LH / FSH showed a significantly negative correlation. Study by Jong Eun Lee et al. (2015)[15] showed a significant partial correlation between the serum LH/FSH ratio and the AMH when adjusted by age ($r = 0.348$, $P < 0.001$). JyotiBala et al. (2018)[13] concluded a significant inverse relationship between serum AMH and FSH concentration ($r = - 0.488$, $P < 0.001$).

CONCLUSIONS

In our study, serum AMH levels and the LH/FSH ratio was significantly decreased in patients with advanced age. Serum FSH and LH levels were significantly increased according to age. AMH and FSH level showed a significantly negative correlation between each other. Serum AMH level and LH / FSH ration showed a positive correlation but not statistically significant. FSH and LH / FSH ratio showed a significantly negative correlation. We consider serum AMH, a better predictor of ovarian reserve as it is relatively stable throughout the cycle and can be assayed at different time during the cycle whereas FSH and LH varies during cycle.

Data sharing statement provided by the authors is available with the full text of this article at jebmh.com.
Financial or other competing interests: None.
Disclosure forms provided by the authors are available with the full text of this article at jebmh.com.

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