

## Biochemical Parameters Serving As Prognostic Indicators of Ovarian Reserve in Females with Unexplained Sub-Fertility

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**Abstract:** Ovarian reserve plays a crucial role in achieving pregnancy following any treatment in subfertile women. The estimation of ovarian reserve is routinely performed through various ovarian reserve tests (ORTs) in an effort to predict the response and outcome in couples prior to In Vitro Fertilization (IVF) and counsel them. Most widely used tests are estimation of basal follicle stimulating hormone (FSH) and anti-Mullerian hormone (AMH) and antral follicle count (AFC). In our routine practice the role of different biochemical parameters to estimate ovarian reserve of subfertile women is discussed in this article.

**Keywords:** Subfertility, ovarian reserve tests (ORT), anti-mullerian hormone (AMH), leutinizing hormone (LH).

### I. Introduction

Delayed child-bearing, voluntary or involuntary, is a common feature in couples visiting fertility clinics. Majority of the fertility clinics perform ovarian reserve tests (ORTs) as part of the evaluation of women with infertility prior to *In Vitro* fertilization (IVF). Diminishing ovarian reserve is a phenomenon noted in women during mid to late thirties and at times earlier, reflecting the declining follicular pool and oocyte quality.<sup>1</sup> The age related decline of ovarian reserve is believed to be more than double when follicle numbers fall below a critical figure of 25,000 at 37.5 years of age.<sup>2</sup> Assuming fixed time differences between reproductive milestones, fertility will not be lost completely for next 4 years, on an average following the onset of this phase.<sup>3</sup> ORTs provide an indirect estimate of a woman's remaining follicular pool. An ideal ORT should be easy to perform, reproducible and the decisions based on their results should help differentiate women with a normal and poor ovarian reserve. Various factors on which ovarian reserve depend are age, concentration of basal serum Follicle Stimulating Hormone (FSH), Estradiol (E<sub>2</sub>), AMH, Inhibin B etc. The various tests done are Clomiphene Citrate Challenge Test, FSH & Gonadotropin Releasing Hormone (GnRH) Challenge test, Ultrasonological estimation of ovarian volume and AFC. Ovarian biopsy is also done. The present study was undertaken to identify the various biochemical and other factors which predict the ovarian reserve in women of unexplained subfertility. The factors to be studied are – BMI, AMH, FSH, LH, TSH and their interdependence on each other.

#### General Objective:

Assessment of ovarian reserve in women with unexplained subfertility.

#### Specific Objective(S):

1. To find out correlates (BMI and basal FSH, LH, TSH and AMH concentration) of infertility.
2. To estimate the interrelationship of these factors.
3. To establish the accuracy of these factors as predictive indicator of infertility

### II. Materials And Methods

A descriptive cross-sectional comparison study was carried out in the Department of Biochemistry, Medical College, Kolkata involving the patients suffering from infertility attending the Out Patient Department (OPD) of Gynecology & Obstetrics of the same medical college during the period of December, 2013 to June, 2014.

**Inclusion Criteria**

Female aged 25 – 35 years with documented history of unexplained subfertility were selected in the ‘study group’.

**Exclusion Criteria**

1. Patients suffering from chronic diseases like Diabetes mellitus, Cancer, Renal Failure, Liver Diseases etc.
2. Undergoing treatment for subfertility.
3. Undergoing treatment for any other endocrine disorder viz. thyroid disorders
4. Ultrasonographically proven absence of either or both ovaries
5. Ultrasonographically or clinically proven infertility due to non-ovarian causes viz. infertility of uterine and or tubal origin
6. Patients on prolonged drug therapy such as methotrexate, phenytoin, theophylline, niacin, fibrates etc.

**Selecting Participants**

Fifty patients of subfertility attending OPD, G & O, Medical College, Kolkata were selected for the ‘study group’. Fifty age-matched fertile women attended same OPD during the same time interval for other morbidities were enrolled into the ‘comparison group’. One infertile patient and one age matched control were selected by simple random sampling technique on infertility clinic day once in a week. After fully explaining the study, an informed consent was obtained from every participant. For the current study, permission from the Institutional Ethics Committee was duly obtained.

**Collection Of Information And Laboratory Sample**

Baseline information was collected by interview using a predesigned and pretested questionnaire. Body weight and height of both cases and controls were taken following standard procedures. The selected subjects were asked to attend the department of Biochemistry, Medical College, Kolkata next day in fasting state. Approximate 5 ml fasting blood sample(s) was collected from each subject by single needle prick. Collected samples were centrifuged and the supernatant was kept for further storage/analysis. Centrifugation and separation were carried out within 30-45 minutes after collection of samples. The sera were stored at minus 20°C for hormonal assay and analyzed within 15 days.

**Biochemical Assays:**

Routine biochemical parameter such as fasting plasma glucose (FPG) was measured for all the subjects under study using automated clinical analyzer (model Daytona, Randox). Hormones like AMH, LH, TSH, FSH levels were estimated by ELISA (Enzyme linked Immunosorbent Assay) Technique.

**Statistical Analysis**

Collected data were analysed by SPSS 22 version. Mean and standard deviation were calculated for describing the variables. Data display was done with the help of charts and tables. Statistical tests like independent ‘t’ test, Pearson correlation coefficient (r), multiple binary logistic regressions, receiver operation curve (ROC) with area under the curve (AUC) analysis; sensitivity, specificity, positive & negative predictive values of test were used for drawing statistical inference about the relationship between the variables as well as the diagnostic predictivity of serum markers of ovarian reserve. P value of <0.05 was considered statistically significant at 95% confidence interval (CI).

**III. Results**

Analysis of data reflected that the cases and control groups were comparable in respect of age, serum level of fT4, TSH, FSH, prolactin, FPG and BMI as per the p value and 95% CI of standard error of difference (Table-1). However, the groups had difference in serum level of AMH and LH. The serum AMH level and serum LH level were found to be significantly low and high, respectively among infertile women compared to their counter- part. (Table-1)

**Table-1: Between Groups Difference In Various Biomarkers**

Markers	t-test for Equality of Means							
	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
							Lower	Upper
AMH	0.076	-6.166	58	.000	-4.25533	.69016	-5.63684	-2.87382
TSH	0.237	2.096	58	.040	1.00033	.47735	.04480	1.95586
FSH	0.001	2.070	58	.043	7.71967	3.72923	.25479	15.18454
LH	0.000	2.650	58	.010	4.27833	1.61468	1.04619	7.51047
BMI	0.010	2.571	58	.013	2.88833	1.12328	.63985	5.13681

Analysis also revealed that serum hormone level as well as BMI had some sort of correlation amongst themselves. AMH showed significant weak to moderate negative linear correlation with age and serum TSH. LH was found to have significant correlation with FSH & BMI. (Table-2)

**Table-2:** Correlations between various attributes of Participants (N=60)

Marker	Test & probability	Age	AMH	TSH	FSH	LH	BMI
Age	Pearson Correlation	1	-0.300	-0.054	0.028	-0.084	0.150
	Sig. (2-tailed)	NA	0.020	0.679	0.830	0.525	0.253
AMH	Pearson Correlation	-0.300	1	-.269*	-.076	.036	-.218
	Sig. (2-tailed)	0.020	NA	.038	.565	.785	.094
TSH	Pearson Correlation	-0.054	-.269*	1	.065	.105	.013
	Sig. (2-tailed)	0.679	.038	NA	.622	.423	.921
FSH	Pearson Correlation	0.028	-.076	.065	1	.666**	.046
	Sig. (2-tailed)	0.830	.565	.622	NA	.000	.727
LH	Pearson Correlation	-0.084	.036	.105	.666**	1	.261*
	Sig. (2-tailed)	0.525	.785	.423	.000	NA	.044
BMI	Pearson Correlation	0.150	-.218	.013	.046	.261*	1
	Sig. (2-tailed)	0.253	.094	.921	.727	.044	NA
*. Correlation is significant at the 0.05 level (2-tailed).							
**. Correlation is significant at the 0.01 level (2-tailed). NA=not applicable							

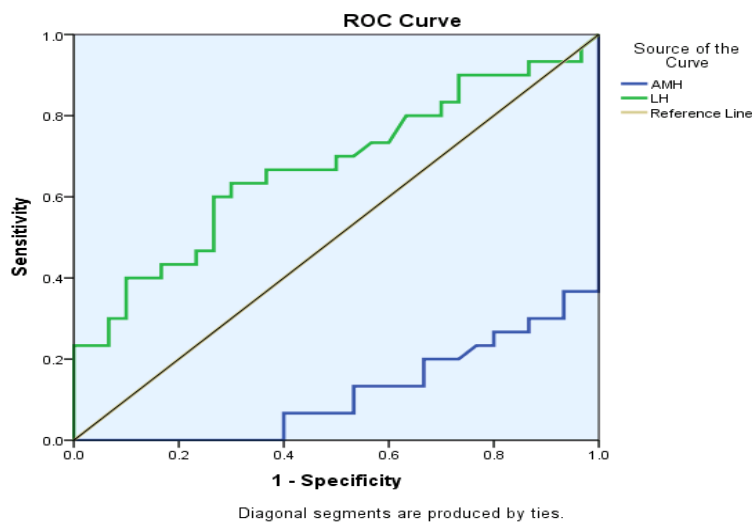
Multiple logistic regressions involving infertility as binary outcome variable [absent (fertile) and present (infertile)] and serum level of AMH, LH, FSH, TSH and BMI (which were shown to be associated with infertility with P value of <0.05 at 95% confidence level) as input variables revealed that infertility had a negative linear relationship with AMH and a positive relationship with the serum LH level. About 66% variation in infertility could be explained by changes in these two serum markers with high significant model fit. (Table-3)

**Table-3:** Multiple logistic regressions showing interrelationship of variables

Variables in the Equation		B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I.for EXP(B)	
								Lower	Upper
Step 1	AMH	-1.568	.539	8.471	1	.004	.208	.073	.599
	TSH	.510	.406	1.582	1	.208	1.666	.752	3.691
	FSH	-.038	.061	.395	1	.530	.963	.855	1.084
	LH	.432	.165	6.867	1	.009	1.541	1.115	2.129
	BMI	.128	.120	1.130	1	.288	1.136	.898	1.438
	Constant	-.314	3.809	.007	1	.934	.731		

a. Variable(s) entered on step 1: AMH, TSH, FSH, LH, BMI.

For resolving the query whether any of these two markers of ovarian function could predict the infertility, receiver operation curve (ROC) analysis was done. In this regard serum LH level was found to be more reliable than serum AMH level as it yielded a higher area under curve (AUC) clearly above the reference level (diagonal line in figure-1). (Fig.1) AUC is an indicator of overall performance of the test/marker. (Table-4)



**Fig. 1:** Results from receiver operation curve (ROC) analysis

**Table-4: Area Under the Curve (AUC)**

Test result variable(s)	Area	Std. Error <sup>a</sup>	Asymptotic Sig. <sup>b</sup>	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
AMH	.117	.042	.000	.035	.200
LH	.672	.070	.022	.535	.810
The test result variable(s): AMH, LH has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased.					
a. Under the nonparametric assumption					
b. Null hypothesis: true area = 0.5					

It was also reflected that at a cut-off of 4.05mIU/ml serum level of LH had an optimum sensitivity and specificity of 66.7% and 63.3%, respectively to predict infertility among the target women. At this cut-off level the predictor had positive and negative predictive values of 64.5% and 65.5%, respectively. (Table-5)

**Table-5: Distribution of cases and control as per the result of serum LH level at or above the cut-off of 4.05mIU/ml**

Test result	Cases	Control	Total
	No. (%)	No. (%)	No. (%)
High LH level	20 (33.33)	11 (18.33)	31 (51.67)
Normal/less LH level	10 (16.67)	19 (31.67)	29 (48.33)
<b>Total</b>	30 (50.0)	30 (50.0)	60 (100)

Sensitivity=20/30=66.7%, Specificity=19/30=63.3%;

Positive Predictive Value = 20/31 = 64.5%, Negative Predictive value = 19/29 = 65.5%

However, this marker may yield a high false positivity and false negativity rate of 11/30=36.7% and 10/30=33.3%, respectively.

#### IV. Discussion

Infertility is defined as the inability to conceive after one year of unprotected intercourse of reasonably frequencies. Most couples are more correctly considered to be subfertile, rather than infertile, as they will ultimately conceive if given enough time.<sup>4</sup> An association between the age of women and reduced fertility is well documented. Women in their mid to late 30s and early 40s constitute an important part of the subfertile population due to decline in oocyte quantity and quality. Disorders of ovulation account for about 20% to 40% of all cases of female subfertility.<sup>5</sup> Many of them require expensive treatments including Assisted Reproductive Technologies (ART). Performing an Ovarian Reserve Test (ORT) is an effort toward not only the estimation of the primordial follicle pool but also for determining how the ovaries will respond to ART. Moreover, the ovarian reserve is determined not only by the size of the ovarian follicle pool and but also the quality of the oocytes therein.<sup>6</sup>

The ideal parameter to estimate ovarian reserve would be easily measurable, minimally invasive, inexpensive, and have good predictive value for the outcome assessed. Biochemical parameters like FSH, LH, AMH & TSH with a special emphasis on AMH as the screening tests for ovarian reserve of the subfertile women attending in a tertiary care hospital was tried to be established in the current study. AMH is one of the basal biochemical markers found to predict the ovarian response to ovulation induction by human gonadotropin therapy, both poor and hyper, with a high sensitivity and specificity.<sup>7</sup> AMH shows distinct age-related declines at a very young age, much earlier than other markers including AFC.<sup>8</sup> Serum AMH levels show minimal intra and inter cycle fluctuations and thus can be performed at any stage of the menstrual cycle.<sup>9</sup> In conditions with high LH and normal or low FSH levels, as in polycystic ovarian syndrome (PCOS), AMH concentrations are positively correlated with LH concentrations.<sup>10</sup>

TSH is included as hypothyroidism is now-a-days common in female and one cause of subfertility.<sup>11,12</sup> In our study, no relationship was found between AMH and BMI, confirming earlier observations in a group of subfertile subjects (Nardo et al., 2007) although this was in contrast to other studies (Freeman et al., 2007; Chen et al., 2008).<sup>13,14</sup> Pigny and colleagues (2003) also found that BMI did not influence the circulating AMH.<sup>15,16</sup> Differences in study populations, clinical setting have to be borne in mind to explain discrepancies between the studies. In this study, AMH showed significant weak to moderate negative linear correlation with age and TSH (Table 2) but no relation with LH and BMI though LH is positively correlated with BMI and FSH. Subfertile group shows higher LH and lower AMH compared to fertile group. As a routine biochemical parameter for investigation of subfertile patient serum LH level was found to be more reliable than serum AMH.

## V. Conclusions

ORTs do have a moderate ability to predict poor and hyper-response of ART. The information can influence the treatment protocol to be chosen for IVF but should not be used to exclude anyone from first attempt at IVF. The present evidence shows that LH and AMH appear to be the most useful biochemical markers of ovarian reserve in addition to chronological age. In addition AMH has the ability to be of diagnostic value and may be of use to ladies to decide to delay pregnancy as an informed consent.

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