INFERTILITY

Multicenter evaluation of the Access AMH antimüllerian hormone assay for the prediction of antral follicle count and poor ovarian response to controlled ovarian stimulation

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Objective: To evaluate a new fully automated antimüllerian hormone (AMH) assay for prediction of poor ovarian response (POR) to ovarian stimulation defined as four or fewer oocytes retrieved.
Design: Prospective cohort study.
Setting: Thirteen private and academic fertility centers in the United States.
Patients(s): A total of 178 women undergoing their first in vitro fertilization (IVF) cycle eligible for the study were consented and enrolled, with data available from 160 women for prediction of POR and 164 women for AMH correlation with antral follicle count (AFC).
Intervention(s): None.
Main Outcome Measure(s): Cutoff point for AMH that predicts POR. Correlation of AMH with AFC, and cutoff point for AMH that correlates with antral follicle count >15.
Result(s): The mean AMH among the poor responders was 0.74 ng/mL, compared with 3.20 ng/mL for normal to high responders. The AMH cutoff at 90% specificity for predicting POR with the use of the receiver operating characteristic (ROC) curve was 0.93 ng/mL, with an associated sensitivity of 74.1%. For prediction of POR, ROC analysis showed that AMH (area under the ROC curve [AUC] = 0.929) was significantly better than FSH (AUC = 0.615; $P<.0001$). AMH was positively correlated with AFC (Spearman rho = 0.756). The AMH at 90% sensitivity for AFC >15 was 1.75, with specificity of 59.1%.
Conclusion(s): A fully automated AMH assay can be a useful biomarker for predicting POR in IVF cycles. Because AMH cutoff points vary depending on the assay used, future studies should continue to calibrate test results to clinically important outcomes. (Fertil Steril® 2018;■:■–■. ©2018 by American Society for Reproductive Medicine.)
Key Words: Antimüllerian hormone, ovarian response, antral follicle count

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Antimüllerian hormone (AMH) is a glycoprotein hormone produced by the granulosa cells of preantral and small antral follicles (1, 2). In the ovary, the physiologic roles of AMH include inhibition of primordial follicle recruitment and inhibition of follicle growth in response to FSH. Serum levels of AMH have been demonstrated to positively correlate with the size of the primordial follicle pool (3) and the number of antral follicles visible by ultrasound (4).

Clinically, AMH can identify infertile patients at risk for poor ovarian response (POR) to gonadotropin stimulation (5–7). AMH can also identify those at greatest risk for ovarian hyperstimulation syndrome (OHSS) (8) and possibly allow dose adjustments to reduce the risk of OHSS (9, 10). Analysis of data collected from randomized controlled trials suggest that AMH performs better than antral follicle count (AFC) in predicting both poor and high ovarian response (11–13). A strong positive age-independent relationship between AMH and the proportion of euploid blastocysts has been recently reported (14).

Despite its demonstrated clinical utility, the interpretation of AMH results has been challenging because of variability between available AMH assays, with cutoff points for predictions of ovarian response differing depending on the AMH assay used (15–18). For prediction of POR, cutoff points used for AMH have ranged from 0.10–1.66 ng/mL, with reported sensitivities of 44%–97% and specificities of 41%–100% (1, 9). Furthermore, most of the literature regarding AMH to date has been reported with the use of manual plate-based ELISAs, which have been associated with concerns about assay reproducibility between laboratories (19), with no international reference standard for AMH available to aid in comparisons between assays or between laboratories (17).

Fully automated assays have been developed that can be performed more quickly than plate assays and do not require manual steps and the use of a plate reader. The Beckman Coulter Access AMH assay is a fully automated electrochemiluminescence sandwich immunoassay that has become available recently to aid in the assessment of ovarian reserve. Given the variability of cutoff points between assays, it is clear that as new AMH assays are developed, assay-specific cutoff points must be reported to accurately guide clinical use of the test result.

The primary objective of the present study was to evaluate the Access AMH assay for prediction of POR to controlled ovarian stimulation defined as four or fewer oocytes retrieved. We compared AMH with FSH and AFC as a predictor of POR. A second aim of this study was to assess the correlation between Access AMH assay and AFC, including the determination of an AMH cutoff point corresponding to an AFC of >15.

MATERIALS AND METHODS

This multicenter, prospective, cross-sectional study was conducted at 13 fertility centers in the United States. The study was Institutional Review Board approved, and subjects provided informed consents. Women 21–45 years of age who were undergoing their first cycle of controlled ovarian stimulation for in vitro fertilization (IVF) and met the eligibility criteria, were invited to participate. Inclusion criteria included regular menses and presence of both ovaries. Oocyte donors and women undergoing oocyte cryopreservation were eligible. Exclusion criteria included polycystic ovary syndrome according to the Rotterdam criteria, previous ovarian surgery, surgically confirmed endometrioma, an ovarian cyst or a follicle measuring ≥ 20 mm, previous ovarian stimulation for IVF, exposure to cytotoxic drugs or pelvic radiation therapy, and hormonal contraceptive use within 2 months before enrollment and study blood drawing.

The dose of gonadotropin was chosen by the treating physician at each center without the knowledge of the study AMH level which is the subject of this report. Defined stimulation protocols were not used, because the goal of this report was to generate results that could be extrapolated to the general practice of IVF, which is the setting in which the test would be used, rather than to be predictive only in the case of a particular defined protocol. Each included participant was undergoing her first cycle of IVF, and therefore there were no data from previous cycles to guide choice of protocol. Factors used in determining medication dose were not set by the study and included AFC and/or results from biomarker assays if previously ordered by the clinician. There were no mild stimulation or natural cycles included. In all cases, the dose of gonadotropin was chosen to optimize the number of oocytes to be collected while minimizing the risk of OHSS.

Blood was collected and AFC determined on day 2–4 of the menstrual cycle. Transvaginal ultrasound was performed by reproductive endocrinologists or sonographers experienced in the performance of AFC at each of the participating sites. AFC was defined as the sum of follicles 2–10 mm in diameter in both ovaries (20).

After collection of blood by means of standard venipuncture, specimens were allowed to clot completely (for a minimum of 30 minutes and not longer than 2 hours) and then centrifuged. The serum was pipetted into 1-mL aliquots, frozen at $-20^{\circ}C$ or colder within 4 hours of blood drawing, and shipped frozen for testing at an independent outside laboratory (ARUP Laboratories, Salt Lake City, Utah) which did not have access to clinical data about the study participants.

Serum and plasma specimens were tested for AMH, FSH, and E_2 with the use of the automated Beckman Coulter Access 2 immunoassay analyzer (21, 22) with published total imprecision for Access AMH ranging from 2.4% to 5.2%. The capture antibody (F2B/12H) is bound on paramagnetic particles, and the second antibody (F2B/7A) is alkaline phosphatase labeled. The concentration of AMH in the sample is proportional to the light production and is determined with the use of a six-point calibration curve. More recent results (https://www.accessdata.fda.gov/cdrh_docs/pdf17/K170524.pdf) show total imprecision for Access AMH for multiple sites (reproducibility) ranging from 2.2% to 3.2%. Access AMH assay sensitivity is reported as 0.01 ng/mL. The automated Access FSH assay used is commercially available with published performance characteristics (https://www.beckmancoulter.com/wsrportal/page/