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Performance of anti-Müllerian hormone (AMH) levels measured by Beckman Coulter Access AMH assay to predict oocyte yield following controlled ovarian stimulation for in vitro fertilization

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Abstract

Purpose: We evaluated the performance of anti-Müllerian hormone (AMH) measured by the Beckman Coulter fully automated Access assay to predict oocyte yield following controlled ovarian stimulation (COS) for in vitro fertilization (IVF).

Methods: The correlation between the Access assay and the pre-mixing method with Generation II ELISA assay (Gen II pre-mix assay) was assessed using 230 blood samples. The relationship of AMH level measured by the Access assay and the actual number of oocytes retrieved following COS was assessed using 3296 IVF cycles. The performances of AMH, follicle stimulating hormone (FSH), and estradiol (E2) in predicting the responses to COS were also evaluated by constructing receiver operating characteristic (ROC) curves.

Results: The AMH levels measured just before oocyte retrieval by the Access assay and the number of oocytes retrieved following COS showed a good correlation with $R = 0.655$. The ROC analysis revealed that the sensitivity of AMH was comparable with or lower than that of E2 but higher than that of FSH.

Conclusions: With the improved Access AMH assays, AMH was as sensitive as E2 and could become an accurate marker of ovarian response to COS in more than 3000 Japanese IVF patients.

KEYWORDS
anti-Müllerian hormone, Beckman Coulter Access assay, mature oocyte, oocyte yield, ovarian reserve

1 | INTRODUCTION

Anti-Müllerian hormone (AMH) has become widely known as a marker of ovarian reserve in reproductive medicine,[1-3] and in Japan, an increasing number of fertility centers are introducing the measurement of AMH. However, sometimes AMH analysis seems to be conducted without a thorough understanding of the significance of AMH measurement and ovarian reserve or adequate explanation to patients. The use of AMH measurement is expanding to a broad range of fields, for example, the assessment of

ovarian reserve in women who will receive or have received cancer treatment.

In 2008, Asada Ladies Clinic started the measurement of AMH for all patients treated for infertility. The AMH assay system we used first was the MBL AMH/MIS kit (an enzyme-linked immunosorbent assay [ELISA] kit made by IMMUNOTECH s.r.o.) which was replaced in 2011 by the Beckman Coulter Generation II ELISA assay (Gen II original assay). An AMH level of 7.14 pM/mL measured by the MBL AMH/MIS kit was initially reported to be equivalent to 1 ng/mL measured by the Gen II original assay; however, it was later found that the Gen II original assay provided considerably lower values because of complement interference, causing massive confusion among physicians. Beckman Coulter revised the Gen II original assay by adding a pre-mix step to eliminate complement interference and released the Gen II pre-mix assay in 2013, which was further developed into the fully automated AMH assay (Access assay) in 2015 for use with their Access Immunoassay Systems. The Access assay incorporates the identical pair of antibodies used in the Gen II assays. Despite the growing clinical importance of AMH, the frequent changes of assay method and measurement unit have generated concern over the past decade.

Nevertheless, AMH is an important marker for the assessment of ovarian reserve, the prediction of ovarian response to hormonal stimulation, and the prior identification of patients with polycystic ovarian syndrome (PCOS) who are at high risk of ovarian hyperstimulation syndrome (OHSS) for in vitro fertilization (IVF) treatment, as well as the early diagnosis of premature ovarian failure. In view of AMH's potential in aiding diagnosis and appropriate treatment, it is critical to be able to interpret and utilize AMH values, regardless of changes in assay method. In this Original Article, we evaluated the correlation of AMH values measured by the Gen II pre-mix assay and the fully automated Access assay, and the associations of AMH values measured by the Access assay with clinical data including patients' age, using samples collected during IVF cycles conducted at our clinics. In addition, the performance of AMH in predicting ovarian response to controlled ovarian stimulation (COS) was compared with that of other hormonal markers.

2 | MATERIALS AND METHODS

The correlation of AMH values measured by the Gen II pre-mix assay and the Access assay was assessed using 230 blood samples collected at our clinics before the introduction of the Access assay. In addition, data with an AMH value of <10 ng/mL (211 blood samples) were evaluated excluding exceptionally high value of AMH.

Of the total of 7164 IVF cycles conducted between January 2015 and June 2017 at Asada Ladies Nagoya Clinic and Asada Ladies Kachigawa Clinic, 3296 cycles (excluding clomiphene-stimulated cycles which have a potential impact on estradiol [E2] levels, or cycles with no follicle stimulating hormone [FSH] measurement conducted in the previous cycle) were used to evaluate the correlation between AMH levels measured just before oocyte retrieval by the Access assay

and the actual number of oocytes retrieved following COS. These IVF cycles were divided by oocyte yield into the following three groups: the poor response group composed of cycles with an oocyte yield of ≤3 (n = 70), the normal response group composed of those with an oocyte yield of ≥4 and ≤14 (n = 1499), and the high response group composed of those with an oocyte yield of ≥15 (n = 1727), to evaluate the performance of AMH, FSH, and E2 in predicting poor and excessive responses by constructing receiver operating characteristic (ROC) curves based on DeLong's method. FSH levels were measured on day 3 of the menses in the previous cycle and E2 levels on the day of ovulation induction, 2 days before ovulation.

In addition, of the 7164 IVF cycles, 3463 IVF cycles (excluding cycles stimulated by clomiphene) were used to assess the associations of E2 levels per oocyte retrieved and per mature oocyte with age, oocyte yield, and AMH level in order to confirm whether the results obtained by the Access assay are consistent with those obtained by the previous methods.

3 | RESULTS

The AMH values measured by the Access assay were well correlated with those measured by the Gen II pre-mix assay. Regression lines were plotted for AMH values measured by the Access assay against those measured by the Gen II pre-mix assay using all the 230 samples and 211 samples with an AMH value of <10 ng/mL. The slope of the regression lines of whole samples was 0.711, indicating that AMH values measured by the Access assay were slightly lower than those measured by the Gen II pre-mix assay (Figure 1). The similar regression lines were obtained using 211 samples with an AMH value of <10 ng/mL (slope = 0.755).

The AMH values measured just before oocyte retrieval by the Access assay and the number of oocytes retrieved after COS showed a good correlation with R = 0.655 (Figure 2).
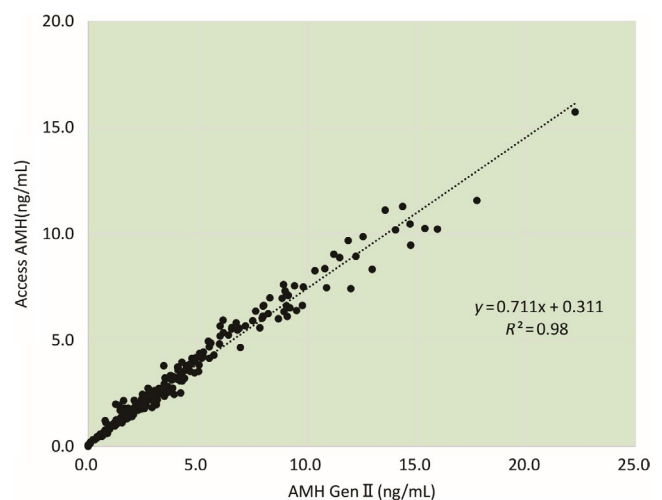

$y = 0.711x + 0.311$
$R^2 = 0.98$

FIGURE 1 Method comparison between the Access anti-Müllerian hormone (AMH) assay and the Gen II pre-mix assay (230 blood samples)
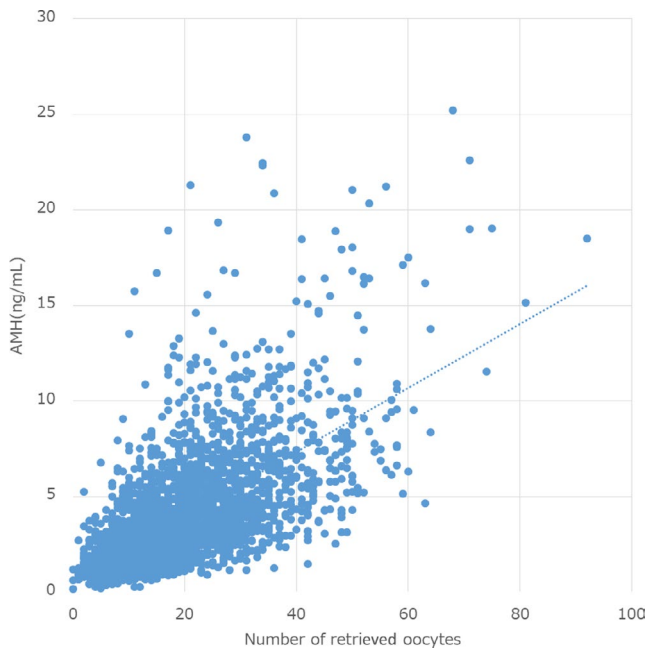
FIGURE 2 Relationship between Access anti-Müllerian hormone (AMH) value and oocyte yield. N = 3296 cycles excluding the cycles stimulated by clomiphene or no follicle stimulating hormone (FSH) measurement in the previous cycle

The AMH, E2, and FSH levels in the poor, normal, and high response groups are presented in Figure 3. The ROC analysis for differentiating the normal response group and the high response group revealed that the AUC for AMH was comparable with that for E2 and larger than that for FSH, indicating that AMH is a good marker of high response to ovary stimulation with sensitivity that was close to that of E2, a direct measure of follicular development, and higher than that of FSH (Figure 4A). The ROC analysis for differentiating the normal response group and the poor response group demonstrated that AMH was a useful marker of poor response to ovarian stimulation, with sensitivity that was lower than that of E2 but higher than that of FSH (Figure 4B).

Anti-Müllerian hormone levels decreased with increasing age, and E2 levels per oocyte retrieved and per mature oocyte increased with increasing age and with decreased oocyte yield. Assessment of the relationship of E2 levels per oocyte retrieved and per mature oocyte with AMH level was highly correlated with oocyte yield and revealed that E2 levels increased as AMH level decreased. The results obtained by the Access assay in more than 3000 Japanese women were similar to those observed by previous measuring methods.

4 | DISCUSSION

After many twists and turns, automated immunoassay systems are becoming mainstream as a method to measure AMH levels.[4] Instinctively, AMH measurement involves a high degree of variability and common measurement errors, as compared with the measurement of other hormones. Even so, AMH is thought to be an accurate predictor of ovarian reserve, because it is relatively stable with no major short-term changes throughout the menstrual cycle, unlike other hormonal markers that dramatically fluctuate within the menstrual cycle, such as FSH, LH, E2, and progesterone.[5]

The most important clinical value in IVF of AMH is the good correlation with oocyte yield after COS. The coefficient of correlation (R) between AMH level measured by the Access assay and oocyte yield was 0.655, which was higher than the previously obtained coefficients of correlation by the MLB AMH/MIS, Gen II original, and Gen II pre-mix assays (0.612, 0.438, and 0.607, respectively; in-hospital data). It is assumed that the accuracy of measurement is improved by the shift from solid-phase ELISA assay detecting antigen-antibody reaction to liquid-phase Access assay.

At our clinics, AMH and age are used for decision-making about shifting fertility treatment to the next step, prediction of oocyte yield following COS, and selection of the type and dose of fertility drugs to conduct ovarian stimulation with the aim of providing maximum
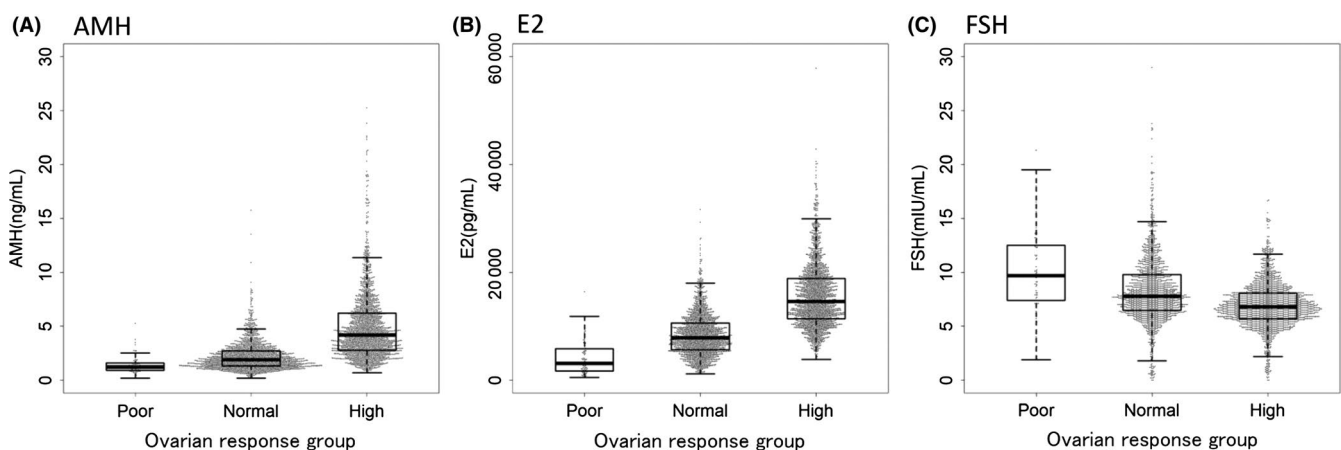

FIGURE 3 Box and whisker plots for anti-Müllerian hormone (AMH), E2, and follicle stimulating hormone (FSH) by ovarian response group. N = 3296 cycles, excluding the cycles stimulated by clomiphene or no FSH measurement in the previous cycle. The poor response group: cycles with an oocyte yield of ≤3 (n = 70), the normal response group: cycles with an oocyte yield of ≥4 and ≤14 (n = 1499), the high response group: cycles with an oocyte yield of ≥15 (n = 1727)

(A) High response group vs Normal response group

(B) Poor response group vs Normal response group
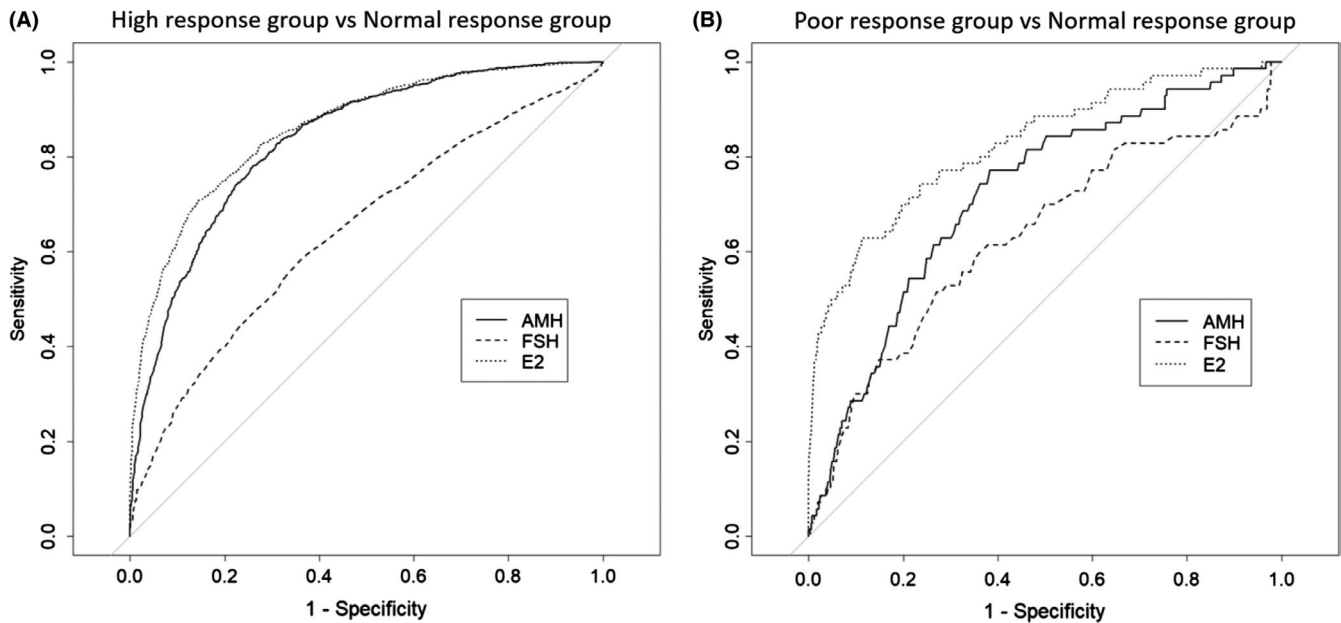

FIGURE 4 Receiver operating characteristic (ROC) curve analysis for differentiating (A) the high response group from the normal response group and (B) the poor response group from the normal response group. N = 3296 cycles excluding the cycles stimulated by clomiphene or no follicle stimulating hormone (FSH) measurement in the previous cycle. A, Among the three parameters in the ROC curve, E2 has the highest sensitivity and specificity value (AUC: 0.86, 95% CI: 0.85-0.87), followed by anti-Müllerian hormone (AMH; AUC: 0.83, 95% CI: 0.82-0.85) and FSH (AUC: 0.64, 95% CI: 0.63-0.66). The AUC of E2 was significantly greater than that of AMH (AMH vs E2: $z = 3.9$, $P < 0.001$) and FSH (FSH vs E2: $z = 39.8$, $P < 0.001$), and the AUC of AMH was also significantly greater than that of FSH (AMH vs FSH: $z = -36.4$, $P < 0.001$). B, Among the three parameters in the ROC curve, E2 has the highest sensitivity and specificity value (AUC: 0.83, 95% CI: 0.77-0.88), followed by AMH (AUC: 0.72, 95% CI: 0.66-0.78) and FSH (AUC: 0.63, 95% CI: 0.55-0.70). The AUC of E2 was significantly greater than that of AMH (AMH vs E2: $z = 3.6$, $P < 0.001$) and FSH (FSH vs E2: $z = 8.7$, $P < 0.001$), and the AUC of AMH was also significantly greater than that of FSH (AMH vs FSH: $z = -6.8$, $P < 0.001$)

therapeutic effect. Therefore, the estimation of the number of retrievable oocytes with a higher degree of precision is essential.

The results of the study demonstrated the good correlation between AMH level and oocyte yield, suggesting that AMH may be very effective in predicting oocyte yield in IVF treatment. It is important to note, however, that AMH is not a good predictor of pregnancy or pregnancy potential as reported elsewhere.[6-8] AMH is an indicator reflecting the degree of primordial follicles remaining in the ovary, which is independent of whether fertilized oocytes can be developed in the uterus. Even so, it may be certain that high AMH levels suggestive of increased oocyte yields are somehow advantageous to the chance of pregnancy per oocyte retrieval; thus, the numerical superiority of oocyte yields may reflect pregnancy potential. The degree of aging and damage of oocytes are biased, and if more oocytes are retrieved, there will be more opportunities to find less damaged oocytes. The impact of high AMH levels, however, may be smaller than that of the aging of oocytes, and accordingly, AMH should not be used to predict pregnancy outcome or response to fertility treatment.

In this study, E2 level per oocyte retrieved was increased to ≈700 pg/mL around the age of 40, as compared with ≈650 pg/mL below the age of 30. Similarly, E2 level per mature oocyte was increased to ≈1000 pg/mL around the age of 40 compared with ≈800 pg/mL below the age of 30. Although it has been stated that

peak E2 level per mature oocyte is 200-400 pg/mL,[9] E2 levels per oocyte retrieved and per mature oocyte observed in clinical situations appear to increase with age. Also in this study, the E2 levels per oocyte were extremely higher. This may be partially explained by the process of dividing E2 levels by the number of oocytes actually retrieved, not oocytes detected, resulting in the higher mean E2 level per oocyte. In our clinical experience, even if 10 retrievable oocytes are found, the number of oocytes that can be actually retrieved is usually seven to nine and it is rare to be able to collect all the 10 oocytes. In any event, it is clear that a high E2 level is required to obtain a mature oocyte in patients of advanced age, especially in those with a low oocyte yield. The similar trend was noted for AMH that correlates well with oocyte yield; namely, a higher E2 level is required to obtain a mature oocyte in patients of advanced age, with a low AMH value.

These findings were similar to the results of a previously reported multicenter, observational study.[10] In this study, we measured AMH by the Access assays in more than 3000 Japanese women and the results indicated that AMH could be an accurate marker of ovarian response to stimulation as observed in the previous Gen II pre-mix assay. This study also demonstrated that AMH levels are as sensitive as E2 levels measured just before oocyte retrieval, which are directly associated with oocyte yield, in predicting ovarian response. As a result, though FSH basal value used to be a good indicator of ovary

stimulation for IVF, AMH measured before ovary stimulation is considered to be a superior marker to FSH.

The improved sensitivity of AMH assays by automatization is good news. If the measurement errors associated with AMH are further reduced, new applications and concepts of AMH would be developed. Further accumulation and utilization of information on AMH are expected.

DISCLOSURES

Conflict of interest: The authors declare no conflict of interest. *Human rights statements and informed consent*: All the procedures accorded with the ethical standards of the relevant committees on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and its later amendments. This study was approved by the institutional review board of Asada Ladies Clinic. Informed consent was obtained from all patients for being included in the study. *Animal studies*: This article does not contain any study with animal participants that have been performed by any of the authors.

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