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New automated antimüllerian hormone assays are more reliable than the manual assay in patients with reduced antral follicle count

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Objective: To compare the strength of the relationship between antral follicle count (AFC) and serum antimüllerian hormone (AMH) concentrations obtained with two automated and one manual AMH assays in three different AFC populations.
Design: Prospective cohort study.
Setting: University-affiliated IVF-ET center.
Patient(s): Frozen–thawed serum samples of 211 assisted conception candidates, aged 24–43 years.
Intervention(s): Serum AMH was measured using one manual (AMH Gen II) and two fully automated (Access AMH and Elecsys AMH) assays. Antral follicle count was performed under strictly standardized conditions and sorted into three groups according to tercile values: low AFC (3–12 follicles; n = 73), intermediate AFC (13–20 follicles; n = 65), and high AFC (21–84 follicles; n = 73).
Main Outcome Measure(s): Strength of correlation between AMH levels and AFC.
Result(s): Overall, AMH levels were lower with Access AMH (−16%) and Elecsys AMH (−20%) than with AMH Gen II. Remarkably, the strength of correlations between AFC and circulating AMH levels was the same with the three assays ($r = 0.83$). Yet in the low AFC group, serum AMH levels obtained by Access AMH and Elecsys AMH showed a stronger correlation with AFC ($r = 0.63$ and $r = 0.65$, respectively) than the AMH Gen II ($r = 0.52$), a phenomenon that was not observed in the remaining AFC groups.
Conclusion(s): As compared with conventional AMH Gen II assay results, [1] serum AMH concentrations were −16% and −20% lower with Access AMH and Elecsys AMH, respectively; and [2] automated assays were more strongly correlated to AFC in the subset of patients with reduced follicle count. (Fertil Steril® 2016;■:■–■. ©2016 by American Society for Reproductive Medicine.)
Key Words: Antimüllerian hormone, antral follicle count, ovarian reserve, poor responders

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Associated or not to ultrasonographic counting of antral follicles, serum antimüllerian hormone (AMH) measurements have become the reference in the clinical appraisal of the ovarian follicular status (1), and its clinical use has been continuously gaining momentum during the last 12 years. Antimüllerian hormone levels reflect the activity of granulosa cells of small antral follicles, thereby providing patients and physicians with invaluable information on ovarian aging and how to individualize controlled ovarian hyperstimulation protocols (2).

Yet from a practical standpoint, in addition to cost, problems related to reliability and consistency of serum AMH measurements have raised doubts about their clinical soundness. These problems essentially are attributed, on the one hand, to an uncoordinated development of AMH assays, which displayed different callibration and standards and, on the other hand, to the compulsory operator- and technique-dependent manipulations. Recently, to overcome these limitations and to improve quality of AMH measurements, fully automated AMH assays (Access AMH [Beckman Coulter] and Elecsys AMH [Roche Diagnostics International]) have been developed and commercialized (3, 4). Yet analytical data available have been limited to the confirmation of an adequate concordance of serum AMH levels obtained by these assays (4–11).

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Unfortunately, only limited and/or indirect data taking the necessary relationship between AMH and the number of AMH-producing antral follicles as a reference have been hitherto published (5–7). Because it is undoubtedly the small antral follicles that produce most of the circulating AMH concentrations (12–14), antral follicle count (AFC), when it is performed under optimal conditions, should be taken as the standard for comparing reliability of AMH assays. Indeed, serum AMH levels have been strongly correlated to AFC by numerous investigators (1, 13, 15, 16). Moreover, the relative performance of new automated and manual AMH assays in subgroups of patients displaying low, intermediate, and high AFC remains undetermined.

Therefore, this insufficient knowledge spurred us to investigate the reliability and concordance of AMH levels obtained by one manual and two automated AMH assays in different AFC populations.

MATERIALS AND METHODS

Subjects and Procedures

We used frozen–thawed serum aliquots obtained from 211 women who were willing to enter our assisted conception program between April 2015 and July 2015. Inclusion criteria were [1] both ovaries present without morphologic abnormalities (menstruating patients with or without polycystic ovary syndrome or polycystic ovary morphology were included); [2] optimum ovarian visualization at transvaginal ultrasound scans; and [3] body mass index ranging between 18 and 25 kg/m^2.

All blood samples were collected between days 1 and 5 of the menstrual cycle and remained frozen from 2 to 4 months before thawing. Serum AMH determinations were performed using three different ELISAs according to manufacturer protocols by a single operator (J.-L.B.): modified AMH Gen II (Beckman Coulter), Access AMH (Beckman Coulter), and Elecsys AMH (Roche Diagnostics International). Detailed AMH assay procedures have been previously described (6). In brief, limits of detection of the three AMH assays tested were, respectively, 0.08, 0.02, and 0.01 ng/mL; limits of quantification, 0.16, 0.08, and 0.03 ng/mL; and maximum imprecision, 8.0%, 4.3%, and 3.5%. Moreover, serum E_2, FSH, and LH levels were determined by an automated multianalysis system using a chemiluminescence technique (Cobas e411 Analyzer, Roche Diagnostics, Mannheim, Germany). For E_2, limit of quantification was 15 pg/mL, and maximum imprecision was 8%. For FSH and LH, limit of quantification was 0.1 mIU/mL, and maximum imprecision was 3%.

In parallel to blood samplings, AFC (antral follicles measuring 3–10 mm in diameter) was carefully and exhaustively determined using a 5–9-MHz transvaginal ultrasound probe (RIC 5-9-D; Voluson E8 Expert, General Electric Medical Systems, Paris, France) by a single operator (T.T.) who was unaware of AMH results. Given that the present investigation was limited exclusively to hormonal measurements in frozen–thawed serum aliquots and ultrasound scan records and that patients had previously given their informed consent for additional hormonal analysis using their stored sera, our local institutional review board advised us that it did not require ethics committee submission.

Definition of AFC Groups

Participants were arbitrarily sorted into three different AFC groups according to the 33rd and 66th centiles of AFC distribution (12 and 20 antral follicles): low AFC (3–12 follicles; n = 73), intermediate AFC (13–20 follicles; n = 65), and high AFC (21–84 follicles; n = 73). These cutoffs were used as an effort to pragmatically identify patients with different ovarian follicular phenotypes.

Statistics

Because data distribution was considered nonparametric, we elected to use the median as the measure of central tendency and minimum–maximum as the measure of variability. Circulating AMH levels were compared two by two among the three different assays tested by using the Wilcoxon signed-rank test. The strength of relationships between AFC and serum AMH levels provided by the three different assays was assessed by the Spearman correlation test in the total population and in the three AFC groups. Comparison of Spearman correlation coefficients was performed using the Fisher *r*-to-*z* transformation, a statistical procedure that can be applied to assess the significance of the difference between two correlation coefficients found in two independent samples. In addition, agreement between the different AMH assays was assessed graphically using the Bland-Altman plots and by Passing-Bablok regression. The Cusum test for linearity was used to test the applicability of Passing-Bablok regression. A P value of $< .05$ was considered statistically significant.

RESULTS

Population Characteristics

Characteristics of individuals included in the present analysis corresponded, as expected, to the profile of women who are usually candidates for assisted conception at our center, encompassing young and aged women and those with small and large counts of antral follicles. Median age (range) was 35 (24–43) years. In the early follicular phase, median serum E_2 level was 45 (15–249) pg/mL, and serum FSH and LH levels were 6.9 (3.0–21.6) and 5.1 (1.1–21.1) mIU/mL, respectively. Overall median antral follicle count was 15 (3–84) follicles.

AMH Levels according to Manual and Automated Assays

Serum AMH levels measured by the three different ELISA assays are depicted in Figure 1. As shown, each one of the tested assays resulted into statistically different AMH levels. Hence, AMH Gen II provided AMH levels at 1.97 (0.04-30.66) ng/mL [median (interquartile range)] that were higher ($P< .001$) than those obtained with Access AMH at 1.66 (0.04–30.46) ng/mL and with Elecsys AMH at 1.58 (0.04–26.17) ng/mL. In other words, AMH Gen II levels are, on average, 16% and 20% as high as Access AMH and Elecsys AMH, respectively. In addition, it is noteworthy that statistically different results were observed between