ORIGINAL ARTICLE

Antimullerian Hormone as a Marker of Ovarian Reserve

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ABSTRACT

Aim: To determine the predictive value of antimullerian hormone as a marker for ovarian reserve and compare it to other markers such as age, FSH and antral follicle count.

Methods: A prospective study carried out at IVF centre from 1st January, 2014 to 30th December 2014. It included 50 patients who were enrolled for IVF/ICSI treatment. All of these patients underwent hormonal profile such as FSH, LH. AMH and estradiol level on 2nd day of menses and transvagainal U.S.G on 5th-8th day of cycle for antral follicle, count of 2-5mm in size. These patients received controlled ovarian stimulation by gonadotrophins. Dose was decided according to age and BMI.Primary outcomes measure was number of oocyte retrieved.

Result: While good responders' achieved higher estradiol level (mean 7103(2594) as compared to group B 5041(3791) and dose of gonadotrophins required was less in group A 2718(2187) as compared to group B 2804(1368) age was comparable 34.1 in both groups. This study did not depicted age and FSH to have any significant correlation with ovarian response. But antral follicle count and AMH emerged as predictor of ovarian reserve significantly P= .002 and P .000 respectively. AMH is even better predictor of ovarian response. AMH had 97% sensitivity and 41.6% specificity in predicting the response

Conclusion:AMH is good predictor of ovarian reserve with high sensitivity. It helps in individualizing the dose for ovarian stimulation and can effectively identify women who will not respond to ovarian stimulation.

Keywords: Antimullerian hormone, ovarian reserve, BMI

INTRODUCTION

Women are very different to men with regard to reproductive aging. A fall in ovarian reserve is inevitable with increasing age. Ovarian reserve is a measure of how well the ovaries are still functioning at a certain point in time[1].

A number of ovarian reserve tests have been designed to determine oocyte reserve and have been evaluated for their ability to predict the outcome of IVF in terms of oocyte yield and occurrence of pregnancy. Currently available tests are early follicle phase blood values of FSH, estradiol, inhibin B, antral follicle count (AFC), ovarian volume and antimullerian hormone (AMH) level and some dynamic tests such as clomiphene citrate challenge test (CCCT)[2].

AMH is a member of transforming growth factors B (TGF-B family). It was identified as a factor that causes regression of mullerian ducts during male fetal development.In females it is produced in granular cells of ovarian follicles. It is expressed in pre antral and small antral follicles and is good indicator of size of the ovarian antral follicle poo[3].It is not cycle dependent and can be measured any day, it is not altered by hormonal therapy even after down regulation by GnRh agonists. As compared to FSH there is no cycle to cycle Variation, but it declines with age[4].

Because traditional assessments of ovarian reserve such as early follicular phaseFSH, Inhibin B, and (CCCT) have low sensitivity in the early stages of reduced ovarian Reserve[5].

There is urgent need for reliable and early marker for detection of declining no. of follicles, and prediction of spontaneous pregnancy potential and assisted reproduction technology outcome[5,6].

Therefore a serum marker that reflects the number of follicles that have made transition from primordial pool into growing follicle pool, and that is not controlled by gonadotrophins would benefit both patients and clinicians. In recent years accumulated data indicate that anti-mullerian hormone(AMH) may fulfill this role[7].

Present study was carried out to determine the predictive value of AMH as marker for ovarian reserve and compare its predictive value to other markers such as, age, FSH, and antral follicle count.

PATIENTS AND METHODS

This is a prospective study carried out at Australian concept fertility centre Lahore between 1st January 2014 till 30th December 2014. It included 50 Patients who were enrolled for IVF-ICSI cycles. Main

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indication for ART were: male infertility 60%, tubal pathology 13%, endometriosis 9%, unexplained infertility 18%.Among 50 patients 3 dropped out before IVF treatment. In 2 patients AMH value could not be carried out because of lack of stored serum, so total 46 entered into analysis.The criteria for inclusion was patients with regular cycle pattern and no endocrinal problem, such as related to thyroid or hyperprolactinemia, if diabetics were present. Their sugar levels were controlled.Patients with age less than 42years were enrolled.

METHODS

After history patients underwent baseline hormonal profile i.e., on day 2^{nd} to 5^{th} of woman's normal cycle serum FSH, LH, estradiol and AMH levels were carried out. On day 5^{th}-8^{th} transvaginal ultrasound for numberof antral follicle count in each ovary was performed. 5-10 follicles of 2-8mm in each ovary was taken as normal antral follicle count.

For AMH measurement a sensitive ELISA kit was used according to manufacturer's instructions. The standards cover a range from 1.05 to 1.5ng/ml. The sensitivity is 00.06ng/ml. Normal level is taken between 2-8ng/ml, low normal range is between 1.5-2ng/ml and below 1.5ng/ml is designated as low and 0.5ng/ml as very low. Serum FSH, LH were performed by Elisa and FSH of less than 12 was taken as normal.Women under 30yrs of age underwent long protocol usingdown regulation from mid luteal phase of previous cycle using 0.5mgof GNRH analogue.Rest of the women underwent short protocol using gonadotrophin antagonist. Ovarian hyperstimulation was carried out using either recombinant follitrophinA(rec, FSH, Puregon) or follitrophin B (Gonal F).Starting dose for patients <35years was 150-200mg and >35 it was 225-300mg or more. After 5days of stimulation, follicular growth was assessed by vaginal ultrasound and estradiol measurement and dose was adjusted accordingly. When at least 3 leading follicles of 18-20mm developed,trigger was carried out using 10,000 IU/LhCG (Pregnyl).After 35-37hours transvaginal ovum retrieval was done. Embryo transfer took place 72hours after this. The primary outcome measure in this study was the number of oocyte retrieved and poor ovarian response. Poor response was defined as fewer than 4 oocytes at follicular puncture or as cancellation due to impaired (fewer than 3 follicles) or absent follicular growth in response to ovarian hyperstimulation[8].

The study group was divided into two subgroups according to number of oocyte retrieved patients with oocyte count of 4 or more were considered good responders and patients with less than 4 as poor responders.

Data was analyzed using SPSS version 15 values were presented as mean and standard devotions. Student ttest was used to compare endocrine profile and basic characteristicsof patients. It was presented in the form of frequencies and percentages. The correlation between different parameters was expressed as spearman's correlation co-efficient. P value less than .05 was considered significant.

RESULTS

The mean age in group A n(33) was 34±5.7 as compared to 34±6.06 years for group B n(12 patient) P=[NS]. Group A (good responders had higher BMI 25.2±4.2 as compared to group B 22.8±6.05. Maximum estradiol levels achieved was higher in group A 7103± 2594 as compared to 5041±3791 in group B Although Higher gonadotrophin dose 2804± 1368 was required in group B as compared to group A.

Table I shows that primary infertility was present in 72.2% in group A as compared to 91.7% in group B (8.3%) one patient with secondary infertility in group B as compared to 9(27.3) in group A.

Table II depicts that main indications for ART n(45) were male sub fertility in 35.6% and tubal pathology 15.6% PCO (17.8%) endometriosis (17.8%) age factor (8.9%) unexplained sub fertility (4.4%) was present.

Table III shows correlation of AMH and other parameters of ovarian reserve such as age, AFC, FSH, and in predicting the poor responders. R is spearman's correlation coefficient followed by P value.Age did not carry statistical correlation in both groups while antral follicle count was significantly correlated with good response (P0.002) Similarly normal AMH level has statistical significant correlation P.000 with good response. On the other hand FSH value of less than 12 IU/L was not significantly different between two groups.

Table IVconcludes that AMH carries 97% sensitivity in detecting the poor response and specificity of 41.6% in detecting good response.

The correlation coefficients for the association between AMH levels on one hand and several ovarian reserve test variables and total number of oocyte retrieved on the other hand are depicted in figure A-D. AMH was significantly correlated with AFC and number of oocytes retrieved after ovarian hyperstimulation R=0.20 in Fig B. As compared to age and FSH. There was inverse co-relation between and age and FSH level and No. of oocyte retrieved. R=.056 and .014 for FSH Fig C and age Fig D respectively.

Table I: Demographic Features

Variable	Good Responders/ mean (St D)(n=33)	Poor Responders/mean (St D)(n=12)
Age	34.1 (5.7)	34.1 (6.06)
BMI	25.2 (4.2)	22.8 (6.05)
Infert. Duration	7.9 (5.07)	7.3 (3.62)
Max Estradiol level	7103 (2594)	5041 (3791)
GnT dose	2718 (2187)	2804 (1368)

Table I: Demographic Features

Variable	Good Responders/ mean (%)(n=33)	Poor Responders/mean (%) (n=12)
Primary Inf	24 (72.7%)	11 (91.7%)
Sec Inf	9 (27.3%)	1 (8.3%)

Table II: Causes of Infertility

Variable	Total (n=45) Frequency (%)	Good Responders Frequency (%) (n=33)	Poor Responders Frequency (%)(n=12)
Tubal Pathology	7 (15.6%)	6(18.2%)	1(8.3%)
Male Factor	16 (35.6%)	13(39.4%)	3(25%)
PCO	8 (17.8%)	8(24.2%)	0(0%)
Endometriosis	8 (17.8%)	2(6.1%)	6(50%)
Age > 38yrs	4 (8.9%)	3(9.1%)	1(8.3%)
Unexplained	2 (4,4%)	1(3%)	1(8.3%

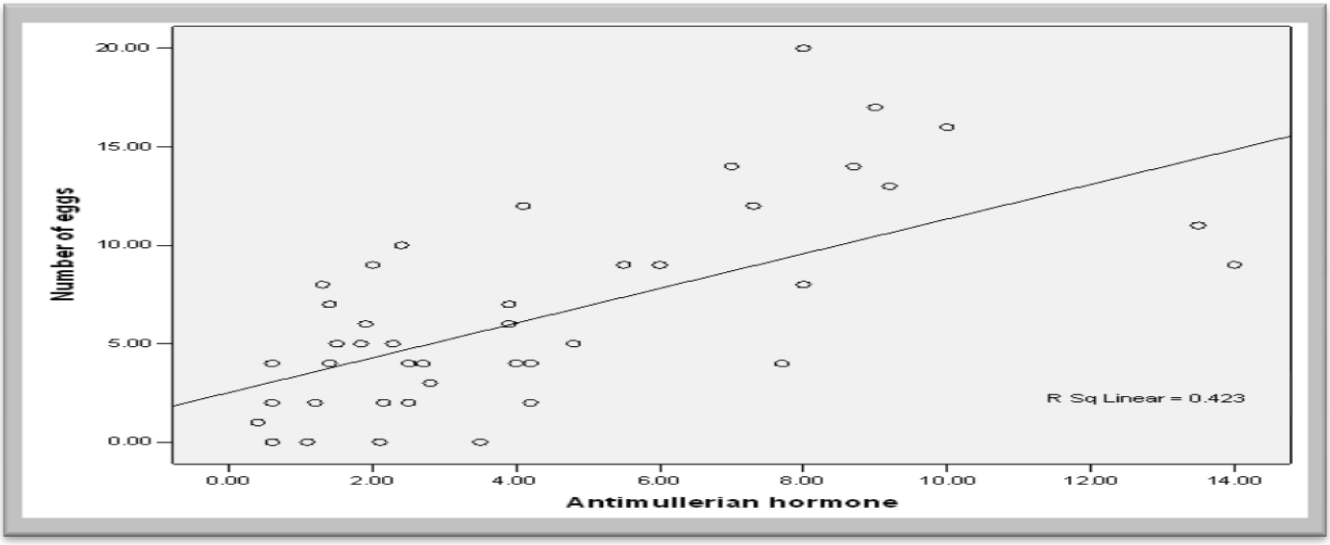
Table III: Correlation of AMH with other parameters

Variable	Good Responders/Mean (St D)(n=33)	Poor Responders/Mean (St D)(n=12)	R value	P value
Age	34.1 (5.7)	34.1 (6.06)	- 118	NS
AFC	7.9 (5.7)	5.4 (1.24)	++ 453	0.002
FSH	8.0 (3.04)	8.48 (2.47)	- 238	NS
AMH	5.5 (3.8)	2.11 (1.37)	+++ 650	0.000

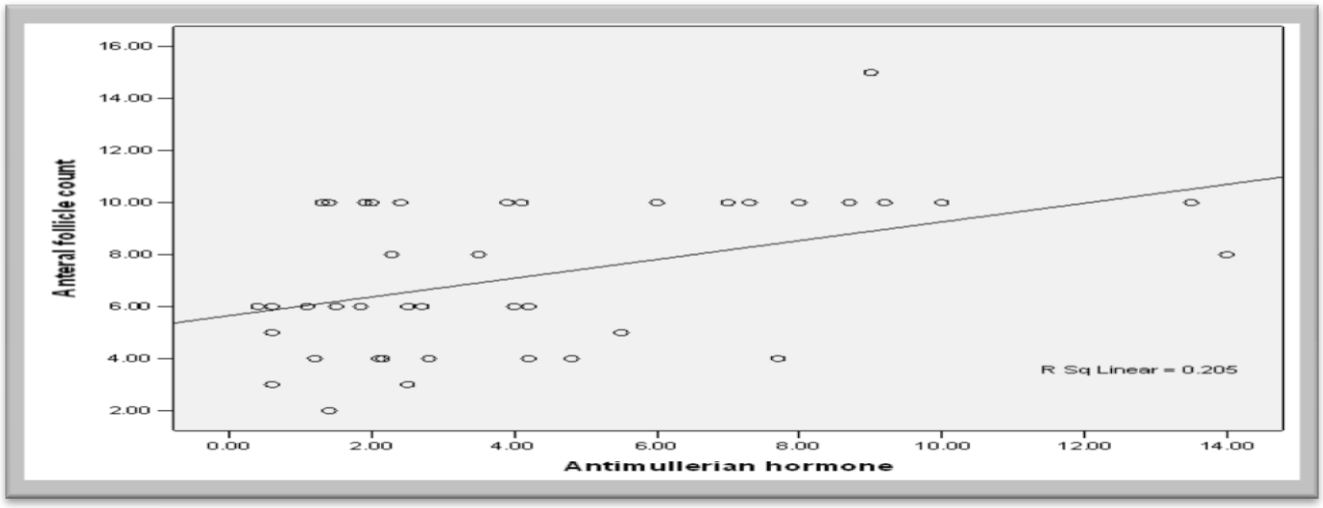
Table IV: Detection & Prediction of Poor Responders (< 4 Oocytes)

Observation	Value (n=12)
Sensitivity (true positive rate)	97%
Specificity (true negative rate	41.6%

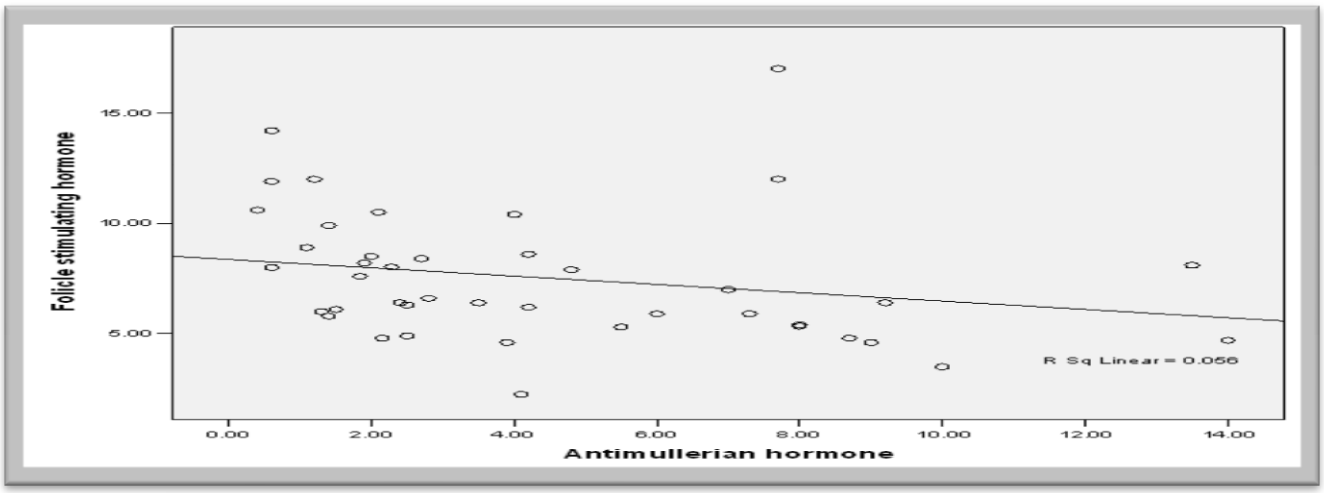
Graphs I: Correlation of AMH with # of oocyte retrieved



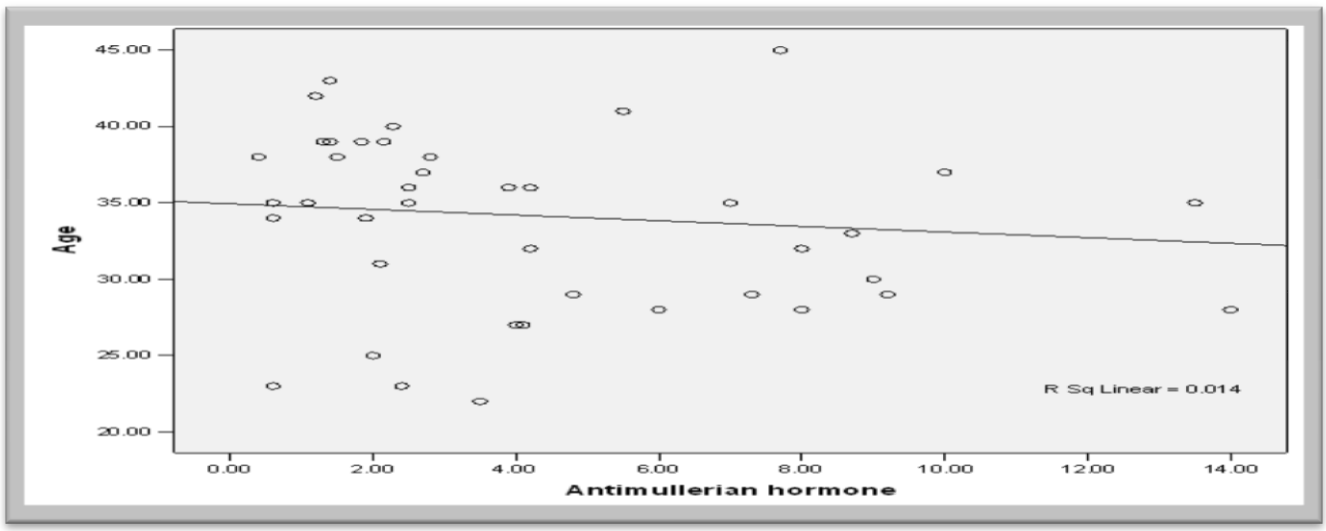
Graphs II: Correlation of AMH with AFC



Graphs III: Correlation of AMH with 3rd day FSH



Graphs IV: Correlation of AMH with age



DISCUSSION

This study was carried to see whether AMH can predicts ovarian response in IVF cycle and comparison was made with other markers of ovarian reserve, age, FSH, AFC test. There was significant correlation with ovarian response, as expressed by number of oocytes retrieved.

Previous studies have shown that AFC gives the best prognostic information with regards to occurrence of poor response in IVF[8]. The present study also depicted significant correlation with antral follicle count.With normal antral follicles count of 5-10 follicles there was good response P=0.002. However AFC is subjective and also might vary between cycles in patients. But AMH is reported to be similar at different stages of cycle[9].

In this study the AMH was found to be even better predictive of good response P=.000. A study by (Van Rooij 2002) also found comparable performance in predicting ovarian response between AFC and AMH. Present study did not predicted age as a superior parameter ofovarian reserve. However, even in women of comparable age,there is wide variation in individual ovarian response[10].

As predicted poor responder had significantly higher level of FSH. But relationship was not significant. Other studies[11,12] had found significant correlation between no. of oocytes retrieved and FSH level below 12miu/L. Diagnostic tests were carried out to assess the predictive value of eachparameter independently to predict ovarian response. A good marker should have high sensitivity (in identifying the true poor responders) and high specifically in identifying the true good responders to precisely identify the good and poor response.

Using cut off value of 1.5 as low normal the sensitivity of AMH in identifying the poor response was 97% and specificitywas 41.6%.Value below 1.5 is low and less than 0.5ng/ml is very low.

Other studies[5,10] have shown AMH levels ≤ 1.26ng/ml as strongindicative of diminished ovarian reserve and AMH level of <0.5ng/ml a correct predictor of very low response with <2 oocytes in 88% of cases supporting the valve of AMH as an early warning test for exhausted ovarian function when FSH level are still within normal range[13].

CONCLUSION

Concluding from this study AMH can be used reliably as screening marker for reduced ovarian reserve in women as previously suggested by others (van Rooij et al, 2005)[5]. AMH can affectively identify women who will not respond to ovarian stimulation, so as a result poor pregnancy rates will occur[14].It helps in counseling as well as individualizing dosing for ovarian stimulation thereby improving the efficiency and safety of IVF. Higher level predicts the chances of hyperstimulation and caution is applied in such cases.

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