Anti-mullerian hormone and antral follicle count as predictors of ovarian response in assisted reproduction

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ABSTRACT

OBJECTIVE: The objective of this study was to test the hypothesis that AMH and antral follicle count (AFC) are good predictors of ovarian response to controlled ovarian stimulation and to compare them. **MATERIALS AND METHODS:** This observational cross-sectional study included 56 subjects aged between 25 and 42 years who were enrolled between 1st January and 31st December 2010 for their first intracytoplasmic sperm injection (ICSI) program. Baseline hormone profiles including serum levels of Estradiol (E2), Follicle-stimulating hormone (FSH), Luteinizing hormone (LH), and Anti-mullerian Hormone (AMH) were determined on day 3 of the previous cycle. The antral follicle count measurements were performed on days 3-5 of the same menstrual cycle. Antral follicles within the bilateral ovaries between 2-6 mm were recorded. The subjects were treated with long protocol for ovarian stimulation. Ovulation was induced with 10,000 IU of human chorionic gonadotropin (hCG) when at least 3 follicles attained the size of more than 17 mm. Transvaginal oocyte retrieval was performed under ultrasound guidance 36 hours after hCG administration. An oocyte count less than 4 and absence of follicular growth with controlled ovarian hyper stimulation was considered as poor ovarian response. Oocyte count of 4 or more was considered as normal ovarian response. **RESULTS:** Statistical analysis was performed using SPSS software trail version 16.0. Subjects were divided into 2 groups, depending on the ovarian response. The mean oocyte counts were 12.27 ± 6.06 and 2.22 ± 1.24 in normal and poor responders, respectively, (P = 001). Multiple regression analysis revealed AMH and antral follicle count as predictors of ovarian response (β coefficient ± SE for AMH was 1.618 ± 0.602 (P = 0.01) and for AFC, it was, 0.528 ± 0.175 (P = 0.004). AFC was found to be a better predictor of ovarian response compared to AMH in controlled ovarian hyper stimulation. **CONCLUSION:** The observations made in this study revealed that both AMH and AFC are good predictors of ovarian response; AFC being a better predictor compared to AMH.

KEY WORDS: Anti-mullerian hormone, antral follicle count, ovarian response

INTRODUCTION

The age-related decline in the success of the assisted reproductive technology programs is largely attributed to progressive decline in ovarian reserve.[1] Adequate follicular development of ovaries in response to gonadotrophin has been referred to as an ovarian reserve. Recently, there has been increasing interest in identification of tests of ovarian reserve as the success of assisted reproductive technology program depends on adequate ovarian reserve.[2] Though ovarian reserve declines with age, it varies between individuals.[3,4] An ideal marker for ovarian reserve is of enormous help to reproductive physicians in predicting the outcome of ART as it involves huge costs and burden to the couples who seek treatment. Over the past two decades, a number of tests of ovarian reserve have been designed. Recent studies indicate that anti-mullerian hormone (AMH) and antral follicle count (AFC) are the two tests of ovarian reserve that are very useful in predicting ovarian response to controlled ovarian stimulation.[5,6] AMH is a glycoprotein and member of the transforming growth factor β family. It causes regression of the mullerian ducts during male fetal development. AMH is also produced by the growing ovarian follicles of women during their reproductive

lifespan.[7] Recent studies indicate that AMH levels decline with increasing female age, and basal AMH levels are associated with ovarian response *in vitro* fertilization (IVF) patients with normal FSH levels. Some studies indicate that AMH is also useful in individualizing stimulation protocols.[8] Antral follicle count involves counting the resting follicles that are found on both ovaries at the beginning of proliferative phase of the menstrual cycle by transvaginal ultrasound. They are approximately 2-6 mm in size and are being shown in recent studies as predictors of ovarian response during controlled ovarian stimulation.[9,10] Some studies suggest that they are useful in determining stimulation protocols.[11] The aim of this study was to test the hypothesis that AMH and AFC are good predictors of ovarian response to controlled ovarian hyper stimulation in assisted reproduction technology programs.

MATERIALS AND METHODS

This observational cross-sectional study includes 56 subjects aged between 25 and 42 years, enrolled for intracytoplasmic sperm injection (ICSI) program from 1st January to 31st December 2010. The study protocol was approved by the research and ethics committee of the institute, and an informed consent of the participants was obtained.

The inclusion criteria were

1. First stimulation cycle for intracytoplasmic sperm injection (ICSI).
2. A long protocol for the use of a Gonadotrophin releasing hormone agonist (GnRHa).
3. The presence of both ovaries.
4. Regular menstrual cycles (cycle length of 25 to 35 days duration).
5. No evidence of endocrine disorders (normal TSH, prolactin, testosterone, and androstanidione).
6. A BMI ranging from 18 to 25 Kg/m^2
7. Not on hormone therapy for previous 3 months.
8. No history of ovarian surgery.
9. Serological markers for hepatitis B, C, and HIV were negative

Antral follicle count and hormonal assay

Baseline hormone profiles including serum levels of FSH, LH, and AMH were determined on day 3 of the previous cycle. The antral follicle count measurements were performed by the same sonologist on days 3-5 of the menstrual cycle. Antral follicles within the bilateral ovaries between 2- 6 mm in diameter were recorded. An 8 MHZ transvaginal probe was utilized for all examinations. Serum FSH and LH were measured using a specific immumetric assay kit (Immulite; Diagnostic products corporation, Los Angeles CA, USA). The minimal detection limits for FSH was 0.1 IU/L, and the intra- and inter-assay coefficients of variation for the FSH assay were 6.6% and 7.8%, respectively. The detection limit and intra- and inter-assay coefficients of variation for the LH assay were 0.1 IU/L and 6.5/7.1%, respectively. Measurement of serum AMH levels was performed using AMH/MIS Elisa kit (Diagnostic systems Lab, Webster Texas, USA). The Minimal detection limit and intra- and inter-assay coefficients of variation for the AMH assay were 0.017 ng/ml and 5% and 8%, respectively.

Ovarian stimulation protocol

All patients were treated with long protocol for ovarian stimulation. In the long protocol, pituitary down regulation was achieved by administering luprolide acetate (Lupride 4 Sun pharma) 0.5 mg subcutaneously daily from day 21 of the previous menstrual cycle. Ovarian stimulation was effected with exogenous gonadotrophin in the form of recombinant follitrophin β (racy-FSH puregon, Organon, Germany) or menotrophin (LG IVF.M, LG Life sciences, Korea) from day 2 of the menstrual cycle. The starting dose of gonadotrophin for subjects under 35 years age was 150 IU/day and for subjects over 35 years age was between 225 and 300 IU/day. After 7 days of stimulation, follicular growth was assessed by transvaginal ultrasound with 8 MHZ probe. The dose of recombinant FSH was adjusted according to the ovarian response. Ovulation was induced with 10,000 IU of hCG (Koragon Ferring pharma, India) when at least 3 follicles attained the size of more than 17 mm in diameter. Transvaginal oocyte retrieval was performed under ultrasound guidance 36 hours after hCG administration, and the number of retrieved oocytes was recorded. ICSI was performed on the retrieved oocytes. An oocyte count less than 4 and absence of follicular growth with controlled ovarian hyper stimulation were considered as poor ovarian response. An oocyte count of 4 or more was considered as good ovarian response.

The primary outcome measures of the study were: (1) Number of oocytes retrieved and the ovarian response, (2) To compare AMH and AFC as predictors of ovarian Response.

Statistical analysis

Statistical analysis was performed using SPSS software trail version 16.0. Fisher's exact test was used to examine the difference in categorical variables. Values are presented as mean ± SD. Students 't' test was performed to compare different groups. Pearson correlation coefficients were calculated to explore the relationship between the measured parameters. Multivariate logistical regression analysis was used to test the association between poor response (oocyte count < 4) and normal response with measured parameters. For all statistical analysis, $P < 0.05$ was considered significant.

RESULTS

The mean age (years) of the subjects studied was 34.61 ± 3.62. The mean AMH levels were 1.79 ± 1.72 ng, and mean AFC was 8.57 ± 5.16. The mean FSH levels were 5.60 ± 3.64, [Table 1]. AMH and AFC levels were higher in normal responders [Table 2]. There were statistically significant positive correlations between the number of retrieved oocytes and AMH (r = 0.543, $P < 0.01$) followed by AFC (r = 0458, $P < 0.01$). Statistically significant but inverse correlations between the number of oocytes and age (r = –0314, $P < 0.01$) were observed. No correlation was identified between the number of retrieved oocytes and FSH (r = –0.179, $P > 0.05$) [Table 3]. Patients were divided into 2 groups depending on the number of oocytes retrieved as poor (<4 oocytes) and normal (more than 4 oocytes) responders. There were 33 normal responders and 23 poor responders. Statistically significant differences were observed in mean AMH levels between normal responders 2.19 ± 1.74 ng/ml and poor responders 1.22 ± 1.55 ng/ml (P = 0.036). Similarly, the mean AFC was 10.42 ± 5.56 in normal responders and 5.91 ± 4.31 in poor responders (P = 0.002). The mean no of oocytes retrieved in normal responders was 12.27 ± 6.06 and 2.22 ± 1.24 in poor responders (P = 0.001) [Table 2]. When multiple regression analysis was used for prediction of ovarian response, AMH levels and AFC were found to be independent predictors of ovarian response (β-coefficient [± SE] for AMH was 1.618 ± 0.602, P = 0.010 and for AFC, it was 0.528 ± 0.175,(P = 0.004) [Table 4].

Table 1: Mean values of ovarian reserve parameters in total subjects

Parameters	No. of subjects	Mean values
Age in years	56	34.61±3.62
AMH (ng/ml)	56	1.79±1.72
AFC	56	8.57±5.16
FSH (IU/L)	56	5.60±3.64
No. of oocytes retrieved	56	8.14±6.84

AMH=Antimullerian hormone; AFC=Antral follicle count; FSH=Follicle-stimulating hormone

Table 2: Ovarian reserve parameters in normal and poor responders

Variables	Group status	*n*	Mean±S.D	*P* value
Age (Years)	Poor	23	35.04±3.624	0.457
	Normal	33	34.30±3.644	
AMH (ng/ml)	Poor	23	1.22±1.55	0.036*
	Normal	33	2.19±1.74	
FSH (IU/L)	Poor	23	6.38±4.66	0.228
	Normal	33	5.06±2.68	
AFC	Poor	23	5.91±4.30	0.002*
	Normal	33	10.42±5.56	
E2 (p mole/L)	Poor	23	35.54±24.27	0.693
	Normal	33	33.19±19.99	

* = Significant; AMH = Anti-mullerian hormone; AFC = Antral follicle count; FSH = Follicle stimulating hormone; E2 = Estradiol

Table 3: Correlations between no. of oocytes with measured parameters

		Age (Years)	AMH (ng)	FSH (IU/L)	E2 (*p* mol/L)	AFC
No. of oocytes	r	–0.314*	0.543**	–0.179	–0.050	0.458**
	Sig. (2-tail *P*)	0.018	0.0001	0.186	0.712	0.0001
	n	56	56	56	56	56

* = Significant; ** = Highly significant; AMH = Anti-mullerian hormone; AFC = Antral follicle count; FSH = Follicle stimulating hormone; E2 = Estradiol

Table 4: Multiple regression analysis of factors predictive of ovarian response

Model	Unstandardized coefficients		Standardized coefficients	*t*	*P* value
	B	SE	Beta		
Constant	1.934	1.880		1.029	0.309
AMH (ng)	1.618	0.602	0.372	2.686	0.010*
FSH (IU/L)	–0.192	0.178	–0.103	–1.082	0.285
E2 (*p* mol/L)	0.008	0.030	0.027	0.285	0.777
AFC	0.528	0.175	0.414	3.013	0.004*

* = Significant; AMH = Anti-mullerian hormone; AFC = Antral follicle count; FSH = Follicle stimulating hormone; E2 = Estradiol

DISCUSSION

In this study, the role of AMH and AFC were evaluated and compared for predicting ovarian response to controlled ovarian stimulation with gonadotrophin. Out of the 56 subjects recruited for the study, 33 subjects had normal ovarian response, 22 had poor ovarian response, and 1 subject had empty follicular syndrome. The mean AMH levels and AFC were significantly high in normal responders compared to poor responders [Table 2]. Similar observations were made in other studies.[2,7] Statistically significant positive correlations were found for AMH and AFC with regard to ovarian response (P = 0.01) [Figures 1 and 2]. Similarly, significant inverse correlations were found with respect to age (P = 0.01). This age-related decline in ovarian reserve was also observed in other published studies.[4,12] Statistically significant correlations were not found with respect to other studied parameters[12] like FSH and estradiol (P =>0.05) [Table 3]. These observations are in accordance with the results of other studies conducted recently.[3-5,13] In two recent studies, AMH levels were correlated with ovarian response, oocyte quality, and cycle cancellation.[3,13] In the first study, the mean AMH levels were 1.26 ng/ml, and in the second study, the mean AMH levels were 1.66 ng/ml. In the present study, the mean AMH levels were 1.22 ng/ml and 2.19 ng/ml in poor and normal responders, respectively. This study shows that fertility is preserved even at lower AMH levels in Indians compared to their western counterparts. Various other studies also correlated AMH levels and AFC with oocyte count and quality.[2,9,14,15,16] When multiple regression analysis was used to compare the measured parameters,

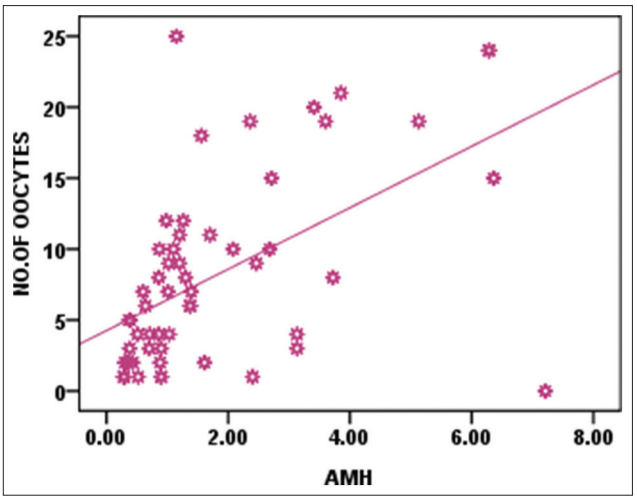

Figure 1: Graphical representation of ovarian response to AMH

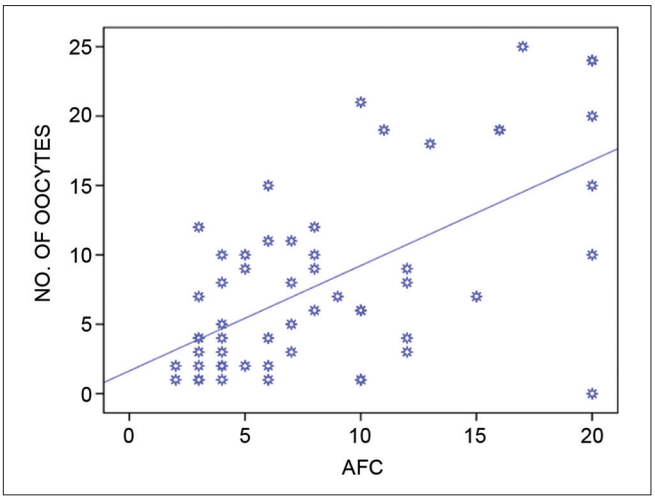

Figure 2: Graphical representation of ovarian response to AFC

both AMH and AFC were found to be independent predictors of ovarian response [Table 4]. The present study indicates that AFC was a better predictor of ovarian response compared to AMH (P = 0.004 for AFC and 0.01 for AMH). This is an important observation as AMH is an expensive test available at few places, whereas AFC counts are measured routinely by infertility consultants. This study demonstrates that AFC can be used as a surrogate for expensive AMH estimation, which can be used for females above 35 years age where it is said to be more reliable for predicting ovarian reserve and pregnancy outcome.[17-21] Recent studies have revealed AMH and AFC as predictors of primordial follicle pool and excessive response to controlled ovarian hyperstimulation.[22,23] Currently, most IVF clinicians determine starting doses of gonadotrophin in the first cycle of IVF, depending principally on age and basal FSH levels.[15] Our study indicates that AMH and AFC are also good predictors of ovarian response to controlled ovarian stimulation. The conventional ultrasound assessment for the determination of ovarian reserve is operator-dependent as it involves subjective interpretation of the images. This can be eliminated by automated evaluation of the antral follicle size and number and thereby improve the outcome. At the present time, one of the principal impediments to the more widespread use of AMH to predict ART outcome is lack of standardization of AMH assay results. Even though AMH and AFC are good predictors of ovarian reserve, they should not be used for excluding subjects from ART programs. They are useful for counseling the infertile couples about realistic outcome of the procedure. Creating realistic expectation is very important in maintaining patient confidence and satisfaction. These tests may also help the clinicians to make adjustments in the starting dose of gonadotrophin, thereby preventing excessive stimulation and ovarian hyper stimulation syndrome.

In conclusion, the observations made in this study reveal that both AMH and AFC are good predictors of ovarian response to controlled ovarian hyper stimulation and that they may supplement the presently available other tests of ovarian reserve. Studies are being conducted to evaluate the role of AMH and AFC as predictors of pregnancy outcome in ART programs.

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