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Research Article

DIAGNOSTIC IMPORTANCE OF SERUM ANTI-MULLERIAN HORMONE IN COMPARISON TO OTHER SEX HORMONES IN IN VITRO FERTILIZATION

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ABSTRACT

Introduction: In vitro fertilization (IVF) is an assisted reproductive technology (ART) technique commonly used for infertile couples. For successful outcome of IVF, optimal evaluation of women and standardized screening protocol is necessary. The success of IVF depends on a good ovarian response. Various endocrine and clinical markers have been proposed to predict the ovarian response though they have limited predictive value. Anti-mullerian hormone, a member of transforming growth family, has recently been suggested as a reliable marker of ovarian response.

Aims and objectives: The present study was planned to assess the reliability of AMH as marker of ovarian response and its comparison with other contemporary endocrine markers.

Materials and methods: Based on standardized initial screening, 155 females were selected for IVF. Day 3 AMH, LH, FSH and Estradiol (E2) levels were estimated. The females were subjected to ovulation induction and grouped on the basis of no. of oocytes retrieved as poor (<5), normal (5-8) and good responders (>8). The hormone levels were presented as mean + SD and subjected to statistical analysis.

Result and discussion: The study proposed that low S. AMH levels can predict a poor ovarian response. A cut off value of 0.6 ng/ml was proposed for S. AMH level as indicative of poor response. AMH was observed to have a strong correlation with age (r = -0.484), no of oocytes retrieved (r = 0.844) and S. FSH (r = -0.342).

Conclusion: The study proposes S. AMH to be a reliable marker of the reproductive potential in females especially in advanced reproductive age and its optimal evaluation can serve as a tool not only for deciding the treatment protocol but also for patient counseling. Baseline estimation of S. AMH can be helpful in decreasing the cycle cancellation rate and increasing the IVF success rate.

INTRODUCTION

Decreased ovarian reserve is one of the most alarming cause of infertility. Ovarian reserve refers to the number of good quality ooytes existing in the ovaries. Women with diminished ovarian reserve are usually not aware of their reproductive potential since their menstrual cycle is quite regular[1]. The success rate of assisted reproductive technology (ART) largely depends on optimal evaluation of the female partner. To obtain successful result, proper evaluation of the ovarian reserve is necessary before planning fertility treatment. Moreover, identification of both poor and high responders before initiating treatment may be helpful in decreasing cycle cancellation. It will also helpful in averting the risk of development of side effects such as ovarian hyperstimulation syndrome (OHSS)[2]. With the advance of reproductive age especially above 35 years, the prevalence unexplained infertility in females increases[3, 4]. Diminished ovarian reserve can be a major contributor for this unexplained infertility. However, there is no obvious correlation between age and decreased reproductive potential as some women remain fertile even with advancement of age[5]. Therefore, emphasis should be laid on finding any correlation between the ovarian reserve markers and the outcome of ovulation induction. Estimation of variables that can predict the ovarian response to gonadotropin is important for optimizing the outcome of treatment and reducing the chances of any complications in IVF. A number of clinical, endocrine and ultrasound parameters have been proposed as markers of ovarian reserve. These parameters, however, have limited predictive value[6, 7] and can be utilized more effectively by combining these

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variables accordingly[8, 9]. In recent years, a new endocrine marker, Anti-Mullerian Hormone (AMH), has been identified as a marker of ovarian response[10, 11]. AMH belongs to the Transforming Growth Factor (TGF) β superfamily which also includes inhibins, activins and bone morphogenetic proteins. The compounds belonging to this family are recognized as peptide growth and differentiation factors and have a wide range of functions such as mesenchymal epithelial interactions, cell growth, extra cellular matrix production and tissue remodeling[12].

In last few years, role of AMH as a marker of ovarian function and dysfunction has gained recognition[6, 13]. AMH is said to have a direct role in initial recruitment of primary follicular development. Further, it has been suggested that serum AMH concentrations may provide useful information about disturbed ovarian function such as anovulation. Though, several studies have recommended AMH as a marker of ovarian function, it's reliability has been debated. Few studies also recommend that baseline assessment of LH and FSH is sufficient for evaluation of the reproductive potential. A perusal of the published literature suggests that very few studies have been conducted on Indian population with reference to AMH. The present study was planned to compare, correlate and verify the role of AMH and other baseline sex hormones in assessing the reproductive potential of infertile women. Further, the study aimed at confirming its significance as a reliable marker of ovarian reserve.

MATERIALS AND METHODS

311 female patients visited the Jaipur Fertility Centre, a unit of Mahatma Gandhi Medical College & Hospital, Jaipur during the study period.

which included complete history, physical examination, routine biochemical, hematological and serology investigations and homone assays viz. Thyroid stimulating hormone (TSH) and Prolactin. Based on the initial screening, a total of 155 women selected for IVF treatment were enrolled for the study. The criteria for inclusion were (i) age between 25 – 45 years, (ii) both ovaries present, (iii) no evidence of endocrine disorder and (iv) not on any hormone therapy. Women with PCOD were excluded from the study. It was an observational study conducted after seeking approval from the Institutional Ethics Committee.

The females selected were subjected to baseline study on day 3 of menstrual cycle that included estimation of serum LH, FSH and Estradiol (E2) by immunofluorescence (ELFA) using VIDAS instrument and Biomerieux, France kits. The intra assay coefficient of variation (CV) was <4.8; <4.9 and <7.5% for LH, FSH and E2 respectively. The interassay correlation was $\geq$0.985. Serum AMH was estimated by ELISA using Beckman Coulter gen II kits. The assay had a sensitivity of 0.08 ng/ml and inter and intra assay CV of <7.7 and < 9.5% respectively.The females were then subjected to ovulation induction as per the IVF protocol. This was followed by ovulation study and on the basis of the number of oocytes retrieved, the females were grouped as poor (< 5 oocytes), normal (5 – 8 oocytes) and high (> 8 oocytes) responders.

Statistical analysis: The hormone levels were presented as mean$\pm$ SD in the three responders groups. Results obtained were analyzed using the statistical package program SPSS 17 Inc; Chicago II, USA. Mean age and hormone levels were compared among the responder groups by applying one way ANOVA. To assess the correlation of S. AMH levels with no. of oocytes retrieved, age and S. FSH levels, Spearman's correlation was applied. P-value $\leq$ 0.05 was considered as statistically significant.

RESULT

The number of oocytes retrieved following induction indicates the ovarian reserve of a female. In case of a poor response the cycle has to be cancelled. Table1 shows the mean and SD values of age and baseline hormones in the 3 groups based on the no. of oocytes retrieved following ovulation induction. On applying one way ANOVA, it was observed that the mean age in the normal and high responders was significantly lower than that of the poor responders (Table 1; Fig. 1). The serum AMH levels were as low as 0.34 $\pm$ 0.24 ng/ml in the poor response group as compared to 1.31 $\pm$ 0.69 ng/ml for the normal response group and still higher i.e. 2.32 $\pm$ 0.94 ng/ml for the high response group (Table 1; Fig. 2).The study also suggests a cut off value of 0.60 ng/ml for a poor ovarian response.

Table 1 Age and baseline hormone levels in the different response groups.

	Poor responders (n=57)	Normal responder (n=50)	High responder (n=48)	Degree of freedom (F)	p-value
No. of oocytes	< 5	5-8	>8		
Age (years)	36.02 $\pm$ 5.32	31.38 $\pm$ 5.15	30.54 $\pm$ 5.40	**16.73**	0.000
AMH (ng/ml)	0.34 $\pm$ 0.24	1.31 $\pm$ 0.69	2.32 $\pm$ 0.94	**114.19**	0.000
FSH(mIU/ml)	8.35 $\pm$ 3.84	6.27 $\pm$ 2.15	6.03 $\pm$ 2.16	**10.49**	0.000
LH (mIU/ml)	3.57 $\pm$ 1.79	3.18 $\pm$ 1.01	3.68 $\pm$ 1.51	1.55	NS
E2 (pg/ml)	44.75$\pm$18.89	45.92$\pm$18.15	43.27$\pm$14.85	0.30	NS

- Values indicated as Mean $\pm$ SD
- **F** and **p** values as obtained on applying **One Way ANOVA test**

Table 2 The correlation coefficient for S. AMH and FSH with other factors

Factors	Correlation coefficient(r)	P	Slope
AMH vs age	- 0.484	0.001	-2.714
AMH vs no.of oocyte	0.844	0.001	3.398
AMH vs FSH	- 0.342	0.001	-1.014
AMH vs LH	0.037	NS	0.024
AMH vs E_2	0.005	NS	-0.073
FSH vs age	0.473	0.001	0.863
FSH vs no. of oocyte	- 0.293	0.001	-0.482

r and P-value as obtained on applying Spearmans rank order correlation

It would be noteworthy that, of the 57 females in the poor response group, 89.5% had a serum AMH level $\leq$ 0.6 ng/ml.On comparing baseline Serum FSH levels (Table 1; Fig. 3) in the three groups, it was found that the levels were higher in the poor responder group i.e. 8.35 $\pm$ 3.84 mIU/ml and fell sharply for the normal responder group i.e. 6.27 $\pm$ 2.15 mIU/ml. However, the difference between the levels of

serum FSH in normal and high responder group was not significant. The above finding again confirms that a poor ovarian response can be predicted by high serum FSH levels. To assess the reliability of AMH as a marker of ovarian reserve, the coefficient of correlation (r) for serum AMH with age, no. of oocytes and with the other baseline hormones was worked out using Spearman's rank order correlation (Table 2). Serum AMH levels showed a strong correlation with the number of oocytes retrieved (r = 0.844, p< 0.001, slope = 3.398) (Fig. 5). Moreover, serum AMH also showed a strong correlation with age (r = - 0.484) (Fig. 4) and with serum FSH (r = - 0.342) (Fig. 6).

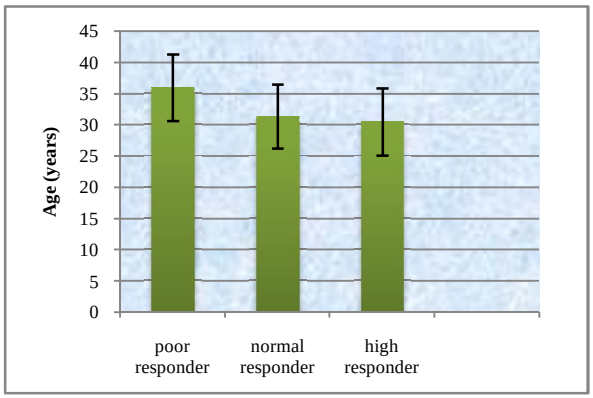

Fig. 1 Comparison of age in the groups according to no. of oocytes retrieved.[F = 16.73; p = 0.000]

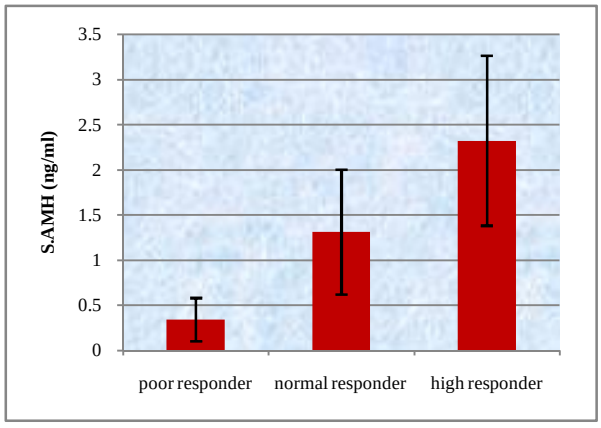

Fig. 2 Comparison of Serum AMH levels in the groups according to no. of oocytes retrieved.[F = 114.19; p = 0.000]

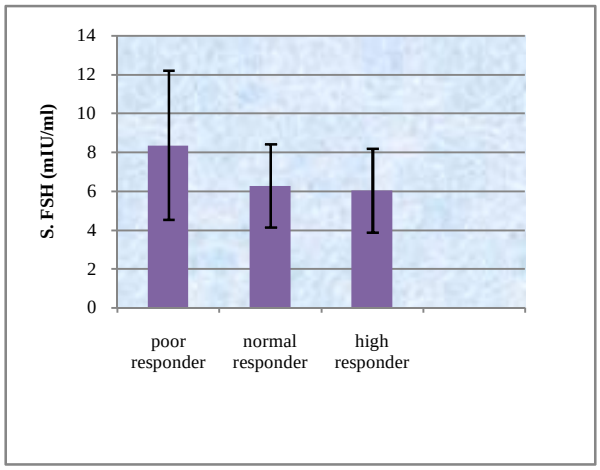

Fig. 3 Comparison of Serum FSH levels in the groups according to no. of oocytes retrieved.[F = 10.49; p = 0.000]

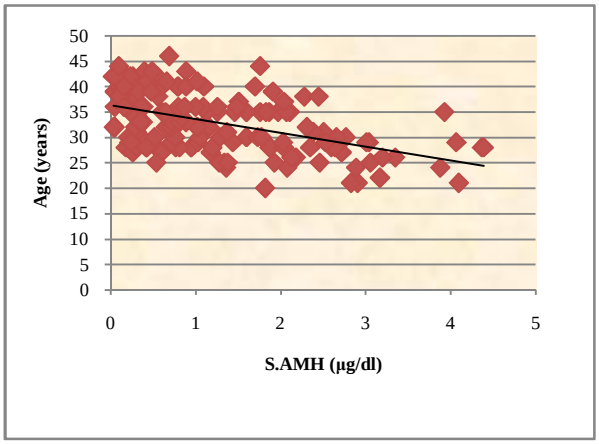

Fig. 4 X-Y Scatter plot for Serum AMH vs age. [r = -0.484; slope = -2.714]

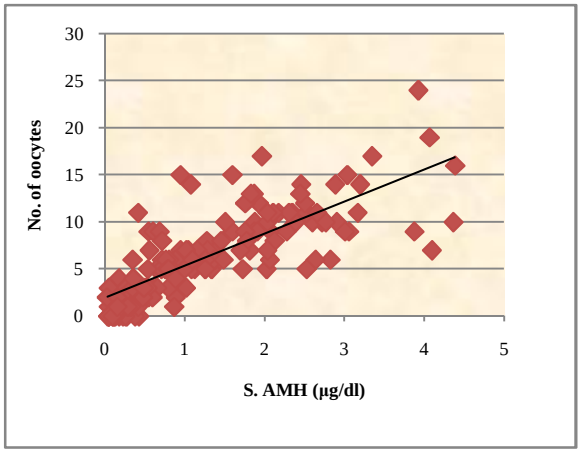

Fig. 5 X-Y Scatter plot for Serum AMH vs no. of oocytes retrieved. [r = 0.844; slope = 3.398]

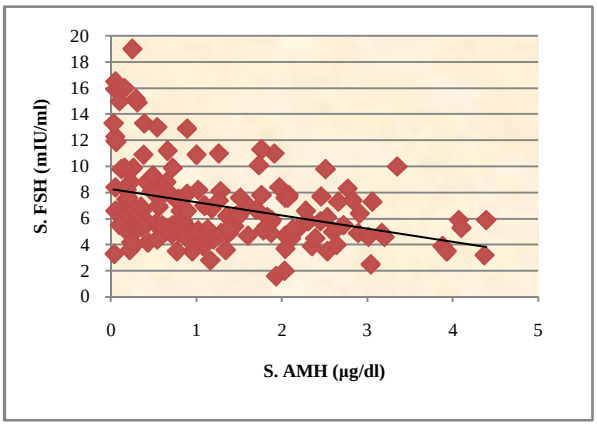

Fig. 6 X-Y Scatter plot for Serum AMH vs FSH. [r = -0.342; slope = -1.014]

DISCUSSION

In the present study, it was observed that age, S. AMH and FSH levels were significantly different with respect to response to ovulation induction and the no of oocytes retrieved thereafter. In a similar study, Van Rooij *et al*., 2002[10] have reported that the poor responders were older than the normal responders. Tremellon *et al*., 2005[1] demonstrated that poor responders (≤ 4 oocytes) were on an average 5.7 years older than the good responders (≥ 8 oocytes). This observation was almost similar to the present study which showed a difference of average 5.48 years between poor and

high responders. Studies of Van Rooij I A *et al*., 2002[10] and Siefer D B *et al*., 2002[14] have linked low plasma AMH levels with poor ovarian response and hence suggest low AMH levels as a reliable indicator of poor ovarian response. In a similar study by Freour T *et al*., 2006[15], the subjects were divided as "adequate", "intermediate" and "inadequate" response with $\geq$ 10, 6-9 and < 6 oocytes retrieved. Serum AMH was significantly higher in the adequate response group which corresponds to the high responder group of the present study.On combining the values of AMH for the intermediate and adequate response group, Freour T *et al*., 2006[15] proposed a normal range of 1.5 to 9.9 ng/ml for a positive ovarian response. Similarly, for the present study, on combining the results of normal and high response, a normal range of 0.6 to 3.3 ng/ml can be proposed. Recent study of Lee R K *et al*., 2011[16] has recommended a serum AMH cut off level of 0.68 ng/ml for IVF cycle cancellation, which is very close to that proposed by the present study.

On comparing the means of serum FSH levels among the groups, a significant variation was observed but the degree of freedom was far lower than that in case of serum AMH. Previous studies have suggested the significance of serum FSH as a marker of ovarian aging and also in deciding the treatment protocol for IVF. Fiza *et al*., 2014[17] have suggested a strong correlation of serum AMH and FSH with the advancing reproductive age. Some investigators consider that FSH inhibits AMH mRNA expression[18] whereas others suggest that AMH might impair[19] or promote[24] FSH induced follicular growth. The actual effect of AMH on FSH and vice versa is not clearly understood. However, it has been demonstrated in several studies that AMH and FSH exhibit a negative correlation[10, 21, 22].

The present study reported a strong positive correlation of S. AMH with no. of oocytes retrieved. On the other hand, a negative correlation was observed between S. AMH vs. age and AMH vs. FSH. The correlation coefficient was highest for AMH vs no. of oocytes retrieved. A similar study by Van Rooij *et al*., 2002[10] has exhibited that AMH correlates with the number of oocytes (r = 0.57). Similarly, the correlation coefficient for AMH vs age was r = - 0.30 and for AMH vs FSH, it was r = -0.54. Fanchin R *et al*., 2003[21] also showed that serum AMH negatively correlates with age (r = -0.22, p < 0.04) and with serum FSH (r = -0.27, p<0.02) but not with E_2 and LH. Similarly, Themmen A P *et al*., 2005[22] have observed a strong correlation of serum AMH with age and FSH. The present study suggests that serum AMH is a more reliable marker of ovarian reserve. Moreover, unlike the other baseline hormones, AMH levels are almost independent of the phase of menstrual cycle. This means that AMH can be measured on any day of the menstrual cycle[2, 13]. AMH levels in healthy women exhibit a continual decline with age and become almost immeasurable after menopause[23]. The diagnostic role of AMH is also identified with elevated levels in PCOD[24, 25] and lower levels in ovarian failure[26]. Despite other identified roles of AMH, its importance and relevance in the IVF treatment has been confirmed and emphasized in recent years. Findings of the present study confirm its role in predicting the response to ovulation induction. The study recommends low S. AMH levels as an easier and more reliable counseling tool for evaluation and identification of females with potential poor outcome, rather than a tool of exclusion. The final decision of whether or not to receive the treatment should be of the patient alone.

CONCLUSION

The present study demonstrates that serum AMH followed by serum FSH have a strong correlation with age and the number of oocytes retrieved following induction. It is therefore recommended that baseline serum AMH and FSH levels can serve as reliable markers of ovarian reserve especially in females of advanced reproductive age. The suggested normal reference range and cut off value can play a decisive role in planning the treatment, counseling the patient and hence in improving the success rate and minimizing the cycle cancellation rate. The study further suggests thorough studies of serum AMH levels in females with varying female causes of infertility and evaluation of its concentration in ratio to that of other baseline hormones.

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