Research Article

(Antral Follicle Count as predictor of ovarian response to controlled ovarian stimulation in IVF)

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Abstract
Objective: The present study aimed to evaluate the performance of uses of the most recently identified marker of ovarian reserve, namely antral follicle count (AFC), 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**: Univariate analyses showed that age, AFC and AMH were significant predictors for poor oocyte yield. AFC presented the highest ROCAUC of ٠.١١, indicating a good discriminating potential for predicting poor ovarian response, followed by AMH with an ROCAUC of ٠.١٥. In the multivariate analysis, the AFC remained significant. **Conclusion:** .Antral follicle count provided no added predictive value beyond AMH in prediction of ovarian response to gonadotropin therapy at the study utilizing GnRH agonist and GnRH antagonist protocols.
Keywords: antral follicle count (AFC), 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 multi follicular 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 chronological 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 <١٠mm 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 during stimulation 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 stimulation owing to poor response and did not proceed to hCG administration and oocyte collection (٣ women).

(b) Group ١B: represents those who proceeded to oocyte retrieval and 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 their cycle canceled before hCG because of risk of OHSS (one women).

(b) Group ٢B: represents those who proceeded to oocyte retrieval and 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 women 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.

The procedure for measuring the AFC was as follows:

A transverse section of the uterus was obtained, one ovary at a time was visualized by moving the probe laterally, adjustments to obtain a clear image of the ovary was done as required by changing the probe frequency, depth of focus or magnification. Each ovary was scanned in a systematic way by panning through the ovary up-down and from side to side. Antral follicles were defined as all echo lucent rounded structures measuring (٢-١٠) mm seen within the ovarian substance. Serial scans were obtained by making a slow sweep with the transvaginal probe from the medial towards the lateral border of the ovary. As this sweep is performed through the whole ovary antral follicles were assessed. Initially a note is made of follicles measuring ١٠ mm or larger. All follicle measurements were made on a frozen image, measuring both the antero-posterior and transverse planes and a mean diameter was calculated. Once a mental note was made of ١٠ mm size, all the antral follicles measuring smaller than ١٠mm (٢-١٠) mm were counted and numbers recorded during a single sweep through the ovary. The procedure was repeated on the contralateral ovary to obtain the Total Antral follicles count (AFC) defined as the count of all antral follicles measuring (٢-١٠) mm in both ovaries at the baseline examination (Bansci et al., ٢٠٠٢).

Results

Table (١): Predictors for poor response in women (≤٤ oocytes) and for negative pregnancy outcome using univariate and multivariate logistic regression analysis.

Data		Odds ratio	٩٥٪CI	P	ROC AUC
For poor response	**Univariate analysis**				
	Age	١.٢	١.١-١.٣	٠.٠٠١*	٠.٧٦
	BMI	١.٠٤	٠.٩٢-١.١	٠.٧	٠.٥١
	AFC	٠.٦٠	٠.٤٨-٠.٧٦	٠.٠٠١*	٠.١١
	AMH	٠.٢٧	٠.١٤-٠.٥١	٠.٠٠١*	٠.١٥
	Multivariate analysis				
	Age	٠.٩٨	٠.٨٣-١.١	٠.٨	
	BMI	٠.٩٢	٠.٨٠-١.٠٦	٠.٢	
	AFC	٠.٦١	٠.٤٤-٠.٨٥	٠.٠٠٤*	
	AMH	٠.٩٣	٠.٣٨-٢.٢	٠.٨	
For negative pregnancy	**Univariate analysis**				
	Age	١.٠٧	١-١.١	٠.٠٥	٠.٦٠
	BMI	١.٠٠١	٠.٩٣-١.٠٧	٠.٩	٠.٥١
	AFC	٠.٨٨	٠.٨١-٠.٩٥	٠.٠٠٢*	٠.٣٠
	AMH	٠.٥٥	٠.٤١-٠.٧٥	٠.٠٠١*	٠.٢٦
	Multivariate analysis				
	Age	٠.٩٧	٠.٨٨-١.٠٨	٠.٦	
	BMI	٠.٩٧	٠.٨٩-١.٠٥	٠.٥	
	AFC	١.٠٠١	٠.٨٨-١.١	٠.٩	
	AMH	٠.٥٤	٠.٣٣-٠.٨٨	٠.٠١*	

Univariate analyses showed that age, AFC and AMH were significant predictors for poor oocyte yield. AFC presented the highest ROCAUC of ٠.١١, indicating a good discriminating potential for predicting poor ovarian response, followed by AMH with an ROCAUC of ٠.١٥. In the multivariate analysis, the AFC remained significant.

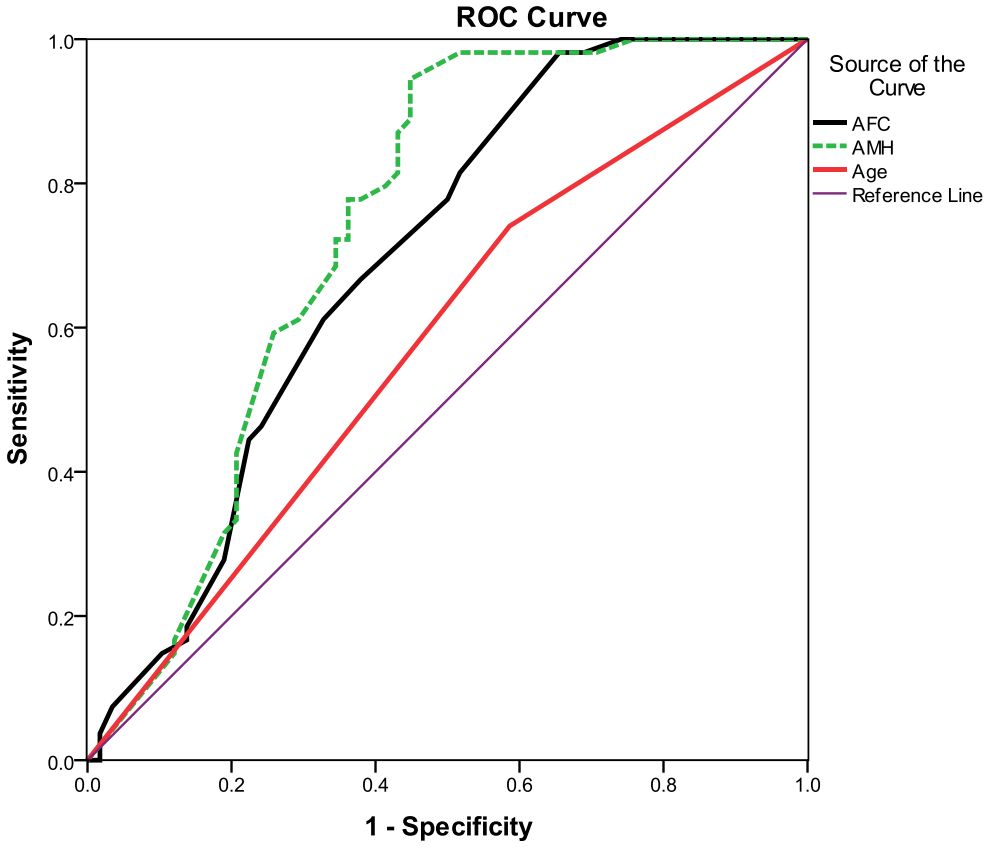
The results of univariate and multivariate logistic regression analyses showed that age was the only significant predictor for negative pregnancy outcome with an ROCAUC of ٠.٦٠

Table (٢): Comparison of performance characteristics for poor responders (≤٤ oocytes) and for negative pregnancy Outcome.

Data	Cut off	sensitivity	Specificity
For poor response			
Age	٣٢	٦٥٪	٨٠.٤٪
BMI	٢٣.٥	٥٥٪	٥٤.٣٪
AFC	٨.٥	١٥٪	٣٣٪
AMH	١.٠٥	٤٥٪	١١٪
For negative pregnancy			
Age	٢٧	٦٤.٤٪	٤٥.٣٪
BMI	٢٣.٥	٤٩.٢٪	٥٤.٧٪
AFC	١٠.٥	٥٠.٨٪	٢٢.٦٪
AMH	١.٦	٤٥.٨٪	١١.٣٪

This table shows that the age was the only significant predictor for negative pregnancy outcome and none of the individual markers of ovarian reserve were able to predict pregnancy. A maximized sensitivity of ٦٥٪ and a specificity of ٨٠.٤٪ .

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

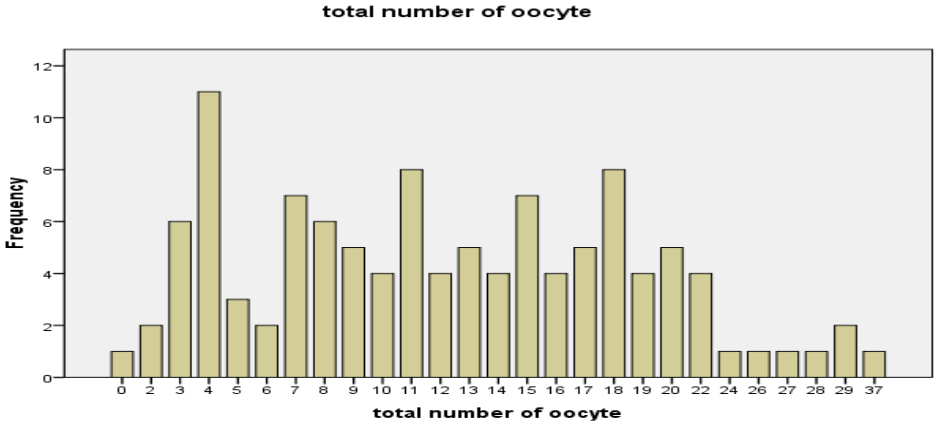


AUC = ٠.٦٩±٠.٠٥ (P=٠.٠٠١*), Cutoff ≥١٠.٥ (sensitivity = ٦٦٪, specificity of ٦١٪).
AUC for AMH = ٠.٧٣±٠.٠٤ (P=٠.٠٠١*), Cutoff ≥٢.٣ (sensitivity =٧٧.٨٪, specificity of ٦٣.٨٪)
AUC for Age= ٠.٥٧±٠.٠٥ (P=٠.١), Cutoff ≤٣٠ (sensitivity =٧٤.١٪, specificity of ٤١.٤٪)

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 (٢) distribution of studied group according to AFC number:



Discussion

The present 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.

AMH, produced by granulosa cells of pre-antral and small an tralfollicles, has emerged as a useful marker of ovarian function. AMH has been used in assessment of ovarian aging, prediction of response to ovulation induction and the assessment of the risk of developing 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) investi-gated the role of AMH in predicting oocyte yield, showing that the use of circulating AMH concentration to individualize treatment 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 the ovarian 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 was constant

for both AMH and AFC, it would not be expected to 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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