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Anti-Mullarian hormone versus day three follicular stimulating hormone and antral follicular count as a predictor for ovarian response to stimulation

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ABSTRACT

Back ground: *Recently, a new marker, the anti-mullarian hormone (AMH), has been evaluated as a marker of ovarian response. Since the number of ovarian follicles decline with increasing age, AMH level might be used as a marker for ovarian aging, and serum AMH can be measured at frequent time-points during menstrual cycle, suggesting the complete absence of fluctuation.*

Aim of study: *To evaluate wither anti-Mullarian hormone is a better predictor of ovarian response to stimulation in comparism to day 3 follicular stimulating hormone (FSH) and antral follicular count (AFC).*

Subjects and methods: *This study was carried out at Bint AL-Huda teaching hospital in Thi-qar and Basrah IVF center during the period from 1st January 2012-1st January 2013 including 60 women with infertility caused by anovulation, and they were assesd by doing day 3 FSH, AMH and AFC, then they were subjected to ovulation induction treatment using clomiphene citrate 50 mg three times daily from day 2-6 of menstrual cycle and then assess at day 7 with transvaginal u/s and serum oestradiol level for the size and numbers of mature graffian follicle, if they were not responding to clomiphene citrate tablet they were given 3 ampules of follicular stimulating hormone (follitropin beta) (50iu/0.5ml) at day 7, 8, and 9 of menstrual cycle, on day 10-11 of menstrual cycle the MGF was assessed for the numbers and size and serum sample obtained for measurement of oestradiol level. The data were analyzed by using t-test, pearson correlation, regression coefficient and area under receiver operating curve (ROC) was calculating as measure of predictive accuracy.*

Result: *From total (60) women. (11) patients have no ovarian response and (49) patients have normal ovarian response, the non responders group have significantly lower day 3 antral follicular count (AFC) <5 compare to normal responders >5 with p value=0.004, and also the non responders group had significantly low anti-Mullarian hormone level (AMH) (0.1±0.1) compare with normal responders the AMH level (1.2±0.6) with p value=0.001, follicular stimulating hormone level (FSH) was significantly higher in non responders (7.6±4.2) compare to normal responders the FSH level was (4.7±1.5) with p value=0.001,and the mean day 11 MGF count and E2 was higher in normal responders group compared with non responders group with p value=0.001. There was a ppositive correlation between AMH and AFC with MGF count while the correlation between FSH and MGF was negative. Multivariate analysis of the*

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variables that predict good ovarian response to ovulation induction treatment demonstrate that the biochemical variable AMH was the most significant predictor as (R2=64. 9%).The AMH level was addressed as the only significant variable in determination of MGF numbers as AMH with a cut-off value >0.37ng/ml has area under the curve (accuracy)(0.96%) with p value <0.001 which was higher than that for AFC and FSH (73.3% and69.8%) respectively.

Conclusion: *the present study concludes that AMH is a promising biochemical marker and it was better than day 3 AFC and FSH for the prediction of ovarian response, and the ability to predict poor response may be a valuable tool for patient counseling, since the poor responders have a lower probability of pregnancy and to estimate the starting dose of gonadotrophins (higher doses are required for poor responders).*

KEYWORDS: *Anti Mullarian hormone, follicular stimulating hormone ,antral follicular count, ovarian reserve.*

I. INTRODUCTION

The WHO defines infertility as two years of exposure to pregnancy without conceiving [1] . Other definitions of infertility include that infertility is the failure of a couple to become pregnant after one year of regular, unprotected intercourse [2] .

The distress and personal devastation experienced by couples suffering infertility has been documented since the beginning of recorded time in cultures throughout the world. Recent data gathered by the World Health Organization (WHO) through Demographic and Health Surveys in developing countries estimates that 186 million married women (excluding China) were infertile in the year 2002 [2]. Previous estimates had the number afflicted with infertility at 60 – 80 million [3]. This represents a substantial increase in the absolute number of people with infertility. Modern medicine has made several advances in the diagnosis, treatment and prevention of infertility over the last fifty years [4].

In both men and women, the fertility process is complex. Infertility affects about 10% of all couples. About a third of infertility problems are due to female infertility and and other third are due to male infertility. In the remaining cases, infertility affects both partners or the cause is un clear.

Causes of female infertility [5]

- CAUSES OF POORLY FUNCTIONING FALLOPIAN TUBES
- ENDOMETRIOSIS
- ADDITIONAL FACTORS

1. Other variables that may cause infertility in women:
2. Behavioral Factors:
3. Environmental and occupational Factors:

Signs and symptoms [6]

Anovulation is usually associated with specific symptoms. However, it is important to note that they are not necessarily all displayed simultaneously.

1. Amenorrhea occurs in about 20% of women with ovulatory dysfunction.

2. Infrequent and light menstruations occur in about 40% of women with ovulatory dysfunction.

3. Irregular menstruation, where five or more menstrual cycles a year are five or more days shorter or longer than the length of the average cycle.

4. Absence of mastodynia (breast pain or tenderness) occurs in about 20% of women with ovulatory problems.

Assessment of current fertility

Current fertility assessment relates to predicting the chances for live birth in natural exposure or in infertility treatment conditions. Outcomes of interest in infertility treatment mainly relate to IVF or intracytoplasmatic sperm injection (ICSI) treatment, such as the response to ovarian hyperstimulation and the chances of ongoing pregnancy. There is a specific urge to identify women of relatively young age with clearly diminished reserve, as well as older women with still an adequate ovarian reserve. Based on the test result, management could be individualized, for instance by stimulation-dose adjustment, by counseling against initiation of IVF treatment, or by indicating the necessity of early initiation of treatment before the ovarian reserve has diminished too far [7].

Individualization of patient management could be more cost-effective, as it could increase the efficacy and reduce the costs of the fertility treatment. So far many studies have been conducted on ovarian reserve tests (ORTs) in IVF/ICSI outcome, but they show contradictory results for both response prediction and pregnancy prediction [8].

Assessment of future fertility

The time interval until natural sterility will have set in, referred to as the reproductive lifespan, will mirror the period in which fertility may be optimal. For the assessment of this reproductive lifespan, tools that closely relate to the future age at menopause may be developed into useful long term predictors. Seen the fixed temporal interrelationship between end of fertility and menopause, correct prediction of menopause may provide valuable information on the individual level [9].

This could open new avenues for the primary prevention of female infertility. Moreover, menopausal age is also related to women's health in general [10].

Predicted early menopause could emphasize the need for timely prevention of bone demineralization, and cardiovascular and neurological disease [11], while the prediction of late menopause would open options for preventive management of breast and intestinal cancer [12].

So far, the time relationship between ovarian reserve tests and menopause has been shown in cross-sectional studies and short term follow up studies [13,14].

Aim of study

To evaluate wether anti-Mullarian hormone is a better predictor of ovarian response to stimulation in comparism to day 3 follicular stimulating hormone (FSH) and antral follicular count (AFC).

II. SUBJECTS & METHODS

This is a prospective study carried out at Bint AL-Huda teaching hospital (infertility unit) and Basrah IVF center , during the period from 1st January 2012 – 1st January 2013 for patients were attending the centers for intra

uterine insemination which was mainly indicated for defect in ovulation. Those patients were fully evaluated (history, examination including pelvic examination and investigations for infertility)

Iniclusion criteria:

1. Regular menstrual cycle
2. Presence of both ovaries
3. No evidence of endocrine disorders normal thyroid function test , prolactin , testerone , androstenadione and no polycystic ovary syndrome
4. Oestradiol level < 50 pg/ml
5. BMI (18 – 30 kg/m^2)
6. Age between (20-45) years

Study protocol :

On day 3 of spontaneous menstrual cycle , the patients underwent a trans vaginal u/s examination to assess the size and the numbers of antral follicules.

The sum of all follicular measures 2 – 10 mm in diameter (mean of two orthogonal diameter) in both ovaries was consider, the FSH on the same day was assessed in plasma using Enzyme linked fluorescent assay (ELFA) technique using (Biomerienx) kit by using MINIVIDAS instrument (Italy), the assay principle combines an enzyme immune assay sandwich method with a final fluorescent detetction , on the same day the serum was separated from all

blood sample and frozen in aliquots at – 20C^o until used for subsequent centralization analysis .Serum AMH level was determined using an ultrasensitive enzyme – linked immunosorbent assay (ELISA) (Biotek) (USA) instrument when two levels of control are available to allow performance monitoring of AMH Gen II ELISA in the A 79765 assay calibrators, controls and samples are incubated in microtitration wells which have been coated with anti – AMH antibody . After incubation and washing , the wells are treated with anti – AMH detection anti body labeled with biotin . After a second incubation and washing step, the wells are incubated with streptavidin - horseradish peroxide (HRP). After a third incubation and washing steps, the wells are incubated with the substrate tetramethylebenzidine (TMB) lastly an acidic stopping solution is added, the degree of enzymatic turn over of the substrate is determined by dual wave length absorbance measurement at 450 nm as primary test filter and 630 nm as primary reference filter. The absorbance measured is directly proportional to the concentration of AMH in the samples . A set of AMH Gen II calibrators is used to plot acalibration curve of absorbance versus AMH concentration the AMH concentrations in the samples can then be caliculated .

Then those patients were subjected to ovulation induction treatment using (clomiphene citrate tablet 50 mg(clomifert ,Dar Al Dawa, Jordan)they were given three times daily from the day 2 – 6 of menstrual cycle and they were assess at day 7 with transvaginal u/s and serum oestradiol level for numbers and size of mature graffian follicle if they were not responding to clomiphene tablet they were given 3 ampules of follicular stimulating hormone(follitropin beta) 50 iu/0.5 ml (puregon,Netherlands) at day 7, 8 and 9 of the cycle , on day 10 -11 of menstrual cycle the mature graffian follicle was assess for numbers and size and serum sample obtained for measurement of oestradiol using

(MINIVIDAS) (Italy) using ELISA technique (Enzyme linked immuno fluorescent Assay). The assay principle combines a competition method with a final fluorescent detection (ELFA).

Statistical analysis

Data were analyzed using statistical package for Social Sciences version 15 (SPSS Inc , Chicago , IL , USA). Continuous variables were expressed as mean ± standard deviation, when there were two independent group, they were compared by Student`s t test. The degree of association between continuous variables was calculated by Pearson correlation and regression (simple and multiple) coefficients . For each single variable used in the univariate analysis and for models, the ability to discriminate between patients with no response and patients with a normal response was assessed by calculating the area under the receiver operating characteristics curves (ROC_{AUC}) [98] .The ROC_{AUC} may vary between 0.5 (no discriminative power) to 1.0 (perfect discrimination). A pvalue < 0.05 was considered to be statistically significant.

III. RESULTS

The present study investigated 60 patients, who were attending Bint AL-Huda teaching hospital (infertility unit) Thi-Qar and Basrah IVF center ,they were subjected to the same ovulation induction regime.

The main cause of infertility was anovulatory infertility problem, other cases with other causes were exclude, 11 (18.33%) of them show no response (no mature graffian follicle), and 49 (81.66%) show normal response (one or more graffian follicle)

Table 1. The characteristics of patients under study

	Total (n=60)	Non responders (n=11) no MGF	Normal responders (n=49) MGF ≥1	P- value
BMI (kg/m2)	26.8±2.05	27.8±2.4	26.2±3.5	0.151
Age (years)	30.2±7.1	40.0±6.1	28.0±5.2	0.001
Day-3AFC(n)	5.8±1.6	4.5±1.0	6.0±1.6	0.004
Day-3FSH(IU/L)	5.3±2.4	7.6±4.2	4.7±1.5	0.001
Day-3AMH(ng/ml)	1.0±0.7	0.1±0.1	1.2±0.6	0.001
Primary infertility	34	9	25	

All of the displayed values are expressed as mean ± standard deviation

Table 1. shows the characteristic of patients under study 34 (56.66%) cases were suffer from primary infertility and 26 (43.33%) cases of them have secondary infertility.

The mean body mass index BMI (kg/m^2) of cases with no response (non responders) was 27.8± 2.4 which is higher than those who had good response (normal responders) 26.2±3.5, but the difference is statistically not significant (p=0.151).

With respect to the mean age (years) it was significantly higher in non responders 40.0±6.1 than that in normal responders 28.0±5.2 with p value equal to 0.001.

Regarding the mean antral follicular count AFC(n) it was significantly higher in normal responders 6.0±1.6 compare to non responders 4.5 ±1.0 with p value equal to 0.004.

The mean Follicullar stimulating hormone FSH (iu/l) level was <8 in both groups but it was significantly higher in non responders 7.3±4.2 compare to the normal responders 4.7±1.5.

The mean anti-Mullarian hormone AMH (ng/ml) was significantly lower in non responders 0.1 ±0.1 compare with normal responders 1.2±0.6 with p value equal to 0.001.

Table 2. Day11 mean oestradiol and mature graffian follicule number

	Total (n=60)	Non responders(n=11)	Normal responders (n= 49)	P- Value
E2(pg/ml)	226.5±128.8	79.2±50.7	258.8±118.5	0.001
MGF(n)	1.2±0.8	0.0±0.0	1.5±0.6	0.001

All of the displayed values are expressed as mean± standard deviaton.

Table 2. shows the number of mature graffian follicle (MGF) and oetradiol (E2) (pg/ml) level at day 11 of the cycle after completing the course of ovulation induction regime.

The mean number of MGF in normal responders group was 1.5±0.6 and mean serum oestradiol level was 258.8±118.5 and they were significantly higher compare with their values in non responders group (0.0±0.0 and 79.2±50.7) respectively with p vlue equal to 0.001.

Table 3. Correlation coefficients (r) between day3 FSH, AFC , AMH and MGF count

Variable / MGF	r	P.value
FSH	-0.373	0.003
AFC	0.426	0.001
AMH	0.769	0.000

Table 3.shows the correlation coefficients of MGF with AFC and AMH were significantly positive ,while that of MGF and FSH was significantly negative.

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Positive correlation mean that the increase in one variable related with increase in the other, where as the negative is visa versa.

Table 4. Simple and multiple regression analysis between MGF and FSH ,AFC and AMH

variable	Regression coefficient (b)	Determinant Coefficient (R2%)	P- value
FSH	-0.124	13.9	0.003
AFC	0.217	18	0.001
AMH	0.847	59.1	0.000
FSH, AFC, AMH		64.9	0.000

Table 4. shows that according to the result in this table multiple regression analysis shows that all the three tests (AFC, AMH and FSH) can predict the ovarian response to stimulation (number of MGF) (the regression was significant and multiple R2 equal to 64.9%) , the most reliable predictor test that the number of mature graffian follicle(MGF) depend on was the anti-Mullarian hormone (AMH) level as the determinant coefficient value for AMH was 59.1 which is higher than those for the antral follicular count (AFC) and follicular stimulating hormone (FSH) (18 and 13.9) respectively.

Table 5. Discriminate analysis (ROC) of FSH ,AMH and AFC for prediction good ovarian response

	FSH ≤7.1	AMH≥0.37	AFC>5
Sensitivity(%)	100	82.61	60.87
Specificity(%)	27.27	100	100
Area under curve%	69.8	96.0	73.3

Using AMH level of >0.37 ng/ml as a cut-off value , AMH has a sensitivity of (82.61) and specificity of (100) as an indicator for good ovarian response to stimulation with area under the curve (accuracy) equal to 96%.

AFC with a cut-off value >5 has sensitivity (60.87) with specificity(100) with accuracy equal to 73.3%.

FSH with a cut-off value ≥7.1 has sensitivity (100) and specificity (27.27) with accuracy equal to 69.8% .

The area under the curve of AMH was 96% which was higher than that for AFC and FSH (73.3 % and 69.8 %) respectively and this indicate that AMH was more accurate than AFC and FSH in predicting ovarian response to stimulation.

IV. DISCUSSION

Ovarian reserve comprises two element: the size of the stock of primordial follicles and the quality of the oocyte [15]. From the primordial follicle pool . primary follicles will start a maturation process and develop through secondary (pre antral) follicles into the pool of antralfollicles from which the monthly follicle to be ovulated is selected [16] .Since the size of the primordial follicle stock is difficult to measure directly, a marker that reflects all numbers of the follicles that have the transition from the primordial follicle pool to the growing pool may have a good indirect measurement. AMH might be such a marker [17]. Previously published data indicate that AMH is somewhat associated with the size of ovarian follicle pool and hence ovarian reserve [18] .

In this study the mean age of the non responders group was (40 ± 6.1 SD) which was significantly higher than that of the normal responders group (28 ± 5.2 SD) and this is goes with the previous claimed that diminished ovarian response to exogenous gonadotrophin stimulation is strongly associated with advanced age and reduced ovarian reserve [19].. Regarding the mean day 3 AFC , we found that it was significantly higher in the normal responders group (6 ± 1.6) compare to than non-responders group (4.5 ± 1.0) and this is similar to the findings reported by (Cigdem K.,O. Gulnur and K.K. Raziyeetal(ArchGyne) [20] and I.A. J.vanRooij, F.J.M.Broekmansetal (Human Reproduction) [21]. This reflects that AFC could be used as a predictor for ovarian response to stimulation. Regarding the mean D3 FSH level, we found that it was significantly lower in the normal responders group (4.7 ± 1.5 iu/l)compare to its level in thenonresponders group (7.6 ±4.2 iu/l) and this is similar to the findings of [20].

But different from that reported by [22] in which there was no significant difference. Regarding the mean AMH level (ng/ml),we foundthat it was significantly higher in the normal respondersgroup (1.2 ± 0.6) compare to thenonresponders group (0.1 ± 0.1 ng/ml). The same finding was reported by the above two studies [23,24] .

Negative correlation between day3 FSH and MGF count (r = - 0.373) and a positive correlation between AFC and AMH and MGF count (r = 0.426 and 0.769) respectively found in our study which is similar to that reported by K.Cigademetal. The correlation was significant between day3 FSH and AFC and MGF but it was highly significant between AMH and MGF count .K. Cigdem reported significant correlation only between (AMH and AFC) and MGF (r = 0.796 and 0.438) respectively but not between FSH and MGF (r = -0.013). J.vanRooijetal also reported significant positive correlation between AMH and number of mature oocytes (R = 0.57).In this study discriminate analysis of (FSH, AMH and AFC) for the prediction of good ovarian response show that the cut – off value for FSH was < 7.1 and AMH was > 0.37 and for AFC was> 5 ,Which were different from those determined by other researchers and these diffrences can be to the demographic and clinical variations among the reviewed populations. The biochemical techniques which were adopted to determine FSH and AMH levels may lead to the documentation of varying results as well.

Using the above cut – off values AMH has good sensitivity (82.61 %) which was lower than that for FSH (100%) and higher than that for AFC . Regarding specificity, AMH was (100%) specific which is similar to AFC and much higher than that of FSH . AMH was the best predictor for normal ovarian response as the area under the curve value washigher for AMH (%96) compare to those values for AFC and FSH (%73.3 and %69.8)respectively with a p value <0.001 and this was similar to that reported by other studies.

In this study multiple regression analysis showed that all the three tests (AFC .FSH and AMH) can be used to predict ovarian response to stimulation and it was also confirmed that AMH was the most reliable predictor for normal ovarian response as the determinant coefficient value was (59.1) which is higher than those for AFC and FSH (18 and 13.9) respectively although it was not investigated in this study.In the prediction of high response , comparable discriminative performance was found for the variables AFC and AMH, supporting the close relationship between these

variables and ovarian response. There are advantages for the use of AMH over AFC in the multivariate model for the prediction of ovarian response, since all the predictive information is obtained with blood sampling and no extra ultrasound is needed Furthermore,since there is no change in AMH level in response to gonadotropins, AMH can be measured throughout the cycle in contrast to the other parameters, which can only be determined during the early follicular phase, an advantage for bothpatients and clinicians.Obviously ,AMH intra – cycle and cycle -to – cycle variation should be furtheranalysed , but the small fluctuation in serum AMH level at three different time points during the menstrual cycle[106], support the feasibility of AMH assessment throughout the cycle.

V. CONCLUSIONS

The present study concludes that AMH is a promising biochemical marker and it was better than day3 antral follicular count (AFC) and follicular stimulating hormone (FSH) for the prediction of ovarian response.

The ability to predict poor response may be a valuable tool for patient counseling, since poor responders have a lower probability of pregnancy.

A further potential application for the prediction of poor response is to estimate the starting dose of gonadotrophins (higher doses are required for poor responders).

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