Evaluation of anti-Müllerian hormone as a test for the prediction of ovarian reserve

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Objective: To compare in an integral way the value of the basal serum anti-Müllerian hormone (AMH) level with most of the established ovarian reserve tests.
Design: Prospective randomized controlled trial.
Setting: Fertility center of a university hospital in the Netherlands.
Patient(s): One hundred ten patients undergoing their first IVF cycle who were randomized, by a computer-designed four-block system, into two groups.
Intervention(s): Fifty-six patients underwent a clomiphene citrate challenge test (CCCT), and 54 patients underwent an exogenous FSH ovarian reserve test (EFORT). In all patients, basal AMH, basal FSH, basal inhibin B, antral follicle count (AFC), and basal volume of the ovaries were measured. In all patients, the test was followed by a standard IVF treatment.
Main Outcome Measure(s): Ovarian response after ovarian hyperstimulation in an IVF treatment, expressed as the total number of stimulated follicles, retrieved oocytes, and ongoing pregnancies.
Result(s): The best prediction of ovarian reserve (Y) was seen in a multiple regression prediction model that simultaneously included AFC, inhibin B increment in the EFORT, and basal volume of the ovaries. Univariate logistic regression showed that the best predictors for poor response were AMH, the CCCT, basal FSH, and the AFC. For hyperresponse, univariate logistic regression showed that the best predictor was AFC. Multiple logistic regression analysis did not produce a better model in terms of improving the prediction of poor response or hyperresponse. The best predictors for the prediction of non-pregnancy were the CCCT and the E_2 increment in the EFORT.
Conclusion(s): Anti-Müllerian hormone is comparable with other commonly used ovarian reserve tests but is probably most applicable in general practice. (Fertil Steril® 2008;90:737–43. ©2008 by American Society for Reproductive Medicine.)

Key Words: AMH, basal FSH, basal inhibin B, CCCT, EFORT, IVF, ovarian reserve

The age-related decline of the success in IVF largely is attributable to a progressive decline of ovarian oocyte quality and quantity (1). Over the past 2 decades, a number of so-called ovarian reserve tests (ORTs) have been designed to give an answer to that particular question. We need to find a minimally invasive, reliable ORT that can give a prediction for a poor response, hyperresponse, or adequate response after ovarian hyperstimulation as well as a prognosis for pregnancy.

A potential new test in this field is measuring the levels of serum anti-Müllerian hormone (AMH); also known as Müllerian-inhibiting substance, this is a dimeric glycoprotein that belongs to the transforming growth factor-β family (2–6). It is involved in the regression of the Müllerian ducts during male fetal development (7) and is expressed in Sertoli cells, from testicular differentiation up to puberty.

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In females, AMH is exclusively produced by granulosa cells of preantral (primary and secondary) and small antral follicles (8) from birth up to menopause. After follicles differentiate from the primordial to the primary stage, production of AMH starts, and it continues until the follicles have reached the antral stages, with diameters of 2–6 mm (9–11). The number of the small antral follicles is related to the size of the primordial follicle pool (12, 13). With the decrease in the number of the antral follicles with age, AMH production appears to become diminished (3, 4, 14), and it invariably will become undetectable at and after menopause.

Studies about IVF stimulations have suggested that AMH as such represents ovarian quantitative reserve and also has some value for the prediction of pregnancy (2, 8, 9). Moreover, evidence is accumulating that AMH, in contrast to FSH, E_2, and inhibin B, can be used as a cycle-independent marker (15, 16).

Recently, we conducted a prospective study that evaluated various established static and dynamic ORTs (17–19), but serum AMH was not included. Given its potential features as a reliable test, we decided to retrospectively measure serum AMH in remaining blood samples, allowing us to make an

integral comparison of serum AMH levels as a test to predict ovarian response to gonadotropin stimulation with most of the established ORTs, including basal FSH (bFSH), basal inhibin B, the clomiphene citrate challenge test (CCCT), the exogenous FSH ovarian reserve test (EFORT), antral follicle count (AFC), and basal volume of the ovaries (BOV).

MATERIALS AND METHODS

Study Population

One hundred ten patients who were 18–39 years of age and who were eligible for treatment by assisted reproduction between June 1997 and December 1999 participated in the study. This study is part of a prospective randomized study on the determination of ovarian reserve in regularly menstruating patients (17). Patient infertility either was idiopathic for >3 years and/or was caused by male factor and/or cervical hostility. Patients had to have regular menstrual cycles, two ovaries, and at least one patent fallopian tube. Patients were excluded who had either polycystic ovary syndrome, defined as a combination of oligomenorrhea or amenorrhea, and an increased LH concentration in the presence of a normal FSH level or who had a severe male factor, defined as [1] <1 million motile spermatozoa after centrifugation (40/90) and/or [2] >20% antisperm antibodies present on the spermatozoa after processing with gradient centrifugation (40/90) and/or [3] >50% of the spermatozoa without an acrosome. Other exclusion criteria were untreated or insufficiently corrected endocrinopathies, clinically relevant systemic diseases, or a body mass index of >28 kg/m^2.

The protocol was approved by the institutional review board and the committee on ethics for research involving human subjects of the Vrije Universiteit Medical Centre (Amsterdam, the Netherlands). All couples participating in the study signed informed consent.

Treatment Protocol

In our studies elsewhere (17–19), 110 patients were randomized into two groups by a computer-designed, four-block system. Fifty-six patients underwent a CCCT, and 54 patients underwent an EFORT. In all patients, the test was followed by IVF treatment. From all blood samples, serum and plasma were separated and stored at –20°C for later estimation of levels of serum AMH.

For the purpose of this study, we analyzed the basal serum AMH (bAMH) on cycle day 3 (CD3; CD1 is the day of onset of menses) and also on CD4, for the EFORT, and on CD10, for the CCCT. The basal FSH level, basal E_2 level, and basal inhibin B level were determined as an integral part of all CCCTs and EFORTs, as described elsewhere (17). On CD3, all patients underwent a transvaginal ultrasound examination to assess the number of antral follicles and the volume of the ovaries.

Clomiphene citrate challenge test Starting on CD5, 100 mg of clomiphene citrate (Serophene; Serono, Geneva, Switzerland) was administered for 5 days. Serum FSH was determined on CD2 or 3 (bFSH) and on CD10 (stimulated FSH, sFSH). Analysis of the CCCT (17) was performed by using the following parameter: bFSH + sFSH.

Exogenous FSH ovarian reserve test On CD3, recombinant FSH (Gonal-F, 300 IU SC; Serono) was administered. In this study, blood samples for the determination of FSH, E_2, and inhibin B were drawn, just before (basal values) and 24 hours after (stimulated values) the administration of FSH. Analysis of the EFORT (17) included the following parameters: E_2 increment and inhibin B increment, 24 hours after administration of FSH.

Transvaginal sonography measurements All ultrasound examinations were performed by two of the authors (J.K. or R.S.) by using an Aloka SSD-1700 ultrasound apparatus (5.0-MHz probe).

The volume of each ovary was calculated by measuring the ovarian diameters (D) in three perpendicular directions and applying the formula for an ellipsoid: $D1 \times D2 \times D3 \times \pi/6$. The volumes of both ovaries were added to obtain the BOV.

To determine the diameter of a follicle, the mean of measurements in two perpendicular directions was taken. The numbers of follicles in both ovaries were added for the total AFC. The follicles visualized and counted by transvaginal sonography in the early follicular phase were 2–10 mm in size.

In vitro fertilization treatment The IVF treatment has been described in detail elsewhere (17–19). The ovarian hyperstimulation protocol was performed according to a long GnRH-agonist protocol.

Ongoing Pregnancy

Ongoing pregnancy was defined as the presence of fetal cardiac activity beyond 12 weeks of gestation. For this study, a multiple pregnancy was regarded as one pregnancy.

Serum Assays

Serum E_2 was determined by a competitive immunoassay (Amerlite; Amersham, Buckinghamshire, United Kingdom). For E_2, the interassay coefficient of variation (CV) was 11% at 250 pmol/L and was 8% at 8,000 pmol/L, and the intra-assay CV was 10% at 350 pmol/L, 8% at 1,100 pmol/L, and 8% at 5,000 pmol/L. The lower limit of detection for E_2 was 90 pmol/L. In the EFORT and CCCT, we measured E_2 by using a sensitive RIA (Sorin Biomedica, Saluggia, Italy). This measurement of E_2 was abbreviated as EE. For EE, the interassay CV was 11% at 60 pmol/L, 8% at 200 pmol/L, 11% at 550 pmol/L, and 8% at 900 pmol/L. The intra-assay CV was 4% at 110 pmol/L and was 5% at 1,000 pmol/L. The lower limit of detection for EE was 18 pmol/L.

Follicle-stimulating hormone was determined by using a commercially available immunometric assay (Amerlite; Amersham). For FSH, the interassay CV was 9% at 3 IU/L

and was 5% at 35 IU/L, and the intra-assay CV was 9% at 5 IU/L, 8% at 15 IU/L, and 6% at 40 IU/L. The lower limit of detection for FSH was 0.5 IU/L. Inhibin B was determined immunometrically by a commercially available assay (Serotec Limited, Oxford, United Kingdom). For inhibin B, the interassay CV was 17% at 25 ng/L, 14% at 55 ng/L, and 9% at 120 ng/L, and the intra-assay CV was 8% at $\leq$40 ng/L and was 5% at >40 ng/L. The lower limit of detection for inhibin B was 13 ng/L.

Halfway through the study (after 62 patients), the Amerlite assay used to assess FSH was withdrawn from the market and was replaced by another commercially available assay (Delfia, Wallac, Finland). The two assays have been compared and have shown excellent linear correlation, although a shift in the values took place (Delfia FSH = 1.28 $\times$ Amerlite FSH + 0.01 [$r = 0.9964$]). For the Delfia FSH, the interassay CV was 5% at 3.5 IU/L and was 3% at 15 IU/L. All FSH determinations were recalculated and are expressed according to the Delfia assay. The lower limit of detection for FSH was 0.5 IU/L.

Values below the detection limit of an assay were assigned a value equal to the detection limit of that assay.

Serum AMH levels were estimated by using an enzyme-immunometric assay (Diagnostic Systems Laboratories, Webster, TX). Inter-assay and intraassay CVs were <7%. The detection limit of the assay was 0.026 μg/L. Repeated freezing and thawing of the samples or storage at 37°C for 1 hour did not affect results of the assay.

Statistical Analysis

The outcome measure of the first part of this study, which will give an answer to the question of which ORT or combination of tests can predict the ovarian response after ovarian hyperstimulation in an IVF treatment, expressed as the number of follicles. By univariate linear regression, we estimated the value of the independent variable bAMH. Subsequent multivariate linear regression analysis was used to develop prediction models for the ovarian response.

The outcome measure of the second part of this study will give an answer to the question of which ORT or combination of tests gives the best prognostic information on the probability of poor ovarian response and hyperresponse in an IVF population, expressed as the number of retrieved oocytes and an ongoing pregnancy.

As described elsewhere (17–19), we arbitrarily defined a poor ovarian response as fewer than six oocytes after ovarian hyperstimulation in an IVF treatment, and we defined a hyperresponse as >20 oocytes after such an IVF treatment. In the analysis of poor ovarian response, patients with cycles that were canceled because of an exaggerated response were included in the group of normal responders. For the analysis of ovarian hyperresponse, patients who had cycles canceled because of an exaggerated response were included in the group of hyperresponders.

We used univariate logistic regression to examine the value of the independent variable bAMH in predicting poor response and hyperresponse, as well as the presence of an ongoing pregnancy after ovarian hyperstimulation in IVF. Subsequent multivariate logistic regression analysis was used to develop prediction models for the ovarian response. The area under the receiver operating characteristic curve (ROC-AUC) was computed to assess the predictive accuracy of the logistic models.

To define a normal and an abnormal test, sensitivity, specificity, positive predictive value, and accuracy were used to find the optimal cutoff level.

Comparison of means was performed by using the unpaired *t*-test and Kruskal-Wallis test. For all tests, the significance level was 0.05.

Statistical analysis of the data was performed by using the Statistical Package for the Social Sciences for Windows (SPSS, Inc., Chicago, IL).

RESULTS

There were no significant differences noted between the groups in baseline characteristics, CD3 measurements, or outcome parameters.

There were no significant changes in serum levels of AMH after injection of the FSH in the EFORT and after 5 days of clomiphene citrate (100 mg/d; Table 1).

In 6 patients, we could not measure serum AMH, because samples were not available anymore.

Prediction of the Number of Follicles After Stimulation

Univariate linear regression analysis Basal AMH was significantly correlated with the number of follicles obtained after stimulation ($r = 0.632$, $P<.001$). The regression line of bAMH vs. the number of follicles (Y) was characterized by the following equation: Y = 7.06 + 2.48 $\times$ bAMH, with a 95% confidence interval (CI) of 1.88–3.08, meaning that each increment of 1 μg of AMH per liter predicts an increment of 2.5 follicles (95% CI, 1.9–3.1; Fig. 1). Table 2 shows the correlations of numbers of follicles after stimulation with the results of EFORT, CCCT, ultrasound, and values of basal estimations, as described in our study elsewhere (14–16), and the additional results of bAMH measurements.

Table 3 shows the characteristics of the poor vs. normal responders.

Step-forward regression analysis In the case of the CCCT group, 51% of the prediction model for ovarian response is explained by the best predictive variable: the total AFC. When the independent variables bAMH, BOV, bFSH + sFSH, bFSH, and age were added in a step-forward regression analysis, the explained variation rose significantly by 6% after the selection of bAMH. The other independent variables did not contribute significantly to the model. The prediction

TABLE 1

Concentrations of AMH, E_2, and inhibin B in CCCT and EFORT.

Parameter	CD3	CD4 or CD10[a]	*P* value
CCCT (n = 53)			
FSH (IU/L)	7.6 ± 2.5	8.3 ± 5.3	.21
AMH (μg/L)	2.6 ± 2.3	3.15 ± 2.71	.95
E_2 (pmol/L)	126.1 ± 53.1	1,387.5 ± 782.9	<.001
Inhibin B (ng/L)	95.0 ± 39.4	317.0 ± 183.3	<.001
EFORT (n = 51)			
FSH (IU/L)	7.4 ± 3.1	12.2 ± 9.2	.04
AMH (μg/L)	3.3 ± 2.9	2.86 ± 2.39	.70
E_2 (pmol/L)	118.6 ± 47.1	288.6 ± 175.2	<.001
Inhibin B (ng/L)	96.3 ± 40.6	211.7 ± 127.4	<.001

Note: Data are mean ± SD.
[a] For EFORT, CD4; for CCCT, CD10.

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of the total number of follicles obtained after stimulation thus increased from 51% to 57%. The regression line of the bAMH and total AFC vs. the number of follicles (Y) was given by the following equation: Y = −1.052 + 1.019 × bAMH (95% CI, 0.227–1.811) + 1.089 × AFC (95% CI, 0.673–1.504; r = 0.756, P<.001).

On the basis of the EFORT group, 63% of the prediction model for ovarian response is explained by the best predictive variable, the total AFC. When adding the inhibin B increment and BOV simultaneously in a step-forward multiple regression prediction model, the explained variation of the best predictive model rose significantly with 9%. The total explained variation thus increased from 63% to 72%. The regression line of the total AFC, inhibin B increment, and total BOV on the number of follicles was drawn by the following regression equation: Y = −3.161 + 0.805 × AFC (95% CI, 0.258–1.352) + 0.034 × inhibin B increment (95% CI, 0.007–0.601) + 0.511 BOV (95% CI, 0.480–0.974; r = 0.848, P<.001).

When we included bAMH, E_2 increment, age, and bFSH as variables in the step-forward regression analysis together with total AFC, inhibin B increment, and the total

FIGURE 1

Plot of the number of follicles obtained after stimulation against the basal AMH. The three lines represent the regression line, with the 95% CI of the mean appearing as well.

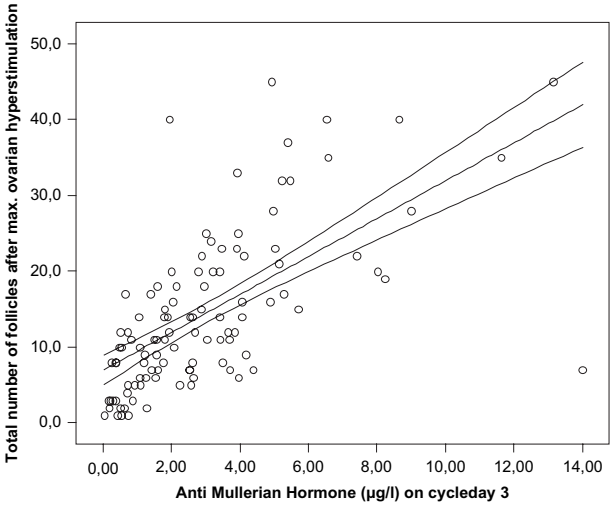

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TABLE 2

Univariate regression analysis of the ovarian reserve tests for the prediction of the stimulable cohort of the follicles in the ovaries (ovarian reserve).

Parameter	n	Correlation	*P*
Age (y)	110	0.423	<.001
Basal FSH (IU/L)	110	0.313	.001
Sum of FSH in CCCT (IU/L)	56	0.496	<.001
E_2 increment in EFORT (pmol/L)	54	0.751	<.001
Inhibin B increment in EFORT (ng/L)	54	0.718	<.001
Total ovarian volume (mL)	110	0.610	<.001
Total antral follicle count	110	0.745	<.001
Basal AMH (μg/L)	104	0.632	<.001

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TABLE 3

Characteristics of poor vs. normal responders.

Parameter	Poor responders (n = 29)	Normal responders (n = 81)	*P*
Age at baseline (y)	35.3 ± 3.0	33.5 ± 4.0	.029
CD3			
FSH (IU/L)	12.0 ± 11.5	6.6 ±1.8	<.001
E_2 (pmol/L)	124.1 ± 54.1	138.4 ± 156.5	.632
Inhibin B (ng/L)	76.0 ± 47.4	93.1 ± 43.0	.077
AMH (μg/L)	1.48 ± 2.59	3.53 ± 2.46	<.001
Endpoints			
Total no. of follicles	4.6 ± 2.6	17.7 ± 9.7	<.001
Total no. of oocytes	3.0 ± 1.6	14.9 ± 8.1	<.001
Ongoing pregnancy, n (%)	2 (10)	22 (24)	.001

Note: Data are mean ± SD unless otherwise indicated.

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BOV, we did not find a significant contribution of these variables.

Prediction of the Number of Retrieved Oocytes After Stimulation

Univariate logistic regression For the prediction of poor response after IVF with ovarian hyperstimulation, the ROC-AUC of logistic regression analysis for bAMH was 0.85 ($P<.0001$), which is comparable with that of the bFSH (ROC-AUC, 0.83; $P<.0001$), AFC (ROC-AUC, 0.83; $P<.0001$), CCCT (ROC-AUC, 0.88; $P<.0001$), and inhibin B increment in the EFORT (ROC-AUC, 0.86; $P<.0001$).

The cutoff level of ≤1.4 μg/L had a sensitivity of 76% and a specificity of 86%. In the population studied, with a prevalence of 27% for a poor response (<6 oocytes after ovarian hyperstimulation in an IVF treatment), the accuracy was 83% (which means that 83% of the patients had a correctly predicted test). In the case of bAMH of ≤1.4 μg/L, the test correctly predicted poor response to stimulation in an IVF treatment in 67% (positive predictive value).

As a single prognostic predictor for the prediction of a hyperresponse in an IVF treatment, bAMH appeared to have a good discriminative potential, as expressed by an ROC-AUC of 0.85 ($P<.001$). Unfortunately, this was not better than the AFC (ROC-AUC, 0.93; $P<.0001$) and inhibin B increment in the EFORT (ROC-AUC, 0.93; $P<.0001$).

The cutoff level of ≥5 μg/L gave the highest sum of sensitivity and specificity. This result had a sensitivity of 53% and a specificity of 91%. In the population studied, with a prevalence of 16% for high response (>20 oocytes after ovarian hyperstimulation in an IVF treatment), the accuracy was 85% (which means that 85% of the patients had a correctly predicted test). In the case of a bAMH of ≥5 μg/L, the test correctly predicted hyperresponse to stimulation in an IVF treatment in 53% (positive predictive value).

Multivariate logistic regression In the CCCT group, multivariate analysis for poor response resulted in a model with one variable: bFSH + sFSH in the CCCT (ROC-AUC = 0.88).

In the EFORT group, multivariate analysis for poor response resulted in a model with only one variable: AFC (ROC-AUC = 0.88).

In the CCCT group, multivariate analysis for hyperresponse resulted in a model with two variables: age and AFC (ROC-AUC = 0.93).

In the EFORT group, multivariate analysis for hyperresponse resulted in a model with only one variable: AFC (ROC-AUC = 0.93).

Prediction of Ongoing Pregnancy After IVF Treatment

Table 4 depicts the statistical significance and areas under the ROC-AUC of logistic regression analysis for all ORTs with a $P<.05$ for the prediction of non-pregnancy after IVF with ovarian hyperstimulation. The CCCT with a cutoff level of 18 IU/L (sensitivity of 25% and a specificity of 100%) and the E_2 increment in the EFORT with a cutoff level of 130 ng/L (18) (sensitivity of 45% and a specificity of 83%) appeared to have the best discriminative potential for the prediction of non-pregnancy.

DISCUSSION

Serum AMH appears able to predict the number of follicles obtained during maximal ovarian stimulation. According to our study, which uniquely allowed direct comparison, bAMH does not appear superior to AFC, BOV, and most of the other commonly used stimulated endocrine ORTs, providing similar ROC-AUC values. When included in the stepwise forward multiple regression model, bAMH did not provide additive value to a combination of the inhibin

TABLE 4

Univariate logistic regression analysis and ROC-AUC of the ovarian reserve tests in the prediction of non-pregnancy in IVF.

Variable	n	*P*	ROC-AUC
Sum of FSH in CCCT (IU/L)	56	.005	0.745
E_2 increment in EFORT (pmol/L)	54	.024	0.709
Basal AMH (μg/L)	104	.023	0.643

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B-increment in the EFORT and BOV, which led to the most optimal prediction model with regard to ovarian response.

The performance of bAMH with regard to the prediction of poor response gave a sensitivity of 76% and a specificity of 86%, which would imply that the test performs moderately. Increasing the threshold of bAMH yields better sensitivity but results in unacceptable specificity, and decreasing the threshold causes the sensitivity to drop in favor of higher specificity. In comparison with CCCT, this sensitivity and specificity are lower, which means that bAMH has no additional value as a test for poor responders.

As a test to predict ovarian hyperresponse, bAMH does not appear appropriate, because the ROC-AUC of the inhibin B increment in the EFORT and the AFC are higher than that for bAMH. The sensitivity is low when using all acceptable threshold levels, which means that there would be many false-negative patients, with potential overtreatment as result.

The results for bAMH in this study confirm the outcome of our recently published systematic review on ORTs (1), that bAMH reasonably predicts ovarian response but unfortunately not pregnancies. With regard to bAMH, the data from this study could not be included anymore but would fit seamlessly into the summary ROC curve of the report.

The CCCT and EFORT had a better predictive value for the prediction of pregnancy. An ideal ORT should identify a substantial percentage of IVF-indicated cases that have a practically zero chance of becoming pregnant in a series of treatment cycles, because of the adverse effects of diminished ovarian reserve. Those women can be prevented from entering an assisted reproductive technology program, because they will incur very high costs for only minimal results. If it is not too expensive and not too demanding for the patient, such a test would be readily embraced by physicians, patients, health politicians, and insurance companies.

In the case of the CCCT, there was a specificity of 100%, which means that there were no ongoing pregnancies above the test result of 18 IU/L, but the sensitivity of 25% was very low.

It should be noted that the use of pregnancy as an outcome parameter for the assessment of ovarian reserve status may be insufficient if only one exposure cycle is taken into account (1). As such, the possibility of misjudgment on the basis of a currently known ORT is hard to rule out. This implies that the use of the test as a basis for denying treatment to assumed ovarian-aged women should be declined, and as a consequence, the test should not be applied on a regular basis or should be used only for counseling or screening purposes.

As shown in Table 3, poor ovarian response has been associated with a reduced chance of pregnancy in the actual treatment cycle. Accurate prediction of poor response could therefore have clinical value if the pregnancy prospects are so unfavorable that a predicted poor responder would be denied treatment.

Accuracy in response prediction, however, will be high only if false positives are prevented by using extreme cutoff levels, which will result in only minor percentages of abnormal tests being found and many future poor responders passing unrecognized. At the same time, it is necessary to know whether the predicted poor responder indeed has very low prospects for success in subsequent cycles. Because much of this is unknown at the present time, women should not be denied from entering IVF treatment on the basis of an ORT.

There are potential advantages of using bAMH over AFC or the CCCT, because AMH can be measured throughout the cycle (15, 16), in contrast to the other parameters, which can only be determined in the early follicular phase. This study supported this phenomenon, because we did not see a change in the level of AMH after an acute endogenous rise in FSH (CCCT) and an acute exogenous rise in FSH (EFORT).

In women, serum AMH expression can first be observed in granulosa cells of primary follicles, and expression is strongest in preantral and small antral follicles. Expression of AMH disappears in follicles of increasing size and is almost lost in follicles of >8 mm, in which only very weak staining remains, restricted to the granulosa cells of the cumulus (11). This expression pattern is in agreement with the observation that AMH plays a role in the initial recruitment of and in the selection of the dominant follicle (20).

Our observations, that serum AMH levels, in contrast to those of inhibin B and E_2, do not substantially alter after injection of FSH (300 IU) or 5 days of clomiphene citrate, support the notion that secretion of this substance is probably a measure of the ovarian follicle population and not of the cyclic gonadotropic hormonal status of the patient.

In conclusion, AMH is comparable with other commonly used ORTs but is probably most applicable in general practice because it can be measured throughout the cycle, which is an advantage for both patients and clinicians. The predictive value of AMH for poor response is comparable with that of AFC, but unfortunately this is not the case with the prediction of hyperresponders. The great advantage of AFC

over any other test is its potential usefulness for the ability to concomitantly predict low and high responders.

REFERENCES

1. Broekmans FJ, Kwee J, Hendriks D, Mol BW, Lambalk CB. A systematic review of tests predicting ovarian reserve and IVF outcome. Hum Reprod Update 2006;12:685–718.
2. Van Rooij IA, Broekmans FJ, te Velde ER, Fauser BC, Bancsi LF, de Jong FH, et al. Serum anti-Mullerian hormone levels: a novel measure of ovarian reserve. Hum Reprod 2002;17:3065–71.
3. Van Rooij IA, Tonkelaar I, Broekmans FJ, Looman CW, Scheffer GJ, de Jong FH, et al. Anti-mullerian hormone is a promising predictor for the occurrence of the menopausal transition. Menopause 2004;11:601–6.
4. Van Rooij IA, Broekmans FJ, Scheffer GJ, Looman CW, Habbema JD, de Jong FH, et al. Serum antimullerian hormone levels best reflect the reproductive decline with age in normal women with proven fertility: a longitudinal study. Fertil Steril 2005;83:979–87.
5. Fanchin R, Schonauer LM, Righini C, Frydman N, Frydman R, Taieb J. Serum anti-Mullerian hormone dynamics during controlled ovarian hyperstimulation. Hum Reprod 2003;18:328–32.
6. Seifer DB, Mac Laughlin DT, Christian BP, Feng B, Shelden RM. Early follicular serum mullerian-inhibiting substance levels are associated with ovarian response during assisted reproductive technology cycles. Fertil Steril 2002;77:468–71.
7. Behringer RR, Finegold MJ, Cate RL. Mullerian-inhibiting substance function during mammalian sexual development. Cell 1994;79: 415–25.
8. Vigier B, Tran D, Legeai L, Bezard J, Josso N. Origin of anti-Mullerian hormone in bovine freemartin fetuses. J Reprod Fertil 1984;70:473–9.
9. Durlinger AL, Kramer P, Karels B, de Jong FH, Uilenbroek JT, Grootegoed JA, et al. Control of primordial follicle recruitment by anti-Mullerian hormone in the mouse ovary. Endocrinology 1999;140: 5789–96.
10. Durlinger AL, Visser JA, Themmen AP. Regulation of ovarian function: the role of anti-Mullerian hormone. Reproduction 2002;124:601–9.
11. Weenen C, Laven JS, Von Bergh AR, Cranfield M, Groome NP, Visser JA, et al. Anti-Müllerian hormone expression pattern in the human ovary: potential implications for initial and cyclic follicle recruitment. Mol Hum Reprod 2004;10:77–83.
12. Kevenaar ME, Meerasahib MF, Kramer P, van de Lang-Born BM, de Jong FH, Groome NP, et al. Serum anti-mullerian hormone levels reflect the size of the primordial follicle pool in mice. Endocrinology 2006;147:3228–34.
13. Gougeon A. Caracteres qualitatifs et quantitatifs de la population folliculaire dans l'ovaire humaine adulte. Contracept Fertil Sex 1994;12: 527–35.
14. de Vet A, Laven JS, de Jong FH, Themmen APN, Fauser BC. Antimüllerian hormone serum levels: a putative marker for ovarian aging. Fertil Steril 2002;77:357–62.
15. Hehenkamp JK, Loomans CWN, Themmen APN, de Jong FH, te Velde ER, Broekmans FJM. Anti-Mullerian hormone levels in the spontaneous menstrual cycle do not show substantial fluctuation. J Clin Endocrinol Metab 2006;10:4057–63.
16. La Marca A, Stabile G, Artenisio AC, Volpe A. Serum anti-Mullerian hormone throughout the human menstrual cycle. Hum Reprod 2006;21:3103–7.
17. Kwee J, Elting MW, Schats R, Bezemer PD, Lambalk CB, Schoemaker J. Comparison of endocrine tests with respect to their predictive value on the outcome of ovarian hyperstimulation in IVF treatment: results of a prospective randomized study. Hum Reprod 2003;18:1422–7.
18. Kwee J, Schats R, McDonnell J, Schoemaker J, Lambalk CB. The clomiphene citrate challenge test versus the exogenous follicle-stimulating hormone ovarian reserve test as a single test for identification of low responders and hyperresponders to in vitro fertilization. Fertil Steril 2006;85:1714–22.
19. Kwee J, Elting M, Schats R, McDonnell J, Lambalk CB. Ovarian volume and antral follicle count for the prediction of low and hyper responders with in vitro fertilization. Reprod Biol Endocrinol 2007;15:5–9.
20. Visser JA, deJong FH, Laven JSE, Themmen APN. Anti-Mullerian hormone: a new marker for ovarian function. Reproduction 2006;1: 1–9.