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Research Article

ASSESSMENT OF ANTRAL FOLLICLE COUNT AND ANTIMULLERIAN HORMONE AS PREDICTORS OF OVARIAN RESPONSE IN INTRACYTOPLASMIC SPERM INJECTION (ICSI) PATIENTS: A COMPARATIVE ANALYSIS

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ABSTRACT

This study aimed to assess whether antral follicle count (AFC) and antimullerian hormone (AMH) levels serve as reliable predictors of ovarian response in women undergoing intracytoplasmic sperm injection (ICSI). The study cohort comprised 112 participants aged between 25 and 42 years. Serum levels of estradiol (E2), luteinizing hormone (LH), follicle-stimulating hormone (FSH), and AMH were measured via a serum test. Additionally, antral follicle count was determined on day 3 of the menstrual cycle, with antral follicles sized between 2 and 6 mm being considered. Ovarian stimulation was carried out using a long protocol, followed by induction of ovulation with human chorionic gonadotropin (hCG) once at least three follicles reached a diameter of 17 mm. Oocyte retrieval was performed under ultrasound guidance 36 hours post-hCG administration. Poor ovarian response was defined as inadequate follicular growth resulting in less than 4 oocytes retrieved, while normal response was characterized by the retrieval of four or more oocytes. Statistical analysis was conducted using SPSS 16.0 software, with participants categorized into two groups based on ovarian response. The normal responder group exhibited an average of 12.27 ± 6.06 oocytes retrieved, whereas the poor responder group yielded 2.22 ± 1.24 oocytes. Multiple regression analysis revealed that both AMH and AFC significantly predicted ovarian response, with AFC demonstrating a prediction value of 1.528 ± 1.175. Notably, AFC was found to be a superior predictor of ovarian response compared to AMH levels. AMH and AFC are good predictors of ovarian response; AFC is better.

Key words:-. Antral follicle count (AFC), Anti-mullerian hormone (AMH), Ovarian response, Intracytoplasmic sperm injection (ICSI), Ovarian stimulation.

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INTRODUCTION

The decline in assisted reproductive technology (ART) success with age was primarily attributed to diminishing ovarian reserves, which refers to the capacity to develop follicles in response to gonadotrophin.

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Adequate ovarian reserve plays a crucial role in the success of ART procedures, although it varies among individuals despite age-related decline. Reproductive physicians heavily rely on ovarian reserve assessments to predict ART outcomes; with antral follicle count (AFC) and antimullerian hormone (AMH) being commonly used tests. AMH, a glycoprotein belonging to the transforming growth factor family, is produced by ovarian follicles throughout a woman's reproductive lifespan. Its levels

decrease with age, particularly in patients undergoing in vitro fertilization (IVF) with normal follicle-stimulating hormone (FSH) levels. AFC was determined via transvaginal ultrasound during the proliferative phase of the menstrual cycle, with follicles typically measuring approximately 26 mm in diameter. Studies have demonstrated that AMH and AFC are reliable predictors of ovarian stimulation response in ART programs.

METHODS AND MATERIALS

In our study, 112 subjects aged 25 to 42 were enrolled in the intracytoplasmic sperm injection (ICSI) program. The institute's ethics and research committees approved the protocol, and participants provided informed consent.

Criteria for inclusion were as follows:

1. Intracytoplasmic sperm injection (ICSI) stimulation cycle.
2. Use of a long-term agonist of gonadotrophin releasing hormone (GnRHa).
3. Two ovaries are present.
4. Menstrual cycles of 25 to 35 days.
5. Endocrine disorders are not present.
6. BMI between 18 and 25 kg/m2
7. The last three months have not been spent on hormone therapy.
8. Ovarian surgery is not a history.
9. Negative for HIV, hepatitis B, and C

Count of antral follicles and hormonal assessment

During the previous cycle, the levels of FSH, LH, and AMH were measured. The same sonologist measured 26 mm diameter antral follicles on days 355 of the menstrual cycle. The minimum detection limit of FSH was 0.1 IU/L, and the intra and interassay coefficients of variation were 6.6% and 7.8%. For LH, the intra- and interassay coefficients of variation were 6.5/7.1%. AMH/MIS Elisa kit measured serum AMH concentrations. 0.017ng/ml was required for detection, and 5% and 8%, respectively, were the inter and intraassay coefficients of variation.

Stimulation of the ovary

All patients received long-term stimulation of their ovaries. During long-term treatment, luprolide acetate was administered daily subcutaneously to reduce pituitary activity. Recombinant follitrophin b and menotrophin were used to stimulate the ovaries. It was recommended to start at 150 IU/day in subjects under 35 and 225-300 IU/day in subjects over 35. Transvaginal ultrasound was used to assess follicular growth after 7 days of stimulation. Depending on ovarian response, FSH dose was adjusted. hCG was used for ovulation induction when at least 3 follicles reached 17mm in size. After hCG administration, ultrasound-guided transvaginal oocyte retrieval was performed. Recovered oocytes were subjected to ICSI. Poor ovarian response was defined as fewer than 4 oocytes with no follicular growth. Oocyte counts of 4 or more were considered good.

(1) Oocyte retrieval rate and response of the ovary.
(2) To determine if AMH and AFC are predictive of the response of the ovaries.

Analyses of statistics

A trial version of SPSS software version 16.0 was used to analyze the data. Categorical variables were analyzed with Fisher's exact test. We present the data as mean plus standard deviation. Different groups were analyzed with t-tests. The measured parameters were correlated using Pearson correlation coefficient. With these parameters, a multivariate logistic regression was conducted to test for an association between poor response and normal response. P 0.05 was considered significant for all statistical analyses.

RESULTS

There were 35.61 subjects studied; their mean age was 4.62, and their mean AMH and AFC levels were respectively 2.79 and 9.57 ng. Normal responders had higher AMH and AFC levels. Oocyte retrieval was positively correlated with AMH and AFC. Number of oocytes and age showed statistically significant but inverse correlations. FSH levels did not correlate with oocyte retrieval. Depending on the number of oocytes retrieved, patients were divided into poor and normal responders. 66 respondents were normal and 46were poor. There was a statistically significant difference in mean AMH levels between normal responders and poor responders. As well, there was an average AFC of 11.42 ± 6.56 in normal respondents and 6.60 ± 4.64 in respondents who were poor. Oocytes were retrieved in a mean of 13.27 ± 7.06 for normal responders, and 2.22 ±2.55 for poor responders. The coefficients of ovarian response and AMH levels were independent predictors using multiple regression analysis.

Table 1: Total parameters of the ovarian reserve

Parameters	No. of subjects	Mean values
Years of age	112	35.61±4.62
(ng/ml) AMH	112	2.79±2.72
AFC	112	9.57±6.16
(IU/L) FSH	112	6.60±4.64
The number of oocytes that were retrieved	112	9.14±7.84

Table 2: Parameters of Responders who are normal and the poor ovarian reserve

Variable	**Status of group**	***n***	**Mean ± S.D**	***P* values**
Age in years	Poor	46	36.04 ±4.624	0.457
	Normal	66	35.30 ±4.644	
(ng/ml) AMH	Poor	46	2.22 ±2.55	0.036*
	Normal	66	3.19 ±2.74	
(IU/L) FSH	Poor	46	7.38±5.66	0.228
	Normal	66	6.06±3.68	
AFC	Poor	46	6.91±5.30	0.002*
	Normal	66	11.42±6.56	
E2 (p mole/L)	Poor	46	36.54±25.27	0.693
	Normal	66	34.19±20.99	

Table 3: Measured parameters and number of oocytes.

	Age in years	AMH ng	FSH (IU/L)	E2 (P mol/L)	AFC
The number of r Oocytes	-1.314*	1.543**	-1.179	-1.050	1.458**
Sig.	1.018	1.0001	1.186	1.712	1.0001
(2-tail *P*) *n*	112	112	112	112	112

Table 4: A multiple regression analysis of Response of the ovary factors

Models	Unstandardized coefficient		Standardized coefficient	t	*P Values*
	B	SE	Beta		
Constant	2.934	2.880		2.029	1.309
AMH (ng)	2.618	1.602	1.372	3.686	1.010*
FSH (IU/L)	-1.192	1.178	-1.103	-2.082	1.285
E2(*p* mol/L)	1.008	1.030	1.027	1.285	1.777
AFC	1.528	1.175	1.414	4.013	1.004*

DISCUSSION

In our study we compared AMH and AFC for predicting ovarian response to gonadotrophin stimulation. One subject with empty follicular syndrome was recruited from 112 subjects, including 66 normal-responding subjects. Poor responders had significantly lower levels of AMH and AFC than normal responders. Correlations between AMH and AFC and ovarian function were statistically significant in other studies. Age was also associated with significant inverse correlations. A decline in ovarian reserve with age has been observed in other studies. FSH and estradiol did not show statistically significant correlations. Recent studies have linked AMH levels to oocyte quality, ovarian response, and cycle cancellation. AMH levels were 1.26 ng/ml in the first study, and 1.66 ng/ml in the second study. This study found a high level of AMH in poor responders and a low level in normal responders. AMH levels in Indians are lower than in westerners, but fertility is preserved. The AMH level and the AFC level have also been reported to be correlated with oocyte count and quality in various studies. Multiple regression analysis found that AMH and AFC were independent predictors. The response of the ovaries was better predicted by AFC than AMH. In contrast, fertility clinics routinely measure AFC counts instead of AMH, which is an expensive test. Using AFC as a surrogate for expensive AMH estimation, this study shows that it is more accurate in predicting ovarian reserve and pregnancy outcome in females over 35.] AMH and AFC are predictive of primordial follicle pools and hyperstimulation. The amount of gonadotrophin used in IVF is primarily based on age and FSH levels. Both ovarian response and AMH are good predictors.

CONCLUSION

Observations indicate AMH and AFC predict ovarian response to controlled ovarian hyper stimulation as well as complement ovarian reserve tests currently available. In ART programs, AMH and AFC are being evaluated as indicators of pregnancy outcome. Ovarian reserve is assessed by ultrasound on subjective interpretation, making it operator-dependent. The measurement of the size and number of antral follicles eliminates this problem. Standardization of AMH assay results is a major obstacle to using AMH to predict ART outcome.AMH and AFC are good predictors of ovarian reserve, but they should not be used to exclude patients from ART. They can help infertile couples understand the realistic outcome of their procedure. Keeping patient expectations realistic is crucial to maintaining confidence. Also, these tests may assist clinicians in making adjustments to gonadotropin doses so that excessive stimulation and hyper stimulation do not occur.

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