Research Article

(Anti mullerian hormone levels as predictor of ovarian response to controlled ovarian stimulation in IVF)

Hossam E. Shawki, Mohammed T. Gad el Rab, Saad A. Ahmed, and Kareem I .Shahin.
Department of Obstetrics and Gynaecology of Minia University Hospital, Minia, Egypt.

Abstract
Objective: The present study aimed to evaluate the performance of uses of the most recently identified marker of ovarian reserve, namely anti-Mullerian hormone (AMH), to categorize women based on their anticipated ovarian response. **Setting:** The study recruited patients referred for assisted reproduction treatment (ART) cycles to Minia infertility research unit (MIRU),Minia University Hospital, Minia, Egypt, during the period from October, ٢٠١٣ to April, ٢٠١٥ according to study protocol. **Study design:** Prospective observational analysis **Study participants:** This prospective study included a total of ١١٩ women undergoing ICSI at Minia infertility research unit(MIRU),Minia University Hospital **Results**: The regression analysis demonstrated significant (P<٠.٠٥) positive correlations between the AMH level and the total number of oocytes collected (r=٠.٦٤), total number of MII oocytes (r=٠.٦٥) and the number of follicles (r=٠.٦٢) on the hCG administration day. **Conclusion:** Antimullerian hormone was a stronger predictor of ovarian response to gonadotropin therapy at the study utilizing GnRH agonist and GnRH antagonist protocols.
Keywords: anti-Mullerian hormone (AMH), Ovarian response, IVF

Introduction
Since the birth of the first IVF baby in ١٩٧٨, IVF results have much improved reaching an average of ٣٠٪ pregnancy rate and ٢٥٪ live birth rate per cycle. Central to this improvement in IVF performance was the shift in paradigm from natural unifollicular IVF cycles to multifollicular IVF cycles as data showed higher pregnancy rates with controlled ovarian hyperstimulation (Van Der vorst et al., ١٩٩٧). However, the patients are exposed to the possibility of a low or excessive ovarian response.

Furthermore, the possibility of a negative impact of supraphysiological levels of estrogen resulting from the large numbers of follicles and oocytes on the embryo quality and/or the endometrium has been repeatedly questioned (Martinez ٢٠٠٧-Rubio C ٢٠١٠). For this reason, knowledge of the patient's potential ovarian response can help clinicians individualize the medication dosage, which may reduce the adverse effects of an excessive ovarian response; decrease the rate of cancelled cycles and ultimately, increase the pregnancy rate. The first indicator of the ovarian reserve taken into account is the patient's age. Although the number and quality of oocytes both decrease with age, the reproductive potential varies drastically among women of similar age; therefore, they might exhibit different responses to ovarian stimulation [Fauser BC et al., ٢٠٠٨].

Consequently, an individual's chrono-logical age may not be as valuable a predictor of fertility as her "biological age", as defined by hormonal and functional profiles [Ezcurra D et al., ٢٠١٢].

In fact, in addition to age, several clinical, endocrine and ultrasound markers, and dynamic tests have been proposed for the prediction of the ovarian response to stimulation [Broekmans ٢٠٠٦-Muttukrishna ٢٠٠٥].

Among these markers, use of the level of anti-Müllerian hormone (AMH) and the antral follicle count (AFC) is of particular

interest [Maheshwari et al., ٢٠٠٦]. The AFC consists of the sum of follicles <١٠ mm in both ovaries on a transvaginal ultrasound and has been used to predict the ovarian reserve and the patient response to ovarian stimulation.

However, there is significant variation among different authors in the limits used to classify antral follicles [Younis et al., ٢٠١٠]. AMH, a member of the transforming growth factor-beta superfamily, is only produced by the granulosa cells surrounding the pre-antral and small antral follicles.

Additionally, AMH is independent of follicle stimulating hormone (FSH), whereby its levels are a direct measure of the follicular pool production. the serum levels of AMH decrease throughout reproductive life and are undetectable in the postmenopausal period [Younis et al., ٢٠١٠]

In this study demonstrate that a derived multi-marker for measuring ovarian reserve was a good predictor for oocyte yield after ovulation induction and also for ongoing pregnancy, facilitate the optimization and individualization of assisted reproductive treatment before the onset of a treatment cycle and need to adapt approaches for patients sub-populations, and finally will consider the use of biomarkers as a tool for implementing an individualized approach to COS treatment protocols

Patients and methods

This prospective study included a total of ١١٩ women undergoing ICSI at Minia infertility research unit (MIRU),Minia University Hospital

Eligability criteria: All patients satisfied to the following Age less than ٣٩ years, Regular menstrual cycle, Both ovaries present, No history of ovarian surgery, No evidence of endocrine disorder and only exclusion was presence of ovarian cysts as assessed by transvaginal ultrasound.

- **Plan of the study:**

The ١١٩ patients who agreed to participate, one withdrewbefore starting stimulation and two patients were canceled duringsti-

mulation for the following reasons: wrong timing of hCG (one) and significant vaginal bleeding duringstimulation (one). The remaining ١١٦ women were classified into twogroups.

(i) Group ١: Total number: ٢٣

(a) Group ١A: represents those who were canceled during stimulationowing to poor response and did not proceed to hCGadministration and oocyte collection (٣ women).

(b) Group ١B: represents those who proceeded to oocyte retrievaland had ≤٤ oocytes (٢٠ women).

(ii) Group ٢: Total number ٩٣

(a) Group ٢A: represents those who were deemed to have anexcessive response to gonadotrophins and therefore had theircycle canceled before hCG because of risk of OHSS (one women).

(b) Group ٢B: represents those who proceeded to oocyte retrievaland had >٤ oocytes (٩٢ women).

Out of ١١٢ who had oocyte retrieval, ٣ women did not proceed to embryo transfer. One patient failed to have any oocytes collected and one woman had complete failure of fertilization. and one women had elective cryopreservation of all embryos because of risk of OHSS.

Clinical work-up:

Written informed Consent was obtained from all patients after giving verbal information about the aim of the study and the scan procedure involved in it.

All patients had comprehensive evaluation including full history taking, thorough physical examination and at the initial

assessment special note was made of the following clinical features (age, cycle length, duration of subfertility, and body mass index (BMI).

Baseline pelvic ultrasound examination and evaluation of baseline hormonal profile namely FSH, LH and estradiol for prediction of ovarian response was done as part of the initial assessment during the follicular phase of spontaneous cycle.

AMH measurement:
A venous blood sample for an AMH measurement was taken before the scheduled treatment (minimum of ٣٠days) during the early follicular menstrual cycle phase in all women. AMH was measured using an enzymatically amplified ٢-site immunoassay kit (AMH Gen II ELISA,

Beckman Coulter Inc.) According to the manufacturer's manual. All clinical, baseline sonographic and hormonal profile data were obtained and recorded prospectively in aspecial database before the start of IVF treatment. This database was routinely updated with Outcome data after the end of first treatment cycle.

Results

The regression analysis demonstrated significant (P<٠.٠٥) positive correlations between the AMH level and the total number of oocytes collected (r=٠.٦٤), total number of MII oocytes (r=٠.٦٥) and the number of follicles (r=٠.٦٢) on the hCG administration day. Additionally, all the other markers of ovarian response showed statistically significant correlations with the variables analysed. However, the association provided by the AMH level improved the correlation because the individual correlation coefficients of each marker of ovarian response (age and AFC) were always lower than that presented by the AMH level.

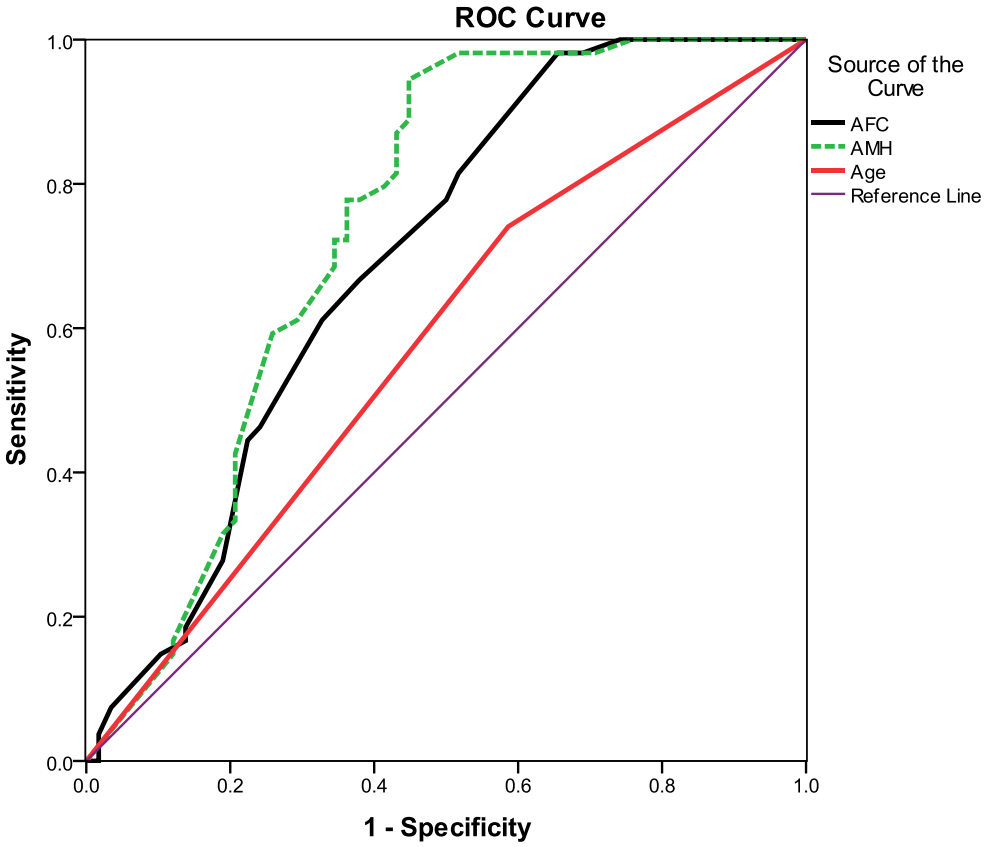
Table (١): Correlation between age, AMH, AFC and the total number of oocytes collected, total number of MII oocytes collected and the number of follicles at the time of hCG administration:

Data	Age r(p)	AFC r(p)	AMH r(p)
total number of oocytes collected	-٠.٣٨(٠.٠٠١*)	٠.٥٨ (٠.٠٠١*)	٠.٦٤ (٠.٠٠١*)
total number of MII oocytes collected	-٠.٣٩(٠.٠٠١*)	٠.٥٩ (٠.٠٠١*)	٠.٦٥ (٠.٠٠١*)
the number of follicles at the time of hCG administration	-٠.٣٩(٠.٠٠١*)	٠.٥٧ (٠.٠٠١*)	٠.٦٢ (٠.٠٠١*)

Regarding the probability of pregnancy occurrence, performance of AMH using the ROC curve showed an area under the curve of ٠.٧٣±٠.٠٤ (P=٠.٠٠١*) , indicating that the AMH had a good prognostic potency for this point. Setting the specificity (٦٣.٨٪) and sensitivity (٧٧.٨٪).

ROC curves also revealed good prognostic potency for all other factors (Age and AFC) analysed. However, the AUC presented by the AMH was always higher than those presented by all other factors. Considering the ROC curves for the AMH level exhibited a good ability to predict clinical pregnancy .

Figure (١): ROC curve analysis for AFC, AMH and Age as a prognostic factor regarding the clinical pregnancy:



AUC = ٠.٦٩±٠.٠٥ (P=٠.٠٠١*), Cutoff ≥١٠.٥ (sensitivity = ٦٦٪, specificity of ٦١٪).
AUC for AMH = ٠.٧٣±٠.٠٤ (P=٠.٠٠١*), Cutoff ≥٢.٣ (sensitivity =٧٧.٨٪, specificity of ٦٣.٨٪)
AUC for Age= ٠.٥٧±٠.٠٥ (P=٠.١), Cutoff ≤٣٠ (sensitivity =٧٤.١٪, specificity of ٤١.٤٪)

Figure (٢): distribution of study group according to AMH level

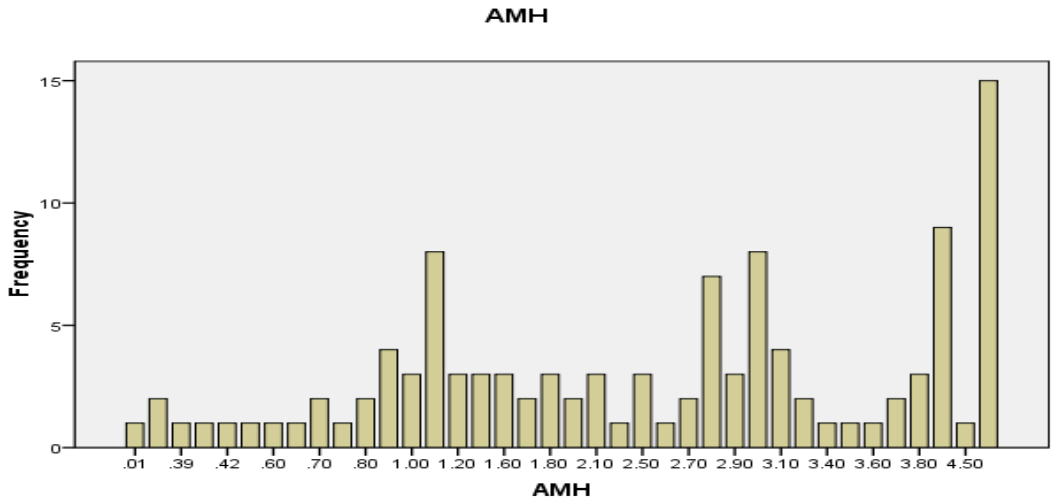


Table (٢): **The development of the ovarian stimulation protocol and doses of follicle-stimulating hormone (FSH) in the groups categorized by the AMH level:**

Data	AMH<٢.٣ N(٤٩)	AMH≥٢.٣ N(٦٣)	P
Total number of oocytes collected ≥٤ (١٠٣)	٤١(٣٩.٨٪)	٦٢(٦٠.٢٪)	٠.٠٠١*
Total number of MII oocytes collected≥٤ (٩٢)	٣٢(٣٤.٨٪)	٦٠(٦٥.٢٪)	٠.٠٠١*
Collected oocytes ≥١٥ (٤٤)	٥(١١.٤٪)	٣٩(٨٨.٦٪)	٠.٠٠٢*
Dose of follicle-stimulating hormone (FSH)	٣٩٨٢.٦±٤٥٨٢.٥	٢٣٠٠±١٢١٠.١	٠.٠٠٦*

Table (٣): characteristics of the study population studied women according to clinical pregnanacy

Data	Negative pregnancy N=٥٩	Positive pregnancy N=٥٣	P
total number of oocytes collected	١١.٣±٧.٤	١٣.٦±٦.٤	٠.٠٧
total number of MII oocytes collected	٨.٤±٦.٥	١٠.٥±٥.٩	٠.٠٨

Table (٤): Correlation between AFC and AMH to ovarian response

Data	Poor response oocytes ≤٤	oocytes ٥-١٤	collected oocytes ≥١٥	P
AMH	٠.٠١-١.١ ٠.٦±٠.١	١.٢-٢.٧ ٢.٢±٠.٥	٢.٨-٥ ٣.٥±١.١	٠.٠٠١*
AFC	٢-١٦ ٦.٠١±٣.٤	٤-٢٢ ١١.١±٤.٤	٨-٣٠ ١٤.٧±٤.٩	٠.٠٠١*

This table shows that statistical significant correlation between AMH level and AFC to outcome of oocytes collected. This allows clinicians to modulate the dose of FSH administered to women according to the number of oocytes they aim to retrieve

Discussion

The present study demonstrate thata derived multi-marker for measuring ovarian reserve was a good predictor for oocyte yield afterovulation induction and also for ongoing pregnancy, facilitate the optimization and individualization of assisted reproductivetreatment before the onset of a treatment cycle and need to adapt approaches for patients sub-populations, and finally will consider the use of biomarkers as a tool for implementing an individualized approach to COS treatment protocols.

AMH, produced by granulosa cells of pre-antral and small antralfollicles, has emerged as a useful marker of ovarian function.

AMHhas been used in assessment of ovarian aging, prediction of response to ovulation induction and the assessment of the risk ofdeveloping OHSS (Van Rooij et al., ٢٠٠٢; Nelson et al., ٢٠٠٧).

Our study clearly demonstrated the superiority of AMH in prediction of good response compared with the other individual markers (AUC for age: ٠.٥٧; AFC: ٠.٦٩; AMH: ٠.٧٣.) Nelson et al. (٢٠٠٩) investigated the role of AMH in predicting oocyte yield, showing that the use of circulating AMH concentration to individualizetreatment strategies for controlled ovarian stimulation reduced clinical risk of OHSS whilst optimizing pregnancy rates.

Our study has shown that AMH in predicting oocyte yield (AUC for AMH:

٠.٨٤) but superior in predicting pregnancy outcome compared to age and antral follicle count.

The finding that AMH was a more robust biomarker of theovarian response to gonadotropins than AFC was also confirmed in the present study, The regression analysis for AMH and number of oocytes retrieved was higher in collecting≤٤ oocytes (OR: ٣.٦; P<٠.٠٠١), ≤٤ metaphase IIoocytes (OR: ٢.١; P<٠.٠١) and ≤١٥ oocytes (OR: ٢.٤; P<٠.٠٠١). Alternativel, regardless ofthe protocol and the different gonadotropin doses used, because the treatment effect wasconstant for both AMH and AFC, it would not be expectedto alter the strength of association for the two biomarkers.

Also we have demonstrated that AMH value was the most accurate predictor of poor response amongst other competing parameters tested. This was followed by age, and AFC. This implies that using AMH and age might improve the possibility of forecasting poor ovarian response in a higher proportion of patients over what is currently possible using the standard predictors as age, basal FSH, E٢. However, on multivariate logistic regression analysis, only AFC stand out as the significant predictors of poor ovarian response after adjusting for potential impact of other confounding predictors whereas other factors as age and AMH levels were insignificant. On the other hand, in our analysis, BMI were inaccurate and insignificant predictors of poor response

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