Case Report:

A Comparitive Study of the Serum FSH, Serum LH, Serum E2, Serum Inhibin B & Serum AMH with the Ultrasonographic Parameters as ORTs in Infertile Women

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Abstract:

Introduction: The human ovary establishes several hundred thousand non growing follicles during the second half of intrauterine life, which is followed by a decline to the menopause.Ovarian reserve collectively refers to the ovarian follicle pool & the quality of the ovarian follicles recruited which is the result of the ovarian pituitary axis. The Follicle Stimulating Hormone (FSH) acts on Ovarian pool as a result of which follicles grow resulting in secretion of Inhibin B & Estradiol by the early antral follicles. With the decline of follicle pool, the Inhibin B & the Estradiol decreases with increase in the FSH levels. Several mathematical models have been proposed relating the decline in follicle number (based on histological analysis) to age but an accurate & non invasive method to assess follicle number that is ovarian reserve for individual women remain elusive.

Aims-: To determine the values of serum AMH, day 3 Serum FSH,LH, Estradiol, Inhibin B, Antral Follicle Count, Ovarian volume to assess ovarian function.

Objectives-: to evaluate direct relationship of the indivisual hormonal parameters to the day 3 ovarian follicle status and to determine the strength of corelation of the AFC and hormonal parameters.

Materials and Methods: - it is a comparative observational study. Our Study includes 120 infertile women between age group of 18 to 43 years who attended the IVF centre NIMS hospital Jaipur and fulfilled inclusion & exclusion criteria were assessed by taking thorough history was elicited from these women and necessary test for exclusion were done. the women were interviewed in their language in full details regarding age, literacy, socioeconomic status, diet, parity, menstrual, sexual and obstetrics history in details, significant past surgical and medical and family history were noted . subjects were followed further by a thorough physical, systemic and obstetrics examinations as per the proforma prepared for study.

Results & Conclusion: - Our study concludes that serum AMH values can be considered for evaluation of ovarian reserve. Serum AMH levels are comparable to AFC values. The results thus concluded in making a genetic marker being useful.

INTRODUCTION

The human ovary establishes several hundred thousand non growing follicles during the second half of intrauterine life, which is followed by a decline to the menopause. Ovarian reserve collectively refers to the ovarian follicle pool & the quality of the ovarian follicles recruited which is the result of the ovarian pituitary axis. The Follicle Stimulating Hormone (FSH) acts on Ovarian pool as a result of which follicles grow resulting in secretion of Inhibin B & Estradiol by the early antral follicles. With

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the decline of follicle pool, the Inhibin B & the Estradiol decreases with increase in the FSH levels. Several mathematical models have been proposed relating the decline in follicle number (based on histological analysis) to age but an accurate & non invasive method to assess follicle number that is ovarian reserve for individual women remain elusive[1].

Being a part of the classical feedback loop of pituitary gonadal axis, composite testing & evaluation of serum levels of FSH,E2,Inhibin B need to be performed. Thus concluding that the serum levels of FSH,E2,Inhibin B are not independent of each others. Moreover the levels are cycle dependent & show great variability at different menstrual cycles. The serum levels of FSH,E2,Inhibin B if evaluated independently, are poor predictors of ovarian reserve as they show variability in assay, laboratory, population & reproductive groups. Furthermore, changes in serum levels of FSH, Inhibin B, & E2 occur relatively late in reproductive aging process. Their serum level changes are evident only when the ovarian reserve is critical & chances of pregnancy are significantly reduced. The purpose of the testing is thus not fulfilled satisfactorily.

The ultrasonographic parameter of measurement of ovarian reserve are antral follicle count (total number of 2 mm to 10 mm antral follicles in both ovaries are measured) & ovarian volume. Antral follicle count is uptill now considered as the best predictor of ovarian reserve in quantitative aspect. In search of a more promising markers, ANTI MULLERIAN HORMONE (AMH) emerged out to be the more appealing one on the basis of the data required. AMH or Mullerian inhibiting substance (MIS) is a glycoprotein hormone, with a molecular weight of 140 kDa, & produced by granulosa cells in ovarian follicles from 36 weeks of gestation until menopause[2]. In human ovary, AMH expression is flanked by two major regulatory steps of folliculogenesis, i.e. initial follicle recruitment & cyclic selection for dominance. AMH is expressed in granulosa cells of primary follicles & being strongest in pre antral & small antral follicles. AMH expression disappears in follicles of increasing size & is lost in large antral follicles, where weak staining only remains present in granulosa cells of the cumulus.

Production of AMH gradually decreases as the follicle grows & then finally stops once the follicle reaches 8 mm diameter. AMH levels do not change significantly throughout the menstrual cycle. This specific expression pattern of AMH in growing non-selected follicles has lead us & others to study whether serum AMH levels decline with increasing age[3] & changes in serum AMH levels were apparent before changes in other serum markers of ovarian aging, such as FSH & Inhibin levels remain relative constant during the menstrual cycle. Furthermore, studies suggest that serum AMH levels are not influenced by gonadotropic status, & only reflect the follicle population. The decline in AMH levels correlates with decrease in the number of growing follicles with aging, & most importantly, with the size of the primordial follicle pool. These findings show that serum AMH levels reflect the quantitative aspect of ovarian reserve. Normal serum AMH level range is 2-6.8 ng/ml (14.28 - 48.55 pmol/l) in any phase of the cycle.

In recent years, accumulated data indicate that serum AMH may fulfill the requirements to be the best test to predict ovarian reserve but due to inter species, inter racial & inter communal variations & various external factors affecting infertility can also emerge, for generalization of the findings, the findings need to be evaluated in various groups of communities.

MATERIALS AND METHODS

Study Population:

- Type of study - Comparative observational study.
- Study design - Cross sectional study.
- Sample size - 120 infertile female.
- Age group - 18-43 yrs
- Centre of Study - IVF Centre, NIMS Hospital, Jaipur.

Inclusion Criteria:

- Regular Menstrual cycles of 21 - 35 days.
- No Current or past diseases.
- Not on any hormonal treatment.
- Body Mass Index (BMI): 18 - 27 KG/M^2
- No evidence of any endocrine disorders.

Exclusion Criteria:

- Any endocrine disorder.
- Abnormal liver functions.
- Abnormal kidney functions
- Genital Tuberculosis.

Study Protocol:

After taking informed consent from all infertile females fulfilling inclusion & exclusion criteria, attending IVF clinic at NIMS Hospital a thorough history was elicited from these women to assess for exclusions chosen for study & necessary tests for exclusion were done. The women were interviewed in their language in full details regarding age, literacy, socioeconomic status, diet, parity, menstrual, sexual & obstetric history in details, significant past medical & surgical history & family history will be noted. subjects ere followed further by thorough physical, systemic & obstetrics examination as per the proforma prepared for the study.

Methodology:

Adequate venous blood samples were drawn from the subjects for measuremnt of Serum FSH, LH, E2, AMH & Inhibin B on day 3 of menses at around 10.00 hrs. Serum FSH, LH, E2 levels were measured by solid phase, two site chemiluminiscent immunometric assay, reagent kit being Abott/ Siemens. Instrument used was Architect/ Avida Centaur. Inhibin B levels were measured by Enzyme Linked Immunosorbant Assay (ELISA) method. Serum AMH levels were measured in Human Serum by two site chemiluminiscent immunometric assay, reagent kit being of BECKMAN COUTLER, Instrument used was BECKMAN COUTLER for in vitro quantative measurement. Antral follicle count & ovarian volume measurement target ultrasonography was done. Follicles measuring 2 - 10 mm were measured by scanning from outer to inner margins of ovaries individually. All the scans were done by a single operator on Voluson E8 (GE Healthcare), with 5 - 9 MHz transvaginal volume probe on day 3 of menses. The addition of the follicles measured in both the ovaries was obtained. The sum was designated as the "Antral Follicle Count". The measurement of ovarian volume is done along with the

measurement of the AFC. The diameter of the ovarian contour is measurd in 3 perpendicular direction as D1, D2, D3 & the ovarian volume of individual ovaries is calculated by using formula D1xD2xD3x0.52. The ovarian volume of the right & the left ovary eas summed up to obtain the total ovarian volume.

Statistical Analysis:

The cases were divided in four groups on the basis of age as <20 as group 1, group 2 as 21- 30, group 3 as 31 - 40, group 4 >40 & also on basis of AFC as <4, 4 - 7, 8 - 12, >12. After compiling the data obtained in a tabulated form, the values of the individual variables were compared & the correlation between the various serum values i.e Serum FSH, AMH, LH,E2, Inhibin B with antral follicle count & ovarian volume was found out. The correlation was evaluated for positive & negative. The correlation as termed significant only if the obtained p value was <0.05 & the rest were termed as not significant. Thus subsequently the results were obtained.

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Table - 1: Study Data

Variables	N	Mean	Std. Deviation
Age	120	27.25	6.96
Serum FSH	120	6.05	0.87
Serum LH	120	5.33	0.75
Serum E2	120	50.71	17.13
Inhibin B	120	61.80	22.26
Serum AMH	120	3.82	1.83
AFC	120	9.86	3.76
Ovarian Volume	120	7.23	1.20

Table - 2: Age

Age Group	No.	%	Mean	Std. Deviation
<20	17	14.17	18.82	0.64
21 - 30	77	64.17	25.19	2.27
31 - 40	15	12.50	36.07	1.91
>40	11	9.17	42.64	1.12
Total	120	100.00	27.25	6.96

Table - 3: Serum FSH

Age Group	N	Mean	Std. Deviation	p Value
<20	17	4.88	0.62	
21 - 30	77	6.03	0.47	<0.001
31 - 40	15	6.31	0.63	
>40	11	7.71	0.84	

Table - 4: Serum LH

Age Group	N	Mean	Std. Deviation	p Value
<20	17	4.00	0.50	
21 - 30	77	5.46	0.44	<0.001
31 - 40	15	5.81	0.46	
>40	11	5.82	0.91	

Table - 5: Serum E2

Age Group	N	Mean	Std. Deviation	P Value
<20	17	23.56	8.34	
21 - 30	77	52.81	13.81	<0.001
31 - 40	15	67.18	9.98	
>40	11	55.46	8.64	

Table - 6: Inhibin B

Age Group	N	Mean	Std. Deviation	p Value
<20	17	82.85	12.90	
21 - 30	77	58.23	18.76	<0.001
31 - 40	15	48.61	22.57	
>40	11	72.19	31.82	

Table - 7: Serum AMH

Age Group	N	Mean	Std. Deviation	p Value
<20	17	5.35	0.55	
21 - 30	77	4.61	1.61	<0.001
31 - 40	15	2.31	1.06	
>40	11	1.00	0.78	

Table - 8: AFC

Age Group	N	Mean	Std. Deviation	p Value
<20	17	16.65	1.06	
21 - 30	77	9.68	2.24	<0.001
31 - 40	15	6.80	1.74	
>40	11	4.82	1.78	

Table - 9: Ovarian Volume

Age Group	N	Mean	Std. Deviation	P Value
<20	17	7.96	0.61	
21 - 30	77	7.41	0.89	<0.001
31 - 40	15	6.34	1.45	
>40	11	5.12	1.94	

Table - 10: Serum AMH

Serum AMH	No.	Percentage
Very Low (0.0 - 0.3)		
Low Fertility (0.3 - 2.2)		
Satisfactory		
Optimal Fertility		
High Level		
Total		

Results:

1. As the age increases, the serum levels of FSH. LH, E2 also increases significantly (p <0.001). Inhibin B serum values also raises with the increasing age.

2. the serum AMH values shows significant decrease with increasing age (p<0.001).

3. The Antral Follicle Count and Ovarian Volume seems to be decreasing with increasing age.(p <0.001).

4. The Serum FSH values seems to be increasing with the decrease in AFC (p<0.001).

5. The Serum LH and serum E2 values also decrease with increase in the AFC (p<0.001).

6. Serum Inhibin B values show variable values with decrease in AFC (p<0.001).

7. The Serum AMH values shows a significant decrease in AFC. (p<0.001).

8. Ovarian Volume shows a decrease in the value with the decrease in AFC. (p<0.001).

9. The Serum FSH, LH, E2 values are negatively related to the AFC status significantly with p value <0.001.

10. The serum AMH values and Ovarian volume are positively related to the AFC status.

11. Age seems to be negatively related to the AFC status.

12. The strength of correlation of the serum FSH, LH, E2, Inhibin B values with the AFC is(r= -0.728, p <0.001), (r=-0.811, p<0.001), (r=-0.759,p<0.001),(r= 0.462, p<0.001) respectively and that of serum AMH values with AFC is(r= 0.357,p<0.001)

13. Age is also strongly correlated to AFC values (r =-0.754, p<0.001).

DISCUSSION:

 our results suggested thet serum AMH also shows a correlation to AFC alongwith the correlation to Serum FSH, LH, Inhibin B, E2 levels. Serum FSH, LH, E2 are negatively correlated to AFC &Serum ANH is positively correlated with AFC.

In a study conducted by **Dayal Meena et al 2013**, which demonstrated that serum AMH levels were correlated to the AFC (p <0.0001) & ovarian volume (p <0.001). Serum AMH values were found to be robustly correlated AFC than Serum FSH, LH,E2 & Inhibin B on day 3 of cycle. This suggested that serum AMH should be taken as single test to reflect ovarian reserve. This study did not suggest the degree of correlation in terms of Pearsons correlation.

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In a study by **Behiye Pinar Godksedef et al 2010**, comparison of the values of basal AMH levels with the most established ovarian reserve tests. serum AMH levels were more tightly correlated (p <0.0001) with number of early antral follicle count (r = 0.467. p <0.0001) than age & serum FSH (r = -0.40, p <0.001: r = -0.299, p <0.001 respectively) No correlation was detected between Serum Inhibin B, LH, E2 and AFC. Hence conclusion was made that serum AMH levels made a strong correlation with AFC our study made correlation as r = 0.357 which was low as compared to the other hormonal parameters.

Sonal Panchal & Chaitanya Nagori 2012, in study of comparison of AMH & AFC for assessment of ovarian reserve in PCOS & non PCOS group. In a non PCOS group, correlation of AFC & ANH with number of ova retrieved is 0.50 & 0.43. In non PCOS, AMH may increase accuracy of estimation of number of ova retrieved on OPU over AFC, but difference is not significant. Variable result may be due to use of 3D ultrasound in 75 subjects with measurement of follicles in 2-9 mm as compared to our study which used 2D ultrasound with measurement of follicles 2010 mm in 120 subjects.

CONCLUSION:

Our study concludes that serum AMH values can be considered for evaluation of ovarian reserve. Serum AMH levels are comparable to AFC values. The results thus concluded in making a genetic marker being useful.

Outcome of the present study showed that AMH help in screening the current status of ovarian function in general sub-fertile population as it has a role in the process of initial & cyclic recruitment. It can be used to identify those patients who are destined to fail induction & ART programs without incurring the financial burden of the interdependent several serum hormonal markers & ART programs.

Serum AMH values with the ultrasonographic markers like AFC can modestly increase the predictability of disease without increasing the cost of the screening process. An AMH test helps to beat the biological clock by predicting how long have left to achieve motherhood with its relation to age, & may also reduce the need for ART in these patients.

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