Comparison of Automated Anti-Müllerian Hormone Assays and Antral Follicle Count in Predicting Ovarian Response During Ovarian Stimulation

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ABSTRACT

Objective: Various parameters had been used to predict ovarian response. Among them, Anti-Müllerian Hormone (AMH) and antral follicle count (AFC) demonstrate the most favourable analytical and performance characteristics. In this pilot study, we aim to determine the cut-off levels of AMH using automated AMH assays and AFC in the prediction of poor and high responders.

Study Design: Prospective study of 43 women between 21 to 45 years old scheduled for assisted reproduction. AMH levels on day 3 of menstruation were analysed using two immunoassay kits, namely the Beckman Coulter Access AMH and the Roche Elecsys AMH on the two automated analysers Beckman Coulter DxI 800 and Roche Cobas e602 respectively. AFC was also assessed on day 3 of menstruation prior to in vitro fertilization (IVF). These were compared with the number of oocytes retrieved after controlled ovarian stimulation.

Results: AMH (Beckman Coulter Access AMH and Roche Elecsys AMH) highly correlated with AFC and the number of oocytes retrieved after ovarian stimulation. Beckman Coulter Access AMH was the better predictor for poor ovarian response with ROC_{AUC} of 0.83. For the prediction of a high response, AFC had a higher ROC_{AUC} of 0.95. Through ROC, the AMH cut-off level for poor ovarian response was 2.23 ng/ml with Beckman Coulter Access AMH and 2.02 ng/ml with Roche Elecsys AMH, while the AMH cut-off for a high ovarian response was 5.19 ng/ml with Beckman Coulter Access AMH and 4.60 ng/ml with Roche Elecsys AMH. For AFC, the cut-off for poor ovarian response was 18 and for high response was 34.

Conclusion: AMH and AFC are reliable predictors of ovarian response. Establishment of specific levels may improve individualised controlled ovarian stimulation and optimise the oocyte yield. Larger studies are required to establish these findings.

Keywords: Anti-Müllerian Hormone; Antral Follicle Count.

INTRODUCTION

Anti-Müllerian Hormone (AMH) is a dimeric glycoprotein member of the transforming growth factor-beta superfamily (Cate et al., 1986; Jost, 1946). It is expressed in granulosa cells from pre-antral and small antral follicles (Durlinger et al., 2002a, 2002b) and continues to be expressed in the growing follicles in the ovary until they have reached the size and differentiation state at which they are to be selected for dominance. In women, AMH appears in serum after birth, increases till puberty and progressively decreases in parallel with ovarian ageing (de Vet et al., 2002; van Rooij et al., 2005). This is because in women AMH is produced by granulosa cells, from pre-antral and antral follicles (Weenen et al., 2004) and its levels reflect the ovarian follicular pool. Therefore, AMH serum levels can be used

as a marker for ovarian reserve, representing the quantity and quality of the ovarian follicle pool (Te Velde and Pearson, 2002).

FSH, inhibin B and antral follicle count (AFC) have been used to measure ovarian reserve and predict ovarian response in In Vitro Fertilization (IVF) patients for decades. However, they fluctuate throughout the menstrual cycle and need to be measured in the early follicular phase of the menstrual cycle for more accuracy. In contrast, there is little intra-cycle variation in AMH levels, supporting feasibility of assessment throughout the cycle (Cook et al., 2000). Furthermore, studies have shown that AMH levels remain unchanged in response to gonadotrophin stimulation (van Rooij et al., 2002).

Many studies have been done to assess the sensitivity of AMH in predicting ovarian reserve and response in IVF patients, especially in

western countries. However, it is unknown whether AMH levels vary between populations. We prospectively assessed the significance of AMH as a marker of ovarian reserve and compared with other parameters (antral follicle count, FSH, E2 and age) to predict the number of oocytes retrieved during IVF in the Singapore population.

SUBJECTS AND METHODS

The study was conducted at the Singapore General Hospital, Centre for Assisted Reproduction. Approval was obtained from the local institutional review board.

Patients scheduled to undergo their first stimulated cycle were recruited. Written informed consent was obtained from all participants.

The inclusion criteria were as follows: 1) age between 21 to 45 years, 2) regular menstrual cycles with cycle length less than or equal to 35 days, 3) BMI < 25, 4) never pregnant or at least 6 weeks from the most recent pregnancy.

The exclusion criteria were as follows: 1) age above 45 or below 21 years old, 2) irregular menstrual cycles with cycle length more than 35 days, 3) current or previous diagnosis of PCOS, 4) current or previous diagnosis of endocrine or metabolic conditions e.g. thyroid disorder, 5) current or prior use of hormonal contraception, 6) postmenopausal status, 7) current or previous chemotherapy recipient, 8) current or previous diagnosis of malignancy, 9) current or ex-smoker, 10) alcohol consumption more than 3 standard drinks per day (a standard drink is defined as one 12-ounce bottle of beer, one 5-ounce glass of wine, or 1.5 ounces of distilled spirits), 11) FSH levels more than 10 U/L on day 3 of menstrual cycle. On day 3 of a spontaneous menstrual cycle, patients underwent a transvaginal

ultrasound examination using a GE Voluson E8 ultrasound system (GE Healthcare, Milwaukee, WI, USA) equipped with a 6–12 MHz 3D endovaginal probe to assess the number of antral follicles measuring 2–10 mm. The scans were conducted by 4 professional sonographers who rotate to our centre every 3 months. Venous blood samples were also obtained on day 3 of menstruation for the measurement of AMH, follicular stimulating hormone (FSH) and estradiol (E2) levels. Samples were centrifuged at 3500 rpm for 5 minutes and serum stored at –20°C until assayed for up to 1 month.

The Beckman Coulter Access AMH and Roche Elecsys AMH immunoassays were compared. Beckman Coulter Access AMH assay is traceable to internal reference standards which consist of recombinant AMH in a BSA matrix. Roche Elecsys AMH assay is traceable to the Beckman Coulter AMH Gen II ELISA (unmodified version without predilution) assay. In Table 1, the assay characteristics of both Beckman Coulter Access AMH and Roche Elecsys AMH are shown. For Beckman Coulter Access AMH, manufacturer's imprecision claim was ≤ 0.032 ng/mL (SD) at AMH < 0.16 ng/mL and ≤ 10.0% (CV) at concentrations ≥ 0.16 ng/mL. For Roche Elecsys AMH the manufacturer's claim for total imprecision was < 0.03 ng/mL (SD) at 0.01–0.5 ng/mL, ≤ 6% (CV) at AMH ≥ 0.5–1 ng/mL and ≤ 5% (CV) at AMH ≥ 1 ng/mL respectively. FSH and E2 were assessed using the Roche Elecsys FSH & E2 assay kits on the Roche Cobas e602 analyser.

All patients had an antagonist cycle. Patient with an AFC of < 14 were given a dose of 450 IU of follitropin alpha or beta. Patients with follicles between 14–24 received 150–300 IU of follitropin alpha or beta based on their BMI. Patients with an AFC > 24 received 150 IU of follitropin alpha or beta. If there were > 19 follicles > 11 mm

Table 1. AMH assay characteristics.

AMH assays	Beckman Coulter Access AMH	Roche Elecsys AMH
Total imprecision	≤ 0.032 ng/mL (SD) at < 0.16 ng/mL ≤ 10.0% (CV) at ≥ 0.16 ng/mL	< 0.03 ng/mL (SD) at 0.01–0.5 ng/mL ≤ 6% (CV) at ≥ 0.5–1 ng/mL ≤ 5% (CV) at ≥ 1 ng/mL
Sample type	Serum	Serum
Minimum sample volume	20 µl	50 µl
Incubation time	39 min	18 min
Limit of Detection (LoD)	≤ 0.02 ng/mL	0.01 ng/mL
Limit of Quantification (LoQ)	≤ 0.08 ng/mL	0.03 ng/mL
Analytical measurement range	0.02–24 ng/mL	0.01–23 ng/mL

Table 2. Patient characteristics (mean of age, AMH, AFC, FSH, E2 and number of oocytes retrieved).

	Total (n = 43)	Poor responder (n = 11)	Normal (n = 26)	High responder (n = 6)	P value	Standard deviation
Age (years)	33.6	35.1	33.4	32.0	0.30	4.08
Beckman Coulter Access AMH level (ng/mL)	3.80	1.59	3.51	9.12	< 0.001	3.44
Roche Elecsys AMH level (ng/mL)	3.17	1.43	2.88	7.61	< 0.001	2.79
AFC (n)	22.86	13.09	21.73	45.67	< 0.001	16.17
Roche Elecsys FSH (IU/L)	6.77	8.76	6.17	5.72	0.119	3.75
Roche Elecsys E2 (pmol/L)	195.06	257.31	181.30	123.26	0.240	162.19
Number of oocytes (n)	10.02	2.55	10.19	23.00	NA	7.00

on trigger day, a lucrin 1 mg trigger was administered. Otherwise all patients received a standard dose of 10,000 IU of HCG as trigger.

When three or more follicles reached >18 mm, HCG or GnRH analogue was administered and follicles were aspirated 36 hours later under sedation. The main outcome measures were the number of oocytes retrieved and the cycle cancellation rate. Poor ovarian response was defined as a fewer than five oocytes or cancellation due to impaired or absent follicular growth in response to ovarian

stimulation. (Bancsi et al., 2002). A normal ovarian response was defined as a collection of 5–16 oocytes. Patients were considered high responders when >16 oocytes were collected at ovum retrieval or when the cycle was cancelled because of exaggerated response (Muttukrishna et al., 2004).

Data were analysed with the Statistical Program for Social Sciences version 22 (SPSS Inc., Chicago, IL, USA). Values are presented as mean. To compare poor, normal and high responders the

Fig. 1. Correlation of age (A), cycle day 3 E2 (B), number of oocytes after oocyte retrieval (C), cycle day 3 number of antral follicles (D) and cycle day 3 FSH (E) with Beckman Coulter Access AMH in IVF patients.

Spearman's correlation coefficient is "r" followed by the "P" value.

ANOVA test was performed whenever appropriate. The correlation between different parameters is expressed as Spearman's correlation coefficient. All variables were considered significant at a P value of less than 0.05. The AMH and AFC cut-offs for poor and high ovarian response of AMH and AFC were derived from ROC_{AUC}.

RESULTS

Forty-three patients who underwent their first stimulated IVF cycle were included in the analysis.

Patient and ovarian reserve test characteristics of poor, normal and high responders are compared in Table 2. AMH levels and AFC measured were significantly lower in the poor responder group compared to the normal and high responders. The numbers of oocytes retrieved in the poor, normal and high responders are also depicted in Table 2. Two patients in the poor responders group had their oocyte retrieval cancelled because of insufficient follicle growth.

The correlation coefficients for the association between automated AMH levels (Beckman Coulter Access and Roche Elecsys) on one hand and several reserve test variables and the total numbers of oocytes retrieved on the other hand are shown in Figs. 1 and 2. AMH (Beckman Coulter Access and Roche Elecsys) were highly correlated with AFC and the number of oocytes retrieved after ovarian stimulation.

In Table 3, the results of the logistic regression analysis for the prediction of poor response are given. Beckman Coulter Access AMH presented the highest ROC_{AUC} of 0.83, indicating a good discriminating potential for predicting poor ovarian response. The ROC_{AUC} for Roche Elecsys AMH was almost identical, followed by the ROC_{AUC} value of AFC. Age, FSH and E2 were not significantly related to poor response.

In Table 4, the results of the logistic regression analysis for the prediction of high response are given. AFC presented the highest ROC_{AUC} of 0.95, indicating a good discrimination potential for predicting high ovarian response followed by the ROC_{AUC} value of AMH (Beckman Coulter Access and Roche Elecsys). Age, FSH and E2 were not significantly related to high response.

Through ROC, it can be derived that the AMH cut-off level for poor ovarian response are 2.23 ng/ml in Beckman Coulter Access AMH with the sensitivity of 81.8% and specificity of 75.0% and 2.02 ng/ml in Roche Elecsys AMH with the sensitivity of 81.8% and specificity of 71.9%. While the AMH cut-off level for high ovarian response are 5.19 ng/ml in Beckman Coulter Access AMH with the sensitivity of 83% and specificity of 89.2% and 4.60 ng/ml in Roche Elecsys AMH with the sensitivity of 83% and specificity of 98.2%. For antral follicle count, the cut-off for poor ovarian response is 17.5 (sensitivity of 81.8% and specificity of 65.6%) whereas the cut-off for high ovarian response is 34 (sensitivity of 83.3% and specificity of 94.6%).

DISCUSSION

In this study we prospectively compared the prediction rate of AMH, AFC, FSH, E2 and age in assessing ovarian response of patients undergoing their first IVF cycle. We evaluated the number of oocytes retrieved during first IVF to evaluate the predictive comparison of AMH and AFC for the identification of a poor or a high responder. Several studies had proposed that among the wide variety of biomarkers used for the prediction of ovarian response AMH and AFC demonstrated the highest reliability (Broekmans et al., 2006; Broer et al., 2009, 2011).

Studies using manual enzyme-linked immunosorbent assays have reported the sensitivity and specificity of AMH to range from 44–97% and 41–100% respectively in predicting poor ovarian response. This was partly attributed to the use of different variants

of AMH assay with variable cut-offs depending on the assay used (La Marca et al., 2010). In this study, we compared the performance of two automated immunoassays (Beckman Coulter Access AMH and Roche Elecsys AMH). Roche Elecsys AMH assay on the Cobas e602 analyser demonstrated a lower imprecision and lower limit of detection when compared to Beckman Coulter AMH on the Beckman Coulter DxI800 analyser (Table 1). Furthermore, our study showed that mean of AMH values obtained from the Beckman Coulter Access AMH are 1.27% higher than Roche Elecsys AMH. Teddy et al. had demonstrated that the Beckman Coulter Access AMH were 5% higher than those obtained with Roche Elecsys AMH (Tadros et al., 2016). It could be due to different antigens are used in both assays. Nonetheless, the excellent correlation between the two assays demonstrated by our study will still allow comparison of results obtained using both assays. In our study, Beckman Coulter Access AMH showed good discriminating potential for the prediction of poor ovarian response with highest ROC_{AUC} of 0.83.

Previous study reported that AMH is cycle independent in the assessment of ovarian response as it had less intra- and inter-cycle fluctuation (Iliodromiti et al., 2015; van Disseldorp et al., 2010). Andersen et al. found that AMH was a significant predictive variable in the assessment of poor and high ovarian response during ovarian stimulation whereas no such independent association was observed with AFC (Andersen et al., 2011). AMH levels do not change in response to an acute endogenous rise in FSH and LH (Durlinger et al., 1999; van Rooij et al., 2002).

However, recent studies have revealed important inter and intra-cycle variations during ovarian stimulation for ART that cannot be explained only by analytical variability. Serum AMH levels are lower in the luteal phase than the follicular phase (Gorkem et al., 2017). The average intraindividual AMH variability in a menstrual cycle was 20% and this biological variation was at least twice the analytical variation (Hadlow et al., 2016). Furthermore, intracycle variation in younger women were different from older women (Overbeek et al., 2012). Short-term inter-cycle variation may be due to the biological variation in the number of AMH-producing follicles in a cycle (Rombauts et al., 2015).

During ovarian stimulation, serum AMH levels gradually decrease in follicular phase irrespective to the type of FSH used or the protocol (Fanchin et al., 2003; Lee et al., 2010; Melado Vidales et al., 2017a; Weintraub et al., 2014). This decline continues till upto 4 days after HCG administration. Then, AMH increases and returns to baseline levels regardless of whether the patient is pregnant or not (Fanchin et al., 2005; Fanchin et al., 2003; Weintraub et al., 2014).

Basal AMH levels on days 1–3 of cycle has shown a good correlation with the number of oocytes retrieved (Lee et al., 2010; Melado Vidales et al., 2017b). Until the dynamics of AMH are further clarified, standardization of measuring AMH during the early follicular phase will provide the most accurate result for hormone stimulation during IVF.

The advantages of AMH is that it is not produced by atretic antral follicles which do not respond to ovarian stimulation which cannot be excluded by an AFC measurement. AMH however has a disadvantage in that, it may overestimate the ovarian response of patients in whom the majority of follicles are 1–2 mm compared to those who have more large antral follicles (> 6 mm). Since the large follicles are most sensitive to gonadotropin treatment while simultaneously losing their ability to produce AMH, AFC might be a better predictor of the immediate ovarian response to gonadotropin stimulation than AMH (Mutlu et al., 2013).

In general, the performance characteristics of AFC in predicting the number of oocytes retrieved is comparable to that of AMH.

Fig. 2. Correlation of age (A), cycle day 3 E2 (B), number of oocytes after oocyte retrieval (C), cycle day 3 number of antral follicles (D) and cycle day 3 FSH (E) with Roche Elecsys AMH in IVF patients.

Spearman's correlation coefficient is "r" followed by the "P" value.

Van Rooij et al. had showed that AFC is a slightly better predictor for poor ovarian response than AMH (manual immunoassay) with ROC_{AUC} of 0.86 compared to 0.85 in AMH (van Rooij et al., 2002). In our study, AMH showed a higher ROC_{AUC} than AFC in predicting poor ovarian response. However, our study also demonstrated that AFC is a better marker for predicting high ovarian response with a ROC_{AUC} of 0.95 compared to 0.88 for AMH.

One advantage of AFC is that the results are immediate whereas AMH may be associated with a turnaround time that is dependent on the local laboratory's workflow. In addition, the observer is able to evaluate many other important aspects of the ovaries, such as their position and accessibility, the presence of endometrioma or other ovarian lesions, concomitant tubal pathology, and uterine abnormalities (endometrial polyps, adenomyosis or leiomyoma).

Table 3. Logistic regression for prediction of poor response following ovarian hyperstimulation.

Univariate analysis	Odds ratio (95% CI)	P value	ROC _{AUC}
AFC (per follicle)	0.88 (0.79–0.98)	0.021	0.79
Beckman Access AMH (per ng/mL)	0.45 (0.23–0.45)	0.022	0.83
Roche Elecsys AMH (per ng/mL)	0.43 (0.20–0.91)	0.026	0.81
Roche Elecsys FSH (per IU/L)	1.20 (0.98–1.47)	0.076	0.65
Roche Elecsys E2 (per pmol/L)	1.00 (1.00–1.01)	0.156	0.66
Age (per year)	1.14 (0.94–1.39)	0.173	0.61

Table 4. Logistic regression for prediction of high response following ovarian hyperstimulation.

Univariate analysis	Odds ratio (95% CI)	P value	ROC _{AUC}
AFC (per follicle)	1.10 (1.02–1.17)	0.008	0.95
Beckman Coulter Access AMH (per ng/mL)	1.62 (1.17–2.23)	0.003	0.88
Roche Elecsys AMH (per ng/mL)	1.91 (1.24–2.94)	0.003	0.88
Roche Elecsys FSH (per IU/L)	0.88 (0.63–1.24)	0.461	0.62
Roche Elecsys E2 (per pmol/L)	0.99 (0.98–1.01)	0.275	0.64
Age (per year)	0.89 (0.73–1.10)	0.295	0.62

The key limitation of AFC however is the lack of standardization for measurement which may underlie its limited transportability across different operators, sites and settings (Iliodromiti et al., 2015). Unlike AMH, AFC exhibits significant variation within and across consecutive cycles (Deb et al., 2013; Elter et al., 2005; Hansen et al., 2003; van Disseldorp et al., 2010). Assessment of the intra-class coefficient (ICC) of AFC showed that it had a modest ICC of 0.71 between two cycles and of 0.69 within one cycle. This was substantially worse than that for AMH which showed an ICC between two cycles and within one cycle of 0.89 and 0.87 respectively (van Disseldorp et al., 2010). Given these concerns a consensus workgroup suggested that AFC should be performed from day 2 to day 4 of an index cycle (Broekmans et al., 2010).

STRENGTH AND LIMITATIONS

This is a prospective study on determining AMH cut-off levels using automated AMH assays and AFC in the prediction of poor and high responders in a Singapore population. The results of this study can be used to guide clinical practice.

The limitation of this study was the small sample size. Hence, further studies with larger sample sizes are needed to prove statistical significance. Furthermore, the AFC scan was conducted by professional sonographers (not MD holders) in our centre with a high-resolution ultrasound machine (GE Voluson E8 ultrasound system). The high resolution of the machine and the experience of our sonographer may explain why our cut-off for AFC for the poor and high responders are relatively higher compared to other studies. Besides, different dosing of gonadotropin was used based on the follicular count and BMI. These confounding factors may have affected the outcomes.

However, there have not been any publications correlating both AFC and AMH to oocyte number. Hence, this novel concept was put forth. Further studies involving a larger number of patients with strict protocols may establish more robust values.

CONCLUSION

AMH and AFC are good predictive markers for ovarian response. Establishment of specific levels may improve individualised controlled ovarian stimulation and optimize oocyte recovery. Larger studies with more patients and strict stimulation protocol can give us a better conclusion of AMH and AFC predictability of oocyte number. This study suggested that both the Beckman Coulter Access AMH and Roche Elecsys AMH assays are concordant with each other and reliably related to the number of oocytes retrieved during IVF. However, clinicians should be aware of variations in absolute values between both assays.

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