Original Article

Ideal value of serum anti-Mullerian hormone as a predictor of ovarian reserve and outcome in assisted reproductive technology

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ABSTRACT

Title of the article: Ideal value of serum anti-Mullerian hormone as a predictor of ovarian reserve and outcome in assisted reproductive technology. **Aims:** To evaluate serum AMH as a marker of ovarian reserve and reproductive outcome. **Settings and Design:** Division of Reproductive Medicine, Kasturba Medical College, Manipal University, Manipal, Karnataka, India. **Methods and Material:** A prospective two year analysis of 84 women undergoing ART with AMH, FSH and AFC measurements was analysed on day 2 of the cycle. The study group was Group I – <0.7, Group II – 0.7 – 3.5, Group III – >3.5 ng/ml. Outcome measures such as the mature oocytes, quality embryos and pregnancy rates were compared between these groups. **Statistical analysis used:** Non parametric test (Kruskal Wallis), Exact test and ROC curves was used. **Results:** AMH levels correlated best with age (P = 0.012), antral follicles (P = 0.001), follicles retrieved (P = 0.002) and oocytes obtained (P = 0.041). The number of mature embryos were higher in Group II & III than Group I (P = 0.312). AMH levels were significantly lower in canceled cycles than completed cycles (P = 0.010). The occurrence of OHSS was higher (61%) in Group III and 10% in in Group II (P = 0.001). **Conclusions:** AMH value of 0.7-3.5 was better than FSH in prediction of number of oocytes. Both FSH and AMH were not good predictors of pregnancy.

Keywords: Anti-Mullerian hormone, assisted reproductive technology outcome, FSH, ovarian reserve

INTRODUCTION

Accurate assessment of ovarian function remains one of the big challenges in fertility practice, and hence a new marker for prediction of ovarian reserve and assisted reproductive technology (ART) outcome has been on search. The present study finds out whether anti-Mullerian hormone (AMH) predicts ovarian reserve and ART outcome.

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Access this article online

Quick Response Code:

Website: www.fertilityscienceresearch.org

DOI: 10.4103/2394-4285.180495

MATERIALS AND METHODS

A prospective observational study carried out between August 2009 and August 2011. A total of 84 women who underwent *in vitro* fertilization (IVF)/intracytoplasmic sperm injection (ICSI) were included in the study regardless of their age or reproductive history. This study received institutional review board approval from the ethical committee of our university before it was executed. Each patient gave informed consent, authorizing the examination.

Controlled ovarian hyperstimulation

The patients followed either long protocol with gonadotropin-releasing hormone (GnRH) agonist or GnRH antagonist protocol.

The long protocol (n = 33)

The stimulation protocol involved down regulation with GnRH analog (inj. Leupride 0.4 ml subcutaneous (sc), Abbott Laboratories) starting from the luteal phase of the previous cycle 1 week before the expected menses, followed by gonadotropin stimulation after ensuring adequate down regulation (estradiol <30 pg/ml and endometrium <5 mm). The follicular development was monitored by transvaginal sonography and serum estradiol levels. Follicular maturation was triggered by

administration of inj. human chorionic gonadotropin (hCG) 13,000 IU (Ovitrelle, Merck Serono) when atleast three follicles reached 18 mm in diameter.

The antagonist protocol (n = 34)

This protocol involved the administration of GnRH antagonist (Cetrorelixor Ganirelix, EMD Serono, Inc) in a daily dose of 0.25 mg on day 5-6 of stimulation when the leading follicle was 12-14 mm. The follicular development was monitored and the antagonist was continued till the day of hCG. hCG of 13,000 IU (Ovitrelle, Merck Serono) was administered when the leading follicles was >18 mm, as per the protocol by Chang *et al*.[1]

Ultrasound-guided transvaginal oocyte retrieval was performed 35 h after the hCG administration. Embryo transfer was done 2 days later.

Data collection

The data including age, basal follicle-stimulating hormone (FSH), AMH level, antral follicle count (AFC) on day 2 or 3, number of patients whose cycle got cancelled, number of follicles, mature oocytes, Grade 3 embryos, and the percentage of pregnancy and ovarian hyperstimulation syndrome (OHSS) were collected. The OHSS was classified as mild, moderate, and severe forms as per Golan *et al*.[2]

Hormone estimation

Blood sample was withdrawn at day 2 of cycle and centrifuged at 3,500 cycles/min for 10 min. Serum was separated and stored in 1.5 ml polypropylene tubes at −80°C. Serum AMH was determined using DSL-10-14400 ACTIVE® ELISA assay kit. Detection limit of the assay is 0.1 ng/ml.

The patients were divided into three groups based on AMH values in ng/ml: Group I: <0.7, Group II: 0.7-3.5, and Group III: >3.5. Outcome measures were compared between these three groups.

Statistical analysis

Results were expressed in median and percentage. The statistical significance was carried out using the Scientific Package for Social Sciences (SPSS version 16.0) by Kruskal–Wallis H nonparametric test and Fischer's exact test. Receiver operating characteristic (ROC) curves were generated for AMH and FSH to compare the predictability of the number of oocytes and pregnancy.

Analysis

A total of 84 women were recruited initially. After evaluation, 17 women did not come back for management and were taken as lost to follow-up, six were cancelled due to poor response and all of them had poor antral follicular counts, one was cancelled due to immature oocytes, and one due to high luteinizing hormone (LH) hormone values. Hence, finally 59 women who completed the ART cycles were analyzed [Table 1].

The statistical analysis was done by Fischer's exact test and *P* - value 0.010 was statistically significant, showing that cycle cancellation rate was higher with lesser AMH value.

As shown in Table 2 and Figure 1, median number of mature oocytes was retrieved in 59 patients; three in Group I, six in Group II, and seven in Group III. The statistical analysis was done using nonparametric Kruskal–Wallis test and *P* - value was 0.149, which was not statistically significant. Hence, there was no significant difference in the median number of mature oocytes between Groups II and III.

As shown in Table 3 and Figure 2, embryos obtained in 59 patients showed that the median number of embryos obtained were two in Group I, four in Group II, and four in Group III. The statistical analysis was done using nonparametric Kruskal–Wallis test and *P* - value was 0.067, which was not statistically significant. Hence it was inferred that even though more number of oocytes were obtained with higher AMH (Group III), there was no significant difference in the embryos obtained in Groups II and III.

As shown in Table 4, out of 59 patients, 21 (35%) became pregnant. None of the patients in Group I with AMH <0.7 ng/ml became

Table 1: Comparison between serum AMH and cycle cancellation

ART cycle	AMH range (ng/ml)			Total *n* (%)
	<0.7 *n* (%)	0.7-3.5 *n* (%)	>3.5 *n* (%)	
Cycle completed	2 (3.4)	40 (67.8)	17 (28.8)	59 (100)
Cycle cancelled	4 (50)	3 (37.5)	1 (12.5)	8 (100)
Lost to follow-up	1 (5.9)	11 (64.7)	5 (29.4)	17 (100)
Total	7 (8.3)	54 (64.3)	23 (27.4)	84 (100)
P - value		0.010		

AMH: Anti-Mullerian hormone, ART: Assisted reproductive technology

Table 2: AMH and median number of mature oocytes

AMH (ng/ml) (*n* = 59)	Group I <0.7 (*n* = 2)	Group II 0.7-3.5 (*n* = 40)	Group III >3.5 (*n* = 17)	*P* - value
Median no. of mature oocytes (IQR)	3 (1, 4)	6 (4, 8)	7 (4, 10)	0.149

AMH: Anti-Mullerian hormone, IQR: Interquartile range

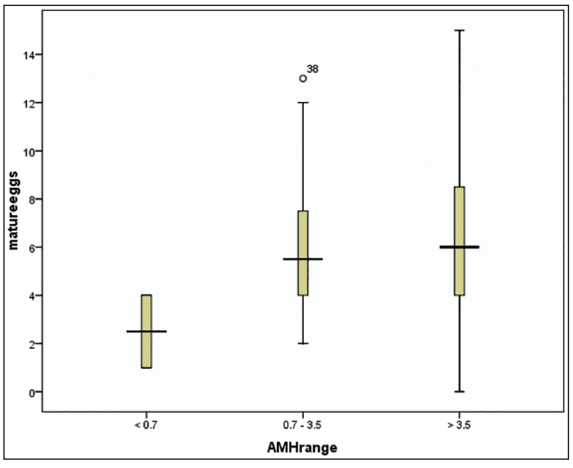

Figure 1: Showing the number of mature oocytes in each group. AMH: Anti-Mullerian hormone

pregnant. Out of 40 patients in Group II (AMH 0.7-3.5 ng/ml), 15 patients (37.5%) became pregnant; and out of 17 patients in Group III (AMH >3.5 ng/ ml), six patients (33.3%) became pregnant. Pregnancy rate was almost similar in Groups II and III, whereas it was nil in Group I. The statistical analysis was done using Fischer's exact test and *P* - value was 0.796, which was not statistically significant.

As shown in Table 5, seven patients with AMH <0.7 ng/ ml (Group I) had median FSH of 8.9, 54 patients with AMH 0.7-3.5 (Group II) had median FSH of 6.8, and 23 patients with AMH >3.5 (Group III) had median FSH of 6.4. The interquartile range of FSH was 7.8-26.5 in Group I. Hence, it was inferred that if the AMH value was less than 0.7, FSH value was higher. Thus, AMH and FSH were inversely proportional. The statistical analysis was done by Kruskal–Wallis nonparametric test and the *P* - value was 0.002, which was statistically significant.

The ROC curve for FSH and AMH for predicting the number of oocytes was drawn. As shown in Figures 3 and 4, the area under the curve (AUC) for FSH was 0.591 and for AMH was 0.790. Hence, it was inferred that AMH was a better predictor of number of retrieved oocytes than FSH.

DISCUSSION

Diminished ovarian reserve is a major cause of infertility. Hence, ovarian reserve testing is crucial in ART. Traditionally, basal serum FSH levels were widely used to assess ovarian reserve, but not considered highly accurate due to intercycle variations. Currently, serum AMH measurements are considered best markers of ovarian reserve as AMH is not affected by pregnancy, oral contraceptive pills, and gonadotropin stimulation.[3]

In our study, we found that serum AMH declined with age; which is consistent with the findings by Lee *et al*.[4] David *et al*.,[5] found that the median AMH levels decreased steadily with an increase

Table 3: AMH and median number of embryos

AMH (ng/ml) (*n* = 59)	Group I <0.7 (*n* = 2)	Group II 0.7-3.5 (*n* = 40)	Group III >3.5 (*n* = 17)	*P* - value
Median no. ofembryos	2	4	4	0.067

AMH: Anti-Mullerian hormone

Table 4: AMH and pregnancy rate

AMH (ng/ml)	Group I <0.7 (*n* = 2)	Group II 0.7-3.5 (*n* = 40) (%)	Group III >3.5 (*n* = 17) (%)	Total (*n* = 59) (%)
Pregnant	0 (0 %)	15 (37.5)	6 (35.3)	21 (35)
Not pregnant	2 (100 %)	25 (62.5)	11 (64.7)	38 (65)
P - value		0.796		

AMH: Anti-Mullerian hormone

Table 5: Comparison between AMH and FSH

AMH (ng/ml) (*n* = 84)	Group I <0.7 (*n* = 7)	Group II 0.7-3.5 (*n* = 54)	Group III >3.5 (*n* = 23)	*P* - value
FSH Median (IQR)	8.9 (7.8, 26.5)	6.8 (6.0, 8.1)	6.4 (4.6, 8.1)	0.002

AMH: Anti-Mullerian hormone, FSH: Follicle-stimulating hormone, IQR: Interquartile range

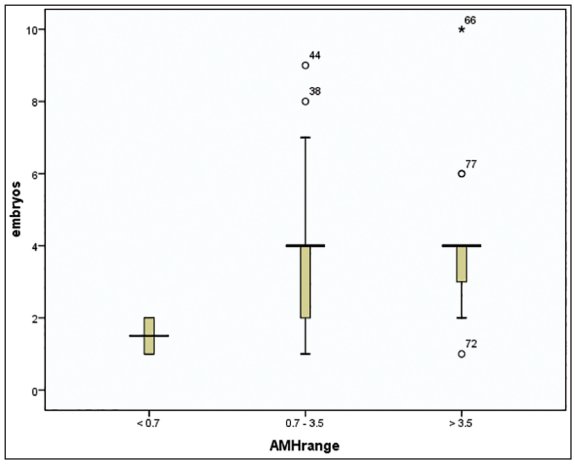

Figure 2: Showing the number of embryos in each group

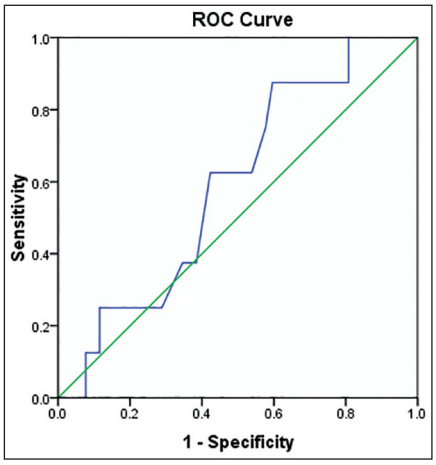

Figure 3: FSH and prediction of less than four oocytes. FSH: Follicle-stimulating hormone

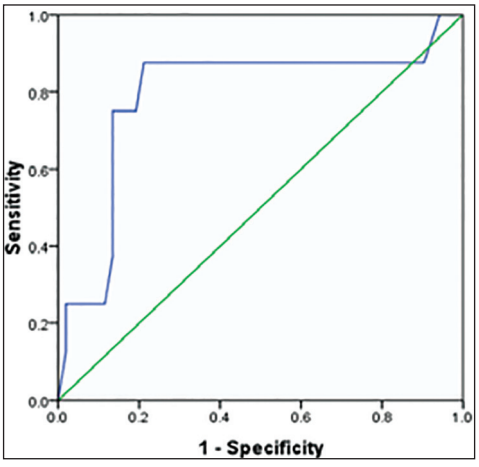

Figure 4: AMH and prediction of less than four oocytes

in age from 24 to 50 years. The predictive value of AMH exceeds the performance of ovarian reserve testing with age, FSH, inhibin B, and estradiol.[6]

As AMH is of ovarian origin, reduction in number of preantral and antral follicles will result in AMH reduction. Various studies showed strong association between serum AMH and ovarian pool.[7-9] This is consistent with our study which showed decline in number of antral follicles, as serum AMH decreased. The AFCs were significantly less when serum AMH was <0.7 ng/ml. Majumder*et al*.,[10] reported that serum AMH and AFC were comparable predictors of the quantity as well as the quality of ovarian responsiveness to exogenous gonadotropins. Plante *et al*.,[11] found out that active smoking was associated with decreased AMH, confirming the effect of smoking on antral follicles.

La Marca *et al*.,[12] reported the AMH level of 0.7 ng/ml had a good sensitivity and specificity of identifying 75% of poor responders. In other studies,[13,14] AMH >3.5 ng/ml predicted hyper response and OHSS. In our study, AMH range of 0.7-3.5 ng/ml had 83.3% sensitivity and 25% specificity for predicting the number of oocytes. Fouda *et al*.,[15] found out in a study that AMH was a reliable predictor for cycle cancellation. Our study also inferred that cycle cancellation rate was higher with lesser AMH value.

Eldar-Geva *et al*.,[16] reported that an additional increase in AMH levels were observed in polycystic ovary syndrome (PCOS) patients due to abnormal activity of granulosa cells, hyperandrogenism, and obesity. In our study, we found out that a significant number of follicles were obtained when AMH value increased.

Nakhuda *et al*.,[17] suggested that AMH was correlated with number of oocytes retrieved; and peak estradiol and AMH appears most useful in prediction of gonadotropin sensitivity, allowing individualization of dosing protocol. Also; Fouda *et al*.,[15] and Ebner *et al*.,[18] claimed a positive association between AMH level, oocyte number, and quality. Similarly our study also found out that more number of oocytes were retrieved with higher AMH (>3.5 ng/ml) and few oocytes with lesser AMH value (<0.7 ng/ml); whereas, an optimal number of oocytes were obtained with AMH between 0.7 and 3.5 ng/ml.

In our study, even though more number of oocytes were retrieved with higher AMH (>3.5 ng/ml), there was no significant difference in the number of mature oocytes and top quality embryos between Group III (AMH >3.5 ng/ml) and Group II (AMH 0.7-3.5 ng/ml). But the total number of oocytes, mature oocytes retrieved, and top quality embryos were significantly less in Group I (AMH <0.7 ng/ml). Hence, the optimal range of AMH was 0.7-3.5 ng/ml for predicting ovarian reserve and optimal ovarian response to gonadotropin stimulation. La Marca *etal*.,[12] also stated that normal level of AMH was between 0.7 and 3.5 ng/ml. The levels between 3.5 and 5 ng/ml and levels >5 ng/ ml showed borderline high fertility and high fertility, respectively.

Nakhuda *et al*.,[19] reported AMH as a good predictor of OHSS and Tremellen *et al*.,[20] reported less AMH levels with poor ovarian reserve. Our study showed that 61.1% of patients with AMH >3.5 ng/ml (Group III) had OHSS; all were of the mild variety. Only 10% in AMH range0.7-3.5 ng/ ml (Group II) and none of them with AMH <0.7 ng/ ml (Group I) had OHSS. Thus, serum AMH strongly predicted OHSS.

Few studies[21,22] showed that higher AMH levels were associated with a greater number of mature oocytes, a greater number of embryos, and ultimately a higher pregnancy rate. Hazeout *et al*.,[23] were the first to demonstrate that AMH was found to have great value in predicting outcome of ART cycles and greater prognostic value than age, serum FSH, inhibin B, or estradiol. Barad *et al*.,[24] concluded that when AMH and FSH were compared, AMH was clearly superior in predicting IVF outcome; also adding FSH to AMH, did not improve results in predicting pregnancy.

In contrast, our study did not show significant association of pregnancy rate and serum AMH levels. Nelson *et al*.,[21] similar to our study concluded that basal AMH had a very good correlation with the number of oocytes retrieved, but like basal FSH, did not seem to predict clinical pregnancy. Similarly some other studies[15,17,23] also showed no significant difference in AMH level between pregnant and nonpregnant cases.

Gleicher *et al*.,[25] compared the concordance between FSH and AMH and found that women with normal FSH and abnormal AMH had reduced oocyte yield; whereas, women with normal FSH and normal AMH had the best oocyte yield, showing that AMH was better marker than FSH for predicting ovarian response. We had noted, with AMH value less than 0.7 ng/ ml, FSH value was higher with an interquartile range of 7.8-26.5 mIU/ ml. Thus, AMH and FSH were inversely proportional.

In this study, ROC curves for FSH and AMH for predicting number of oocytes and pregnancy were drawn. The AUC for FSH was 0.591 and for AMH was 0.790 for predicting the number of oocytes and the AUC for FSH was 0.457 and for AMH was 0.556 for predicting pregnancy. AMH was better predictor of number of retrieved oocytes than FSH and both AMH and FSH were not good predictors of pregnancy. These findings were in accordance with those of earlier studies.[15,25]

ROC curve analysis[16] showed that estimation of AMH levels before IVF/ICSI was a fair test for discrimination between cancelled and completed cycles with highly significant value (AUC-0.747), but it was a poor test for discrimination between nonpregnant and pregnant cases (AUC-0.659). Our study could not establish a definite cutoff value to predict pregnancy due to overlapping values in pregnant and nonpregnant cases.

Broer *et al*.,[26] performed a review of role of AMH in ART outcome and concluded that AMH was an excellent predictor of ovarian response to controlled ovarian stimulation, but could not predict pregnancy after ART.

CONCLUSION

Serum AMH accurately predicts ovarian reserve and oocyte retrieval number in ART. AMH is a better predictor of ovarian reserve than FSH. The optimal range of AMH to get good ovarian response is 0.7-3.5 ng/ ml. AMH predicts the development of

OHSS; whereas, AMH like FSH is a poor predictor of pregnancy. Higher was the cancellation rate when AMH was very low.

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Cite this article as: Kumar P, Ravichandran SS. Ideal value of serum anti-Mullerian hormone as a predictor of ovarian reserve and outcome in assisted reproductive technology. Fertil Sci Res 2015;2:24-8.

Source of Support: Nil, **Conflicts of Interest**: None declared.