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Original Research Paper

Biochemistry

AMH CORRELATION WITH ENDOGENOUS HORMONES IN FEMALES: INDIAN PERSPECTIVE

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ABSTRACT

Introduction: AMH is an indicator of functional ovarian reserve, it is used in combination with other biochemical and radiological markers in assessing fertility status in women, selecting candidates for IVF stimulation, predicting menopause, diagnosis and management of PCOS. AMH alone may be considered for a good ovarian reserve, either low or high will give an indication of primary ovarian failure or anovulatory cycles as seen in polycystic ovarian syndrome respectively. With this basic investigation all the other hormones are added on to rule out the spectrum of differential diagnosis and plan IVF treatments.

Materials and methods: A retrospective observational study was conducted in 38811 female patients 18 to 45 years of age, divided into 2 groups; abnormal AMH (Group I), and normal AMH values (Group II) as per biological reference ranges for age. Group I was further sub divided into Low AMH Group Ia, and High AMH group Ib, (lower and higher than the cut off for age) respectively. FSH, LH, LH/FSH ratio, E2, Progesterone, DHEAS, Free testosterone, FT3, FT4, TSH, and fasting insulin were studied in these groups.

Results: Statistical analyses were performed using "R Studio version 1.4.1103". A two-tailed p value of <0.05 was considered statistically significant. Kruskal Wallis test was used for comparison of continues variables (Hormones) between the groups. A chi-square categorical test shows statistical significance of difference in values of FSH, LH, LH/FSH ratio, Free and total testosterone, progesterone, fasting insulin, DHEAS and Free T3. No statistical significance was seen with Prolactin, TSH, Free T4, and E2.

Conclusion: Variation of normal and abnormal AMH levels with endogenous hormones plays a vital role in better interpretation of AMH. FSH, LH, free testosterone, fasting insulin, Free T3, significantly correlate in patients with Normal AMH levels.

KEYWORDS : Anti-mullerian hormone, infertility, In Vitro Fertilization, ovarian reserve, PCOS

Introduction and Background

Antimullerian hormone, AMH is an indicator of functional ovarian reserve, it is used in combination with other biochemical and radiological markers in assessing fertility status in women, selecting candidates for IVF stimulation, predicting menopause, management and response to treatment in PCOS. The majority of decrease in AMH is with age, and also to some extent with certain medications such as OCPs, (30-40%). This study is designed, to explore the association of decreased or increased AMH levels with endogenous hormones.

Composition and clinical role of AMH:

Antimullerian hormone AMH is a glycoprotein, belongs to the TGF beta family of proteins. It is secreted upon gene expression from the granulosa cells of the pre-antral or growing follicles (2-8mm in size) of each ovary, which are closest to the primordial follicle pool, which amounts to around 400-500 oocytes. [1]

Low levels of AMH <0.5-1.1ng/ml when interpreted along with AFC count of <5-7 follicles is considered as low ovarian reserve according to the Bologna Criteria [2]

Physiology of AMH and hormonal balance:

As the size of the follicles grows towards selection and formation of the dominant graffian follicle which subsequently undergoes ovulation, AMH value decreases in the sense these follicles do not secrete AMH, but now start secreting E2 which signals the FSH and LH to be secreted by the pituitary, on 3rd-4th day of the cycle. Drop in AMH when the preantral follicles mature into the bigger follicles >7mm in size on there way to ovulation sensitizes E2 and FSH to be secreted which is a good indication for a successful ovulation. The LH peak marks ovulation about day 14 of the cycle after which the remnants of the follicles turn into the corpus luteum which starts secreting progesterone about day 21 when progesterone is at its peak, all these hormones at specific stages and days of menstrual cycle are clinically measured and used to assess fertility.

AMH has been studied in conjunction with FSH, and E2 in many studies, which are routinely used by fertility specialists. In this study we see the variation and impact of other hormones with AMH.

MATERIALS AND METHODS:

An observational retrospective study was conducted with data collected over 1.5 years at the Global Reference Laboratory of Metropolis health care with the aim to explore the association of AMH in patients with abnormal and normal AMH levels and endogenous hormones with AMH in women. Data was collected from the laboratory information system, from patient samples from various parts of India.

Inclusion Criteria:

Female patients in the age group 18-45 years undergoing AMH estimation for investigation of infertility, selection of suitable cases for IVF, Polycystic ovarian syndrome, family planning, estimation of menopause, were included. Exclusion criteria: Male patients and female patients below the age of 18 yrs and above the age of 45 years were excluded.

A total of 38811 patient samples were divided into abnormal AMH (Group I) and normal AMH values (Group II) as per age adjusted reference levels for AMH. Group I was further sub divided into 2 sub categories into Low AMH Group Ia, (lower than the cut off for age) and High AMH group Ib Values (higher than the cut off for age) as per published biological reference ranges for age given by Roche assay. Keeping these groups as fixed variables, we have studied the statistical correlation of FSH, LH, LH/FSH ratio, E2, Progesterone, DHEAS, Free testosterone, FT3, FT4, TSH, fasting insulin.

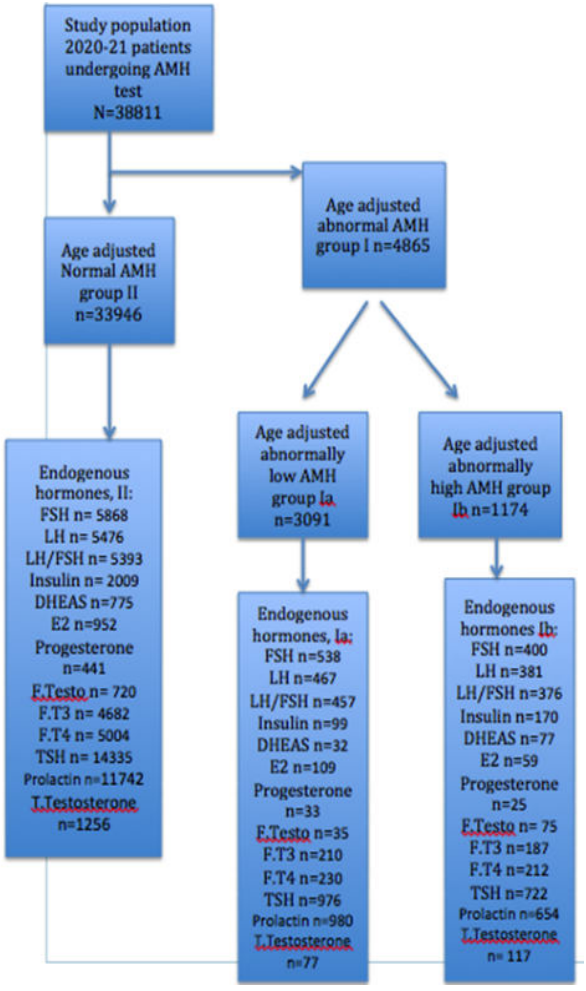

Figure 1: Flowchart of study population:

Retrospective cohort of patients undergoing AMH test at Global reference laboratory of Metropolis health care collected over 1.5 years (January 2020 to July 2021)

Patient consent was taken as per institutional guidelines. All samples were collected by veniputure in gel tubes transported to the central laboratory at 2-8C and analyzed within 24 hours of collection. Samples for FSH and E2 were taken on day 3-5 of menstrual cycle, 8-12 hours of Fasting samples were taken for insulin.

All hormonal tests were analyzed by GEN II Elecsys, by Roche diagnostics. Normal and abnormal levels of AMH were segregated based on Biological expected reference intervals as per Roche kit/ (Roche study No. RD001727) for ages above 20 years. For ages 18 to 20 years, published guidelines reference ranges were used [3]

The analytical senstivity of AMH was 0.01ng/ml. Interassay CV was 3.5%

Results And Statistics:

Data recording was done in MS Excel. Continuous variables are reported as Mean + Standard deviation (SD), median {Interquartile range (IQR)} and range. Categorical variables are summarized in terms of frequencies and percentages. Shapiro-Wilk test was used to determine the difference from a normal distribution. For comparison of continues variables (Hormones) between three groups (AMH [low, high, normal group]) Kruskal Wallis test were used and Mann Whitney U test for two groups (AMH [Normal, Abnormal group]). Pearson's correlation coefficient was used to analyze AMH values and Hormones and chi square test is used for categorical variables. Statistical analyses were performed using "R Studio version 1.4.1103". A two-tailed p value of <0.05 was considered to be statistically significant.

Variation of AMH with hormones:

Comparing AMH with hormones, we have found significant variation in both groups normal II and abnormal I and subgroups Ia, Ib with FSH, LH, and LH/FSH ratio. When correlated with individual hormones, group Ia low AMH levels showed significantly negative correlation with FSH, p value 0.0001 95% CI (-0.51 to -0.38) LH, p value 0.0001 95% CI (-0.43 to -0.27) LH/FSH ratio p value 0.0001, 95% CI (0.10 to 0.28). Group Ib high AMH levels showed significant negative correlation with FSH, p value 0.0002, 95% CI (-0.28 to -0.09), and fasting insulin p value 0.0335 95% CI (-0.31 to -0.01) , and a positive correlation with Free T3 p value 0.0046, 95% CI (0.06 to 0.34) and LH/FSH ratio p value 0.0002 95% CI (0.09 to 0.28). Group II normal AMH levels showed significant negative correlation with FSH p value 0.0001 95% CI (-0.26 to -0.21), progesterone p value 0.0004 95% CI (-0.2587 to -0.0773, TSH. A significant positive correlation was seen with LH p value 0.0001 95% CI (0.05 to 0.10) LH/FSH ratio p value 0.0001 95% CI (0.28 to 0.33), DHEAS p value 0.0003 95% CI (0.06 to 0.20), Free testosterone p value 0.0008 95% CI (0.05 to 0.20), Free T3 p value 0.0001 95% CI (0.06 to 0.12) and Total Testosterone p value 0.0001, 95% CI (0.199 to 0.30) There was no correlation of normal AMH values with TSH, E2 Free T4, fasting insulin. Prolactin and TSH were not found to be significant in both normal and abnormal groups.

Table 1: distribution of Age and AMH values in the current study

	n	Mean±SD	Median(IQR)	Range
Age (years)	38811	31.13±5.93	31(27 - 35)	18 – 45
AMH (ng/ml)	38811	3.24±3.05	2.43(1.09 - 4.44)	0.01 – >23

Table 2: Age wise distribution of samples

Age group	n	%
18 – 25	7064	18.20%
26 – 35	22523	58.03%
36 – 45	9224	23.77%

Table 3: Age wise frequency and percentage Distribution of Group 1 (a Low, b High) and Group II

Age group	AMH					
	High (Ib) n=1774(4.57%)x		Normal n=33946(87.46%)		Normal n=33946 (87.46%)	
	n	%	n	%	n	%
18 – 25	425	23.96%	511	16.53%	6128	18.05%
26 – 35	1189	67.02%	2048	66.26%	19286	56.81%
36 – 45	160	9.02%	532	17.21%	8532	25.13%

Table 4: Distribution of AMH values in Normal, High and low AMH groups

Age group	AMH	AMH		
		Mean ± SD	Median (IQR)	Range
18 – 25	High	14.12±3.30	13.20 (11.86 – 15.74)	9.58 – 24
	Low	0.56±0.37	0.582 (0.142 – 0.872)	0.01 – 1.21
	Normal	4.66±2.39	4.21(2.75 – 6.15)	0.92 – 11.70
26 – 35	High	11.98±3.39	11.05(9.57 – 13.50)	7.54 – 24
	Low	0.33±0.22	0.299(0.10 – 0.491)	0.01 – 0.889
	Normal	3.23±0.1.99	2.79(1.66 – 4.37)	0.15 – 9.85
36 – 45	High	8.84±2.90	8.33(7.42 – 9.79)	2.27 – 24
	Low	0.09±0.03	0.10(0.10 – 0.10)	0.01 – 0.334
	Normal	1.42±1.38	0.992(0.375 – 2.02)	0.01 – 7.47

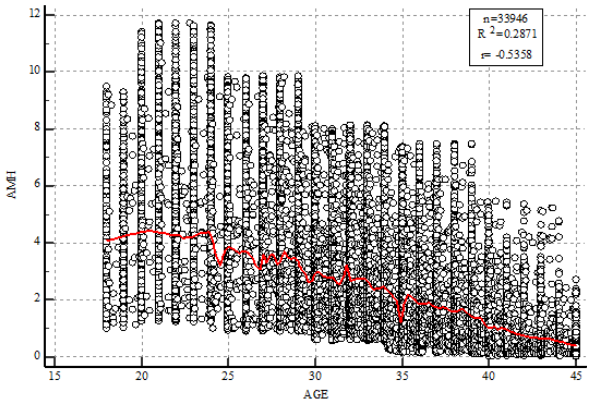

Fig 2 : Group II, Normal AMH levels with Age distribution

Table 5: Correlation of Group Iα (Low) AMH value with hormones

Parameters	correlation coefficient (r)	95% CI of r	p value
FSH n=538	-0.4528	-0.5175 to -0.3829	0.0001
LH n=467	-0.3592	-0.4357 to -0.2775	0.0001
LH/FSH n=457	0.1926	0.1027 to 0.2794	0.0001
Fasting Insulin n=99	-0.0040	-0.2013 to 0.1935	0.9680
E2 - Estradiol n=109	-0.01541	-0.2029 to 0.1732	0.8736
DHEAS n=32	-0.0095	-0.3570 to 0.3403	0.9588
Testosterone (Free) n=35	0.1065	-0.2351 to 0.4247	0.5424
Progesterone n=33	-0.03901	-0.3773 to 0.3084	0.8294
Free T3 n=210	-0.0159	-0.151 to 0.1198	0.8189
Free T4 n=230	-0.04403	-0.1724 to 0.08581	0.5064
TSH n=976	-0.04240	-0.1049 to 0.02041	0.1857
Prolactin n =890	0.03524	-0.0305 to 0.1007	0.2936
Testosterone (Total) n=77	0.03218	-0.1932 to 0.2543	0.7812

Table 6: Correlation of Group Ib (High) AMH value with hormones

Parameters	Correlation coefficient (r)	95% CI of r	P value
FSH n =400	-0.1858	-0.2788 to -0.0893	0.0002
LH n=381	0.06475	-0.0359 to 0.1642	0.2073
LH/FSH n = 376	0.1885	0.0890 to 0.2842	0.0002
Fasting Insulin n=170	-0.1632	-0.3062 to -0.0129	0.0335
E2 - Estradiol n =59	-0.0187	-0.2735 to 0.2385	0.8881
DHEAS n =77	0.01381	-0.2108 to 0.2371	0.9051
Testosterone (Free) n=75	0.1294	-0.1005 to 0.3462	0.2685
Progesterone n=25	0.05547	-0.3473 to 0.4409	0.7923
Free T3 n=187	0.2062	0.06458 to 0.3396	0.0046
Free T4 n=212	-0.01435	-0.1488 to 0.1206	0.8354
TSH n=722	-0.01001	-0.08292 to 0.063	0.7882
Prolactin n =654	-0.007848	-0.0844 to 0.0688	0.8412
Testosterone (Total) n=117	0.1732	-0.0085 to 0.3439	0.0618

Table 7: Correlation of Group II (Normal) AMH value with hormones

Parameters	Correlation coefficient (r)	95% CI of r	p value
FSH n=5868	-0.2373	-0.2613 to -0.2130	0.0001
LH n=5476	0.07224	0.04584 to 0.0985	0.0001
LH/FSH n=5393	0.3062	0.2818 to 0.3301	0.0001
Fasting Insulin n=2009	0.009343	-0.03440 to 0.0530	0.6756
E2 - Estradiol n=952	-0.05995	-0.1230 to 0.00360	0.0645
DHEAS n=775	0.1303	0.06041 to 0.1989	0.0003
Testosterone (Free) n=720	0.1250	0.05236 to 0.1962	0.0008
Progesterone n=441	-0.1695	-0.2587 to -0.0773	0.0004
Free T3 n=4682	0.09090	0.06242 to 0.1192	0.0001
Free T4 n=5004	0.005453	-0.02226 to 0.0331	0.6998
TSH n=14335	-0.006990	-0.02336 to 0.0093	0.4027
Prolactin n =11742	-0.01592	-0.034 to 0.0021	0.0845
Testosterone (Total) n=1256	0.2518	0.1993 to 0.3029	0.0001

DISCUSSION:

A total number of 38811 female patients were studied, amongst this, 33946 (87.46%) had normal AMH values. The remaining n=4865 (12.54%) were abnormal of this n=3091 (63.53%) accounted for low AMH reflecting a low ovarian reserve, and 1774 (36.46%) were above the normal reference levels of AMH. Ovulatory dysfunction according to few studies accounts for about 40% of cases in women, in which PCOS has been one of the etiological causes. [4]

The mean AMH levels in the high abnormal group were 14.12, 11.98 and 8.84 ng/ml in the groups 18-25, 26-35 and 36-45 years respectively. Biochemical cut off for PCOS have been assigned as over 4ng/ml. These patients are also poor responders to IVF and or may result in OHSS in IVF stimulation procedures.

Our study shows the age related decline in both abnormal and normal groups, with 54.5% in Group 1α , 43.7% in Group Ib) and 53.6% in-group II, which is statistically significant with p value of 0.0001, {Table 4}. A peak in AMH levels is seen 20-25 age group followed by a decline in trend, which is similar to the observations in other studies.(fig 2)

Few prospective studies have show an early age related decline in AMH may lead to early menopause. Our data shows a decline in AMH in-group Iα levels below 1ng/ml are seen in the age group as early as 18 years upto 40 years as do evidences in other studies. [5], [6], [7].

Protocols for infertility investigations include serum FSH, LH, E2 in early follicular phase, and progesterone at day 21, DHEAS, TSH, prolactin, androgens such as free testosterone in cases of irregular cycles.

Ovarian reserve tests are ideally performed on a sample collected on day 3 of the cycle due to fluctuation of hormones during menstrual cycle, except for AMH which is stable during menstrual cycles. FSH, AMH, E2, inhibin B along with Ultrasound estimation of AFC have been the gold standard to derive the ovarian reserve required for fertility as well as probability or likelihood of pregnancy. AMH alone may be considered for a good ovarian reserve, either low or high will give an indication of primary ovarian failure or anovulatory cycles as seen in polycystic ovarian syndrome respectively. With this basic investigation all the other hormones are added on to rule out the spectrum of differential diagnosis and plan IVF treatments.

Role of FSH in female reproductive cycle is more when the dominant follicle is to be released by the ovary for ovulation. The signal released by this follicle should be enough for FSH to be released. The lower the reserve the higher the FSH required for the ovary to ovulate. FSH assesses the possibility of pregnancy with levels as much as >10 IU/ml which indicate a lower reserve, and levels around 20 IU/ml, diminishing possibility of pregnancy to negligible. Studies have shown

that women with low ovarian reserve may still show a normal FSH or lower which may be favorable towards achieving pregnancy. [8]

Hence, although an inverse relationship of AMH with FSH exists, the degree of this negative correlation may reflect on the over all fertility in women.

Our study shows, significant negative correlation (p=0.0001) of FSH with AMH i.e 24%, 45%, 19% in normal, abnormal Low and high groups respectively. The negative correlation is profound in low AMH group. The mean AMH in the low group was 0.56+/-0.37 ng/ml, 0.33+/-0.22, 0.09+/-0.03 in the age groups 18-25yrs, 26-35yrs and 36-45 years respectively. Studies have also shown a decline in ovarian reserve way before FSH levels start reflecting this biochemically [9]

However, AMH is a more reliable and earliest marker of declining ovarian reserve in fertile women. [10] Clinical utility of FSH also lies in diagnosis of primary ovarian failure, where there is a Primary ovarian insufficiency, which affects 1 in 10,000 women by age 20, 1 in 1000 by age 30, and 1 in 100 by age 40.[11]

Primary ovarian insufficiency resulting in a diminished or premature exhaustion of the primordial follicular pool is diagnosed in women below 40 yrs of age with 2 consecutive FSH levels corresponding to menopausal age. These patients also have decreased AMH levels of below 1.1 ng/ml.

E2 in infertility, plays more of a role towards planning adequate IVF dosage. Few studies have postulated that expression of AMH in the pre-antral stages is maximal due to the effect of androgens on FSH and growth factors in the granulosa cells. As the follicle grows in size this effect wanes off and a switch to E2 production is seen which in turns switches off AMH production from these follicles. [12] E2 may not represent the ovarian reserve, however it does give an assessment of follicular growth.

Basal levels of E2 are used in clinical practice primarily to assess the response of IVF treatments. A high basal level may indicate a poor responder of oocyte retrieval or pregnancy rate. Low levels of E2 and FSH on day 3 were reported to have better response to pregnancy then higher levels.[13]

As the preantral follicle transforms into antral follicle, E2 starts increasing however, since AMH is secreted by the pre antral pool it remains stable in circulation albeit no longer secreted by the growing antral follicles that release E2. In our study, we did not find any statistical significance of E2 with AMH. A study to evaluate the repressive effect of AMH on E2 by, La Marca et al. (2004) [14] and Liberty et al. (2010), showed no correlation of E2 with AMH except in a group of patients who underwent ovarian stimulation.[15]

Other studies done by (Lee et al., 2010) and Andersen and Byskov, 2006; Andersen and Lossl, 2008; Nielsen et al., 2011). showed a negative correlation of AMH with E2 respectively in patients receiving hCG stimulation.[16] [17]

Progesterone is measured as an indicator of ovulation and values greater than 3 ng/mL are considered as evidence of recent ovulation. Our data shows significant negative correlation of progesterone in patients with Normal AMH values (p=0.0004), 95% CI -0.26 to -0.08. And no correlation in patients with low or high abnormal AMH levels.

Women with infertility over 30 years of age, with a history of hypothyroidism, or autoimmune thyroid disease, or signs and symptoms suggestive of hypothyroidism with TSH>2.5mIU/L are usually prescribed thyroxine treatment; TSH is monitored in these patients to check for response of treatment and maintenance of thyroid function. Our study shows TSH levels shows no correlation with AMH in both normal and abnormal AMH groups. [18]

Few studies have shown the relevance of automimmune hypothyroidism with premature ovarian failure, hence monitoring TSH, Free T4, antithyroid antibodies is recommended along with treatment. Our study shows a significant positive correlation of Free T3 with AMH (p=0.0046), with a 95% CI of 0.065 to 0.34 in patients with high abnormal AMH and in patients with normal AMH (p=0.0001) 95% CI 0.06-0.12

As per Keiji et al study done on 66 Japanese infertile women of reproductive age AMH levels inversely correlated with patient age and TSH levels in patients with decreased ovarian function without other factors affecting thyroid and ovarian function. [19]

In a study of 2155 Infertile women aged between 26 to 46 years a weak positive correlation was observed between AMH and DHEAS levels. Serum DHEA-S values were positively associated with serum AMH levels for all subjects, for women aged below and above 35 years.[20]

Our study shows a positive significant correlation (p=0.0003) of AMH with DHEAS in group II, 95% CI (0.060-0.198).

Androgens mainly testosterone play a role in folliculogenesis by inducing follicular growth through FSH and IGF-I. As per a retrospective study of 1300 patients between 16-43 years for AMH and androgen profile analysis showed no correlation between testosterone and AMH.

Nardo et al. demonstrated a direct but weak relationship between testosterone and AMH levels both in PCOS and non PCOS groups. Few studies demonstrated a direct relation between AMH and testosterone merely in PCOS group. [21]

Our study shows a significant (p=0.0008) 95% CI (0.052-0.196) positive correlation and p value 0.0001 95% CI (0.1993 to 0.3029) of free testosterone and Total testosterone with AMH in normal group II respectively. Whereas no significant correlation was found in low Ia (n=35), and high Ib (n=75) group with free testosterone or Total testosterone Ia (n=77), and Ib (n=117).

In a retrospective study of women diagnosed with PCOS (461) 31.24% of patients were diagnosed with insulin resistance. A negative relationship between AMH and insulin level was observed and higher AMH levels were associated with decreased risk of insulin resistance in this study.[22]

In our study we found a negative correlation of fasting insulin 16% in high abnormal group Ib (n=170) with AMH (p=0.0335), 95% CI (-0.306 to -0.012). No correlation was found in normal group II (n=2009) and abnormal low group Ia (n=99).

In a prospective study done on 200 PCOS women in Egypt AMH was measured along with other hormones FSH, LH, SHBG, Testosterone and Prolactin. Serum AMH levels showed a statistically significant positive correlation with Testosterone. A weak negative correlation between serum AMH levels, fasting glucose levels and serum insulin [23]

A prospective study done on 18 PCOS and 18 Normal women showed higher levels of LH, in women with PCOS compared to levels in normal women. Our study shows a significant negative correlation of AMH with LH 36% in Low abnormal group Ia (n=467) p= 0.0001 95% CI -0.44 to -0.28, and normal group II (n=5476) p=0.0001 95% CI 0.045-0.098. No correlation in high abnormal Ib (n=381). [24]

CONCLUSION:

AMH plays an important role as an indicator of ovarian reserve at a particular age in the female reproductive stage. It is important to correlate hormones with AMH at different stages of folliculogenesis and also the effect of these hormones on fertility in order to interpret ovarian reserve and selection of patients for adequate dosage and type of IVF treatments. The panels of hormones included in AMH profiles may significantly impact clinical care.

Ethical Approval

The study was conducted retrospectively from the data available in laboratory information system of the laboratory. We had obtained approval to use this data for publication from Conscience Independent Ethics Committee wide letter dated 2nd June 2021.

Limitations:

Clinical condition and history of medication and sample collection dates with respect to menstrual cycle were not available for the samples in this study.

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