Human Reproduction Vol.23, No.6 pp. 1359–1365, 2008 doi:10.1093/humrep/den108
Advance Access publication on April 2, 2008

Relevance of anti-Mullerian hormone measurement in a routine IVF program

C. Gnoth[1,2,5], A.N. Schuring[3], K. Friol[1], J. Tigges[1], P. Mallmann[2] and E. Godehardt[4]

[1]Center for Family Planning, Gynecological Endocrinology and Reproductive Medicine, Rheydter Strasse 143, 41515 Grevenbroich, Germany; [2]Department of Obstetrics and Gynecology, University of Cologne, Cologne, Germany; [3]Department of Obstetrics and Gynecology, University of Muenster, Muenster, Germany; [4]Biometric Research Group, Clinic for Thoracic and Cardiovascular Surgery, Heinrich-Heine University of Duesseldorf, Duesseldorf, Germany

[5]Correspondence address. Tel: +49-2181-491513; Fax: +49 2181-491534; E-mail: dr.christian.gnoth@rmz-nrw.de

BACKGROUND: Diminished ovarian reserve has become a major cause of infertility. Anti-Mullerian hormone (AMH) seems to be a promising candidate to assess ovarian reserve and predict the response to controlled ovarian hyperstimulation (COH). This prospective study was conducted to evaluate the relevance of AMH in a routine IVF program. METHODS: Three hundred and sixteen patients were prospectively enrolled to enter their first IVF/ICSI-cycle. Age, FSH-, inhibin B- and AMH-levels and their predictive values for ovarian response and clinical pregnancy rate were compared by discriminant analyses. RESULTS: A total of 132 oocyte retrievals were performed. A calculated cut-off level ≤1.26 ng/ml AMH alone detected poor responders (≤4 oocytes) with a sensitivity of 97%, and there was a 98% correct prediction of normal response in COH if levels were above this threshold. With levels <0.5 ng/ml, a correct prediction of very poor response (≤2 oocytes) was possible in 88% of cases. Levels of AMH ≥0.5 ng/ml were not significantly correlated with clinical pregnancy rates. CONCLUSIONS: AMH is a predictor of ovarian response and suitable for screening. Levels ≤1.26 ng/ml are highly predictive of reduced ovarian reserve and should be confirmed by a second line antral follicle count. Measurement of AMH supports clinical decisions, but alone it is not a suitable predictor of IVF success.

Introduction

Anti-Mullerian hormone (AMH) belongs to the transforming growth factor-β superfamily. In the female, it is a product of the granulosa cells from pre-antral and small antral follicles. Together with two other key players (growth and differentiation factor-9 and bone morphogenetic protein-15), it inhibits the initiation of premature follicle growth and decreases the sensitivity of follicles for the FSH-dependent selection process (Gruijters *et al*., 2003; Visser and Themmen, 2005; Knight and Glister, 2006; Visser *et al*., 2006). AMH levels are characterized by a steady decline with age from adulthood toward menopause reflecting the size of the ovarian follicle pool (van Rooij *et al*., 2005). Therefore, AMH seems to be a promising parameter for early detection of reduced ovarian reserve as well as ovarian dysfunction (Visser *et al*., 2006). The loss of follicles with increasing female age is variable and the chronological age of the ovary does not always reflect its biological and reproductive age (Fleming *et al*., 2006). Nowadays, an increasing number of female patients in their late 30s seek help for infertility problems, meaning that diminished ovarian reserve and age-related ovarian dysfunction have become a major cause of infertility. Furthermore, epidemiological surveys suggest (Nikolaou and Templeton, 2003) that approximately as many as 10% of women in their early 30s could be approaching their perimenopause transition. At a high risk for early ovarian exhaustion are those of our patients with a history of repeated ovarian surgery because of functional cysts and endometriosis. Numbers of such, mostly laparoscopic, interventions seem to increase continuously. Most women with reduced ovarian reserve are still displaying regular menstrual cycle characteristics that will continue normally with deterioration only becoming clinically apparent when ovarian function is already severely impaired (Burger *et al*., 2005). Thus ovarian aging and a reduced ovarian reserve can become a critical factor in infertility, without any obvious clinical symptoms. Furthermore, a substantial rise in FSH levels occurs relatively late when the perimenopausal transition is already present (Burger *et al*., 1999). On the other hand elevated FSH levels in younger women may be related to receptor polymorphisms and not reduced ovarian reserve (Schipper *et al*., 1998). Because traditional assessments of ovarian reserve such as early follicular phase FSH, inhibin B and the clomifen challenge test have low sensitivity in the early stages of reduced ovarian reserve (van Rooij *et al*., 2006), there is an urgent need for a reliable and early marker for the detection of a declining number of follicles, and prediction of

spontaneous pregnancy potential and assisted reproduction technology outcome (Muttukrishna *et al*., 2005; van Rooij *et al*., 2006; La Marca *et al*., 2007; Smeenk *et al*., 2007).

From the current literature, AMH is such a promising and reliable marker, corresponding to the number of small antral follicles (Pigny *et al*., 2006; Visser *et al*., 2006) with essentially constant levels across the cycle (La Marca *et al*., 2006) and a superior intercycle reproducibility compared with that of FSH and early antral follicle count (Fanchin *et al*., 2005; Feyereisen *et al*., 2006; Hehenkamp *et al*., 2006). Very recently some studies reported a possible benefit of serum AMH-measurement in assisted reproductive programs (Tremellen *et al*., 2005; Ebner *et al*., 2006; Ficicioglu *et al*., 2006; La Marca *et al*., 2007). However, urgently needed cut-off levels of AMH for supporting clinical decisions are still missing (Freour *et al*., 2006).

We therefore carried out a larger prospective trial calculating cut-off levels and evaluating the relevance of AMH-measurement for treatment strategies and outcome in a routine IVF program of a private institute.

Materials and Methods

Between 1 June 2005 and 30 June 2006, all women visiting our center of reproductive medicine for the first time, because of involuntary childlessness with at least 1 year of unprotected intercourse without pregnancy, underwent a basic infertility investigation. On Day 3–5 of the women's normal cycle, we performed a routine gynecological examination and a basic vaginal ultrasound took a basal hormone profile (FSH, LH, Prolactin, testosterone, DHEAS, TSH and SHBG) and measured AMH and inhibin B. Other variables such as socioeconomic status, medical history, parity, duration of involuntary childlessness, age and BMI were recorded in a standardized manner.

Out of all examined women, 316 patients were prospectively enrolled in our assisted-reproduction techniques (ART) program to enter their first IVF- or ICSI-cycle. The criteria for inclusion were:

(i) early follicular phase Day 3–5, FSH and AMH levels available;
(ii) regular menstrual cycle pattern (cycle length >25 and <35 days);
(iii) presence of both ovaries;
(iv) age <45.

Main indications for ART were: male subfertility (65%), tubal pathology (12%), endometriosis (12%), idiopathic infertility (9%) with at least two unsuccessful cycles of intrauterine insemination (IUI) and repeated polyfollicular development with gonadotrophin stimulation for IUI (2%). All included patients suffered from primary infertility with an average of 3.4 years of involuntary childlessness.

On Day 3–5 of a spontaneous cycle within 3 months following the start of controlled ovarian hyperstimulation (COH), patients underwent another transvaginal ultrasound examination to exclude ovarian cysts and to count the number of antral follicles, measuring 2–5 mm as described before by others (Scheffer *et al*., 1999, 2003).

This study was conducted in accordance with the principles of the declaration of Helsinki and women were asked for their consent for AMH-measurement and link to their treatment outcome.

For AMH measurement, we used a sensitive ELISA (Enzyme-Linked Immunosorbent Active MIS/AMH ELISA Kit®, DSL-10-14400, Diagnostic System Laboratories, Inc./Beckman-Coulter) according to the manufacture's instructions. This highly specific mono/mono two-site ELISA uses detection and capture antibodies, with results available within 3 h. The standards cover a range from 0.05 to 15 ng/ml (Freour *et al*., 2007). The sensitivity is 0.006 ng/ml. Intra- and inter-assay coefficients of variation were <5% and <8%, respectively. Inhibin B was measured using the DSL inhibin B ELISA test using two highly specific monoclonal antibodies (sensitivity 0.007 ng/ml with intra- and inter-assay coefficients of variation <6% and <8%, respectively, DSL-10-84100, Diagnostic System Laboratories, Inc./Beckman-Coulter).

In brief, the IVF/ICSI-treatment was as follows: all patients started with a monophasic oral contraceptive pill on Day 3–5 of their cycle. Starting of COH was scheduled according to the patient's possibilities and the center's necessities. Down-regulation with either nafarelin acetate (0.4–0.6 mg/day) or triptorelin acetate (0.05 mg/day) was started with the last 3–5 pills, 10–14 days before the start of COH. Ovarian hyperstimulation was performed with either recombinant follitrophin β (rec-FSH, Puregon®, Organon/Germany) or Menogon HP® (Ferring Pharmaceuticals, Kiel/Germany). Starting doses for patients under 35 years of age were 150 IE/ml; for patients 35 and older, it was 225–300 IE/ml. After 5 days of stimulation, follicular growth was assessed by vaginal ultrasound, estradiol measurement and control of pituitary desensitization was assessed by LH measurement. The dose of rec-FSH was adjusted according to ovarian response. Patients received ovulation induction with 250 μg recombinant human choriongonadotrophin (rec-HCG, Ovitrelle®, Serono/Germany) when at least three leading follicles were ∼20 mm in size. Transvaginal oocyte retrieval was performed 35 h later. Luteal support was provided with vaginal application of micronized progesterone (Utrogest®, 400 mg/day, Kade-Pharma/Germany). A maximum of two embryos were transferred in all women in their first attempt. Supernumerous zygotes were frozen according to the German embryo protection act.

The primary outcome measured in this study was the number of oocytes retrieved. According to a widespread definition, a cut-off of four or less oocytes at retrieval was regarded as poor or low ovarian response. With a mean fertilization rate of ∼60%, a minimum of four oocytes is necessary to have a possible transfer of at least two embryos. Approximately 30% of the ART cycles were conducted as IVF and 70% were conducted as IVF/ICSI because of male infertility. Clinical pregnancy was considered to be the secondary outcome measure defined as a gestational sac assessed by vaginal ultrasound 1–2 weeks after a positive pregnancy test.

Data were analyzed using the SAS package, version 9, of statistical procedures (SAS Institute Inc., Cary, USA). Student's *t*-test was used to compare endocrine profiles and basic characteristics of the patients. To compare the relevance of different parameters for predicting ovarian reserve and clinical pregnancy rate, we first performed a stepwise discriminant analysis, followed by linear discriminant analysis to calculate the sensitivities and specificities as well as the percentages of positive and negative predictions. For AMH alone, cut-off values and receiver operating characteristic (ROC) curves were calculated (Fawcett, 2004) to minimize both the false positive and false negative rates in order to find an optimal threshold for the discrimination between women with poor and normal response (>4 oocytes) and clinical pregnancy versus no pregnancy. The calculation of the ROC curves was based on the statistical package R. Statistical significance was considered to be reached at *P*-values of <0.05.

Results

In this prospective study, 316 patients were admitted to our IVF program with the aim of having their first IVF or IVF/ICSI attempt between 1 June 2005 and 30 June 2006. Out of these,

132 patients had their first oocyte retrieval in this time interval. There were no cycle cancelations because of impending ovarian hyperstimulation syndrome or no ovarian response. For 119 patients, pregnancy tests and assessments for clinical pregnancy (completed cycles) could be performed before 1 October 2006. From these, 56 clinical pregnancies were diagnosed corresponding to a clinical pregnancy rate per completed cycle of (56/119) 47%. Thirteen tests were performed after the 1st of October and not included in the further analysis. A flow chart of the 316 patients admitted and further details are presented in Fig. 1. The remaining 184 couples did not start their first cycle because of personal reasons (mainly: clarification of the coverage of treatment costs and/or occupational activity) and were treated later according to a changed protocol (see below). There was no statistical difference in the composition of these non-starters (length of infertility, age, cause of infertility and hormonal levels) and the 119 starters. No pregnancy test was missed in the 119 completed cycles. As expected, non-pregnant women and those with less than five oocytes were significantly older than pregnant women or normal responders.

The mean AMH level of all treated patients was 1.7 ± 2.9 ng/ml. To investigate the correlation of AMH with poor ovarian response, we used step-wise and linear discriminant analyses and calculated an AMH cut-off level for poor ovarian response of ≤ 1.26 ng/ml with minimized false positive and false negative results. Figure 2 shows the typical ROC for AMH indicating poor ovarian response and clinical pregnancy.

Table I gives the results for the stepwise discriminant analysis for poor responders (≤ 4 oocytes, cut-off level AMH ≤ 1.26 ng/ml). AMH alone showed the best performance by detecting 32 of true 33 poor responders (sensitivity). Age, FSH, inhibin B alone or in combinations were less precise. As to be expected, the specificity for identifying poor responders was improved by the use of a combination of parameters. In correctly predicting 'normal' ovarian response, AMH alone, using a calculated cut-off of AMH >1.26 ng/ml, was superior to the other parameters or their different combinations. Applying this model, only 2% of women with an AMH >1.26 ng/ml will have four or less oocytes in IVF following COH. Using a cut-off level of AMH ≤ 1.26 ng/ml, a correct prediction of 'poor' response was only possible in 36% of the cases mainly because of the age-related high heterogeneity between the poor and normal responder groups and probably dose adjustment during COH (see Discussion). To address this problem, both groups were adjusted for age in an additional analysis with a different statistical approach. In this way, the specificity for AMH alone increased to 62% and the correct prediction rate for poor response increased to 88%. Both sensitivity and prediction of normal response with AMH alone were much more robust and remained unchanged with or without adjustment for age (Table I). Using a level of AMH <0.5 ng/ml, a similarly robust and correct prediction of very poor response (≤ 2 oocytes retrieved) was possible in 88% of cases regardless of adjustment for age (Table I).

Table II gives the results of the stepwise discriminant analysis for age, FSH and AMH for 'not' achieving pregnancy. The cut-off level minimizing for false positive and false negative failures was calculated as ≤ 1.8 ng/ml (Fig. 2). Neither AMH alone nor one of the other analyzed parameters alone or their combinations were suitable for predicting the success of ART cycles.

Table III shows the results of the logistic regression analysis for low and normal ovarian response in ART cycles and for AMH with a cut-off level of 1.26 ng/ml. As expected, low responders were significantly older and had a significantly lower clinical pregnancy rate with a significantly lower AMH, than normal responders. In the poor responder group, FSH was elevated but still within the normal range and was weakly achieving statistical significance ($P = 0.034$) in discriminating between low and normal responders. The overall clinical pregnancy rate in the low-AMH group (AMH $\leq$ 1.26 ng/ml) was not significantly different from the group with levels above the cut-off.

Discussion

This prospective study was conducted to evaluate the relevance of routine AMH measurements before IVF. Therefore, 316 patients were prospectively enrolled to enter their first IVF or IVF/ICSI-cycle. Age, FSH-, inhibin B- and AMH-levels and their predictive values for ovarian response and clinical pregnancy rate were compared by discriminant analyses. A total of 132 oocyte retrievals were performed. Hundred and eighty-four couples did not start their first cycle in the scheduled observation period because of individual reasons and were, after a preliminary analysis of the data already collected, treated later following a changed protocol (see below), facilitating our clinical management. Although the number of patients of the study group was relatively small, the results seemed to be promising and, according to power calculations, would not be significantly changed with increasing size of the treatment group.

A significant correlation of AMH levels with ovarian response as expressed by the number of oocytes retrieved during the first IVF or IVF/ICSI treatment cycle has been observed. Cut-off levels for clinical decisions were calculated. The ovarian response to high levels of gonadotrophins reflects the size of the cohort of selectable antral follicles. Because the number of antral follicles is related to the size of the primordial follicle pool (Gougeon *et al*., 1994), the ovarian response to stimulation reflects the ovarian reserve (Lawson *et al*., 2003). Our results show that age and AMH levels are the superior parameters predicting ovarian response. However, even in women of comparable age, there was a wide variation in the individual ovarian reserve (te Velde and Pearson, 2002; Gnoth *et al*., 2003; van Rooij *et al*., 2005). For them, AMH is a very promising and possibly the best actual candidate to evaluate their individual ovarian response to gonadotrophin stimulation and to detect poor responders with levels of AMH ≤ 1.26 ng/ml (97% sensitivity). With an AMH >1.26 ng/ml, a correct prediction of normal response (>4 oocytes) to gonadotrophin stimulation of 98% can be achieved. Combinations of age and serum parameters FSH, inhibin B and AMH are less useful in predicting ovarian response in a subsequent

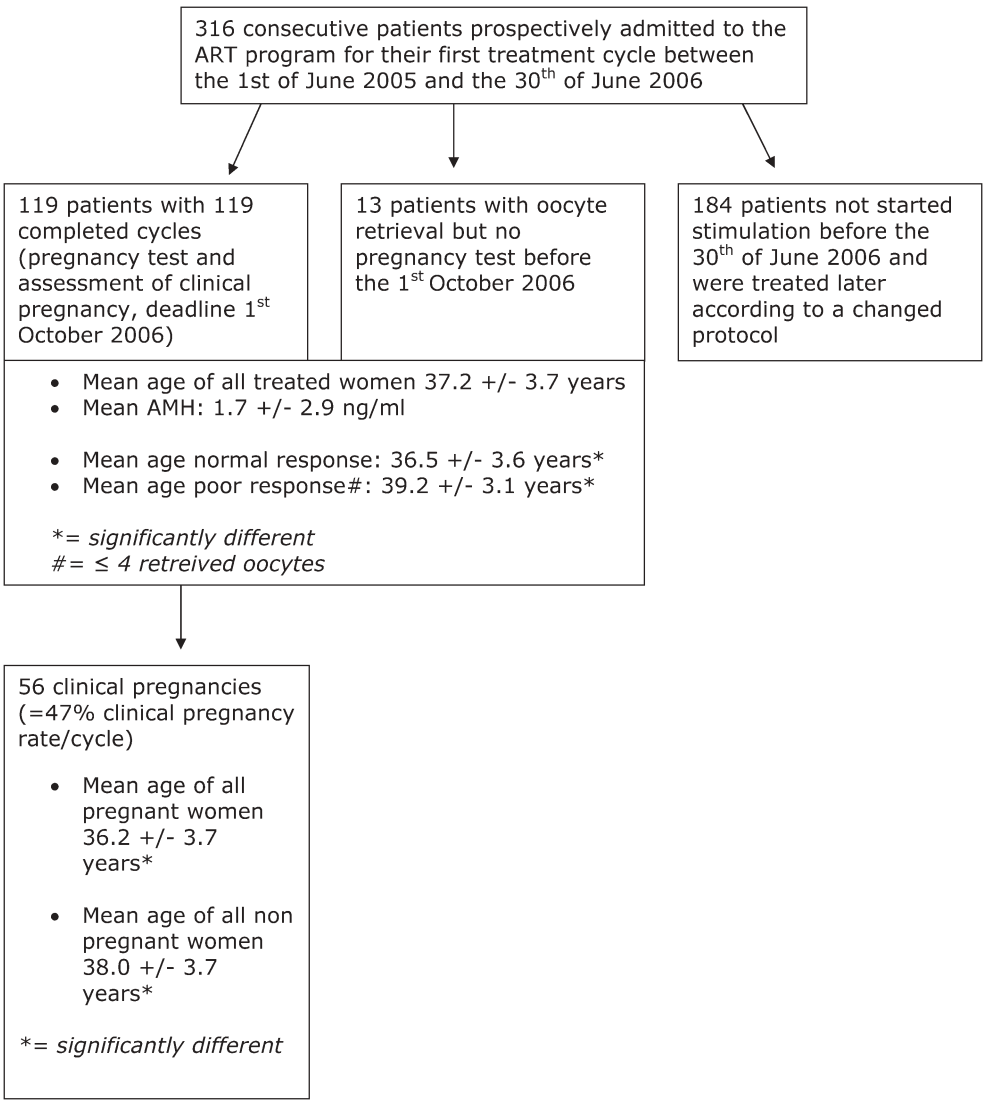

Figure 1: Flowchart of 316 ART patients with 132 oocyte retrievals and 119 completed cycles (means and standard deviations).

ART-cycle than is AMH alone. As a result of the low specificity for AMH alone, the age-related high heterogeneity between the normal and poor responder group, and probably an effect of dose adjustment during COH in this study, the correct overall prediction rate for poor response during COH only reaches 36%. If groups are of comparable age, specificity increases to 62% and a correct prediction of poor response will be possible in 88% of the COHs. Sensitivity and prediction of normal response with AMH alone are much more robust and remain unchanged even if there is a high age-related heterogeneity. Levels of AMH <0.5 ng/ml robustly predicted very poor response (≤ 2 oocytes retrieved) with impaired IVF success.

The antral follicle count is still regarded as a relatively good marker to predict poor ovarian response in ART programs providing better information than the patient's age alone or several endocrine markers (Scheffer *et al.*, 2003). However, in our routine clinical setting, we found a high inter-observer variability of antral follicle count due to the duration of the vaginal ultrasound examination, observers experience and, notably, expected ovarian reserve due to patient's age. We therefore excluded the 'first line antral follicle count' from further analysis and focused on objectively assessed endocrine markers avoiding observer related variability. Although we found a strong correlation of AMH levels and subsequent ovarian response in ART-cycles by using the cut-off level of AMH ≤ 1.26 ng/ml, we only have a correct prediction rate of 36%. By using groups of comparable age, the specificity and 'correct prediction rate of poor response' will increase significantly, although sensitivity and 'correct prediction rate of normal response' are much more robust and will remain unchanged whether or not a high age-related heterogeneity is given. Therefore, AMH measurement and a subsequent antral follicle count should be combined in unselected groups of patients, typical for a clinical setting, to minimize false positive results, as has also been proposed by others previously (Muttukrishna *et al.*, 2005; Tremellen *et al.*, 2005). But the antral follicle count should be performed accurately as a confirmatory test only in the second line and not as a screening method.

According to the current literature, existing assessments of ovarian reserve have limited predictive properties especially for the occurrence of pregnancy (Broekmans *et al.*, 2006). Newest investigations see AMH as a superior predictor of

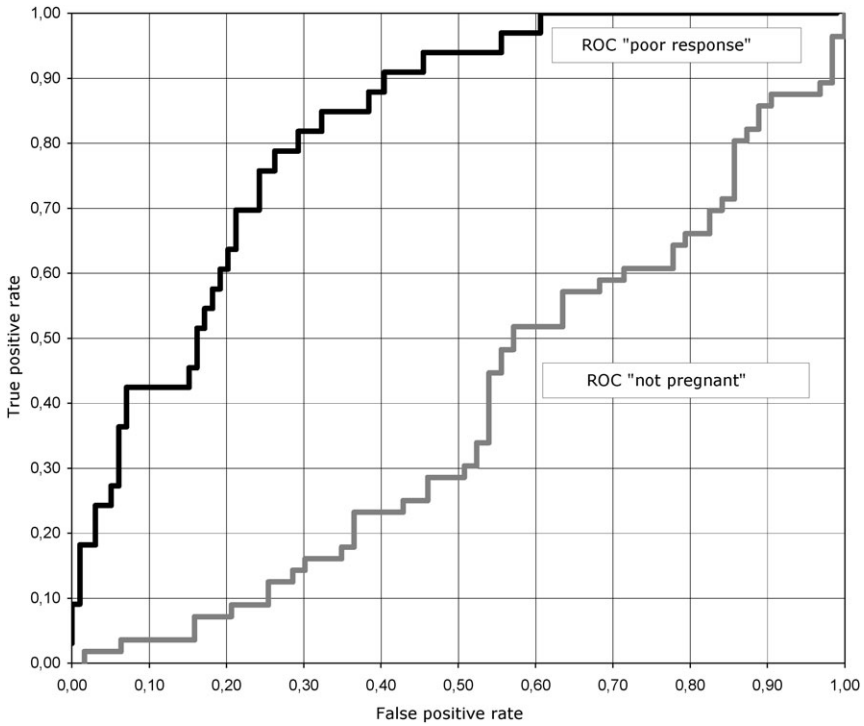

Figure 2: Receiver operating characteristic (ROC)-curves for AMH as an indicator for poor ovarian response and clinical pregnancy.

Table I. Detection and prediction of poor responders ($n = 33$ with $\leq$4 oocytes, calculated cut-off AMH $\leq$1.26 ng/ml) in 132 oocyte retrievals.

Observations	Age, FSH, inhibin B, AMH (n = 60)	Age, FSH, AMH (n = 120)	Age, AMH (n = 132)	AMH (n = 132)
Sensitivity (true positive rate)	0.79	0.69	0.76	0.97*
Specifity (true negative rate)	0.83	0.66	0.65	0.41*
Correct prediction 'poor response'	0.58	0.42	0.42	0.36*
Correct prediction 'normal response'	0.93	0.85	0.88	0.98*

Stepwise discriminant analysis of age, FSH, inhibin B and AMH.
*If, in a further analysis with a calculated cut-off of AMH $\leq$ 1.26 ng/ml, the poor and normal responder groups are adjusted for age, specificity increases to 62% and the correct prediction rate 'poor response' to 88%. Sensitivity and correct prediction rate of 'normal response' remain unchanged. If levels of AMH are $<$0.5, there is 88% correct prediction of $\leq$2 oocytes whether or not groups are adjusted for age.

IVF success (live birth rate) (Nelson *et al*., 2007) which cannot be supported by our findings. In our study, AMH and the other examined markers were not predictive for the success of the subsequent IVF treatment, confirming the results of others (van Rooij *et al*., 2006). However, a case wise analysis of all IVF treatments in this study revealed that in all cases with an AMH $\leq$1.26 ng/ml, a dose adjustment after 5 days of gonadotrophin stimulation was necessary. In this way, we could obviously increase the number of retrieved oocytes and improve treatment outcome. This bias may also explain the low correct prediction rates for poor response in the 'AMH only' analysis (Table I).

Recent studies have shown that a low response to gonadotrophin stimulation is associated with early menopause (Nikolaou *et al*., 2002; Lawson *et al*., 2003). AMH also seems to be a promising candidate for the occurrence of menopausal transition (van Rooij *et al*., 2004). Our results show that AMH levels $\leq$1.26 ng/ml are strongly indicative of significantly diminished ovarian reserve and AMH levels $<$0.5 ng/ml robustly and correctly predict a very low response ($\leq$2 oocytes) in 88% of cases, supporting the value of AMH as an early warning test for exhausted ovarian function when FSH levels are still within the normal range (Feyereisen *et al*., 2006). The low correlation of inhibin B with poor ovarian response as well as with the success of a subsequent IVF treatment may be explained by the fact that inhibin B not only represents the number of antral follicles, but is closely related to functional changes of the follicles during the cycle and with advancing age (Knight and Glister, 2006).

Concluding from our results, we now use AMH as a reliable pre-warning and screening marker for reduced ovarian reserve in women as previously suggested by others (van Rooij *et al*., 2005). In consideration of our results, we generally adapted our treatment strategies for all patients from 30 June 2006 onwards. Regardless of age, women with an AMH $>$1.26 ng/ml receive a starting dose of 150 mU/ml recombinant FSH or HMG. Patients with an AMH $<$0.5 ng/ml are started with 375 mU/ml recombinant FSH or HMG and counseled about their impaired prognosis, independently of age. In cases of AMH levels $\geq$0.5 ng/ml and $\leq$1.26 ng/ml, the start dosage is fixed

Table II. Prediction of 'no pregnancy' in 119 complete ART cycles (cut-off: AMH $\leq$ 1.8 ng/ml, $n = 63$ unsuccessful cycles).

Observations	Age, FSH, inhibin B, AMH ($n = 58$)	Age, FSH ($n = 108$)	Age, AMH ($n = 119$)	AMH ($n = 119$)
Sensitivity (true positive rate)	0.50	0.52	0.60	0.83
Specifity (true negative rate)	0.54	0.60	0.64	0.34
Correct prediction 'not pregnant'	0.60	0.60	0.60	0.58
Correct prediction 'pregnant'	0.43	0.52	0.56	0.63

Stepwise discriminant analysis of age, FSH, inhibin B and AMH.

Table III. Logistic regression analysis for low and normal ovarian response in ART cycles and for AMH using 1.26 ng/ml as a cut-off level.

Observations	Groups					
	low responder ($n = 33$)	normal responder ($n = 99$)	Significance low versus normal	AMH $\leq$ 1.26 ng/ml ($n = 91$)	AMH>1.26 ng/ml (n=41)	Significance AMH $\leq$ 1.26 versus AMH > 1.26
Ages	39.2 $\pm$ 3.1	36.5 $\pm$ 3.6	$P < 0.05$	38.0 $\pm$ 3.0	36.0 $\pm$ 4.0	$P < 0.05$
Clin. Preg. rate	12% (n = 24)	42% (n = 94)	$P < 0.05$	35% (n = 79)	41% (n = 39	ns
AHM	0.32 $\pm$ 0.33	2.20 $\pm$ 3.1	$P < 0.05$			
FSH	9.94 $\pm$ 6.39	7.34 $\pm$ 3.27	$P < 0.05$	8.67 $\pm$ 4.9	6.36 $\pm$ 2.0	$P < 0.05$
Retrieved oocytes	2.0 $\pm$ 1.0	10.0 $\pm$ 5.91	$P < 0.05$	6.0 $\pm$ 4.0	14.1 $\pm$ 6.7	$P < 0.05$

Values are given in mean $\pm$ standard deviations.

to 300 mU/ml recombinant FSH or HMG if an antral follicle count showed <5 follicles/ovary (Kline *et al*., 2005). Women with an AMH $\leq$0.1 ng/ml are treated only in exceptional cases (trial stimulation) and seriously informed about their bad prognosis (Meduri *et al*., 2007) and the probably costineffective stimulation. Women with an AMH >6 ng/ml are at risk of polyfollicular development and ovarian hyperstimulation (Pigny *et al*., 2003, 2006) and are therefore started prudently with 100 mU/ml recombinant FSH. Future studies will be analyzed to see if a further decrease in cycle cancellation rate because of impending hyperstimulation or insufficient ovarian response to gonadotrophin stimulation is possible although in the latter case, past studies have shown disappointing results (Klinkert *et al*., 2005).

In summary, our results confirm the relevance of AMH measurement as an important screening test for reduced ovarian reserve in women. Using AMH alone with a cut-off level $\leq$1.26 ng/ml, we will identify 97% of all women with a reduced ovarian reserve and correctly predict low response to gonadotrophin stimulation in 88% of cases in groups of comparable age. Furthermore, a level of AMH <0.5 ng/ml correctly predicts very poor response with $\leq$2 oocytes in 88% of cases and poor outcome even in groups of high age-related heterogeneity. If AMH levels are $\geq$0.5 ng/ml and $\leq$1.26 ng/ml, an antral follicle count should be added to exclude false positive results and increase specificity especially in a routine clinical setting with heterogeneous groups of patients.

Chronological age fairly well predicts ovarian response, upcoming perimenopausal and menopausal transition. Important studies have shown that AMH is a predictor for the occurrence of perimenopausal transition (cycle length irregularity) within 3–5 years (de Vet *et al*., 2002; van Rooij *et al*., 2004) at levels <0.92 ng/ml. Our results show that by early detection of women with a reduced ovarian response, using a cut-off level $\leq$1.26 ng/ml, treatment options are still available (high-dose stimulation in IVF, polyovulation) with favorable pregnancy rates, not statistically different from those women with 'normal' ovarian reserve. Therefore, females should be advised to get AMH levels determined if they do not achieve pregnancy after six cycles of regular intercourse (Gnoth *et al*., 2005). AMH is suitable as a screening test and may replace FSH which gains relevance only in the late reproductive phase.

Acknowledgements

The authors wish to thank Mrs Silvia Heil for recording and preparing the row data and Mrs Andreja Daniels, Mrs Claudia Hammelstein and Mrs Claudia Monissen for all her efforts performing the laboratory essays.

Conflict of interest: The first author was supported by small grant for statistical analysis (software adaptation) by Diagnostic System Laboratories, Inc./Beckman-Coulter, Germany.

Funding

Small grant for statistical analysis (software adaption) by Diagnostic System Laboratories, Inc./Beckman-Coulter, Germany.

References

Broekmans FJ, Kwee J, Hendriks DJ, Mol BW, Lambalk CB. A systematic review of tests predicting ovarian reserve and IVF outcome. *Hum Reprod Update* 2006;**12**:685–718.

Burger HG, Dudley EC, Hopper JL, Groome N, Guthrie JR, Green A, Dennerstein L. Prospectively measured levels of serum follicle-stimulating hormone, estradiol, and the dimeric inhibins during the menopausal transition in a population-based cohort of women. *J Clin Endocrinol Metab* 1999;**84**:4025–4030.

Burger HG, Robertson DM, Baksheev L, Collins A, Csemiczky G, Landgren BM. The relationship between the endocrine characteristics and the regularity of menstrual cycles in the approach to menopause. *Menopause* 2005;**12**:267–274.

de Vet A, Laven JS, de Jong FH, Themmen AP, Fauser BC. Antimullerian hormone serum levels: a putative marker for ovarian aging. *Fertil Steril* 2002;**77**:357–362.

Ebner T, Sommergruber M, Moser M, Shebl O, Schreier-Lechner E, Tews G. Basal level of anti-Mullerian hormone is associated with oocyte quality in stimulated cycles. *Hum Reprod* 2006;**21**:2022–2026.

Fanchin R, Taieb J, Lozano DH, Ducot B, Frydman R, Bouyer J. High reproducibility of serum anti-Mullerian hormone measurements suggests a multi-staged follicular secretion and strengthens its role in the assessment of ovarian follicular status. *Hum Reprod* 2005;**20**:923–927.

Fawcett T. *ROC Graphs: Notes and Practical Considerations for Researchers*, 2004. http://home.comcast.net/~tom.fawcett/public_html/papers/ROC101.pdf.

Feyereisen E, Mendez Lozano DH, Taieb J, Hesters L, Frydman R, Fanchin R. Anti-Mullerian hormone: clinical insights into a promising biomarker of ovarian follicular status. *Reprod Biomed Online* 2006;**12**:695–703.

Ficicioglu C, Kutlu T, Baglam E, Bakacak Z. Early follicular antimullerian hormone as an indicator of ovarian reserve. *Fertil Steril* 2006;**85**:592–596.

Fleming R, Deshpande N, Traynor I, Yates RW. Dynamics of FSH-induced follicular growth in subfertile women: relationship with age, insulin resistance, oocyte yield and anti-Mullerian hormone. *Hum Reprod* 2006;**21**:1436–1441.

Freour T, Mirallie S, Colombel A, Bach-Ngohou K, Masson D, Barriere P. Anti-mullerian hormone: clinical relevance in assisted reproductive therapy. *Ann Endocrinol (Paris)* 2006;**67**:567–574.

Freour T, Mirallie S, Bach-Ngohou K, Denis M, Barriere P, Masson D. Measurement of serum anti-Mullerian hormone by Beckman Coulter ELISA and DSL ELISA: comparison and relevance in assisted reproduction technology (ART). *Clin Chim Acta* 2007;**375**:162–164.

Gnoth C, Frank-Herrmann P, Freundl G, Godehardt D, Godehardt E. Time to pregnancy: results of the German prospective study and impact on the management of infertility. *Hum Reprod* 2003;**18**:1959–1966.

Gnoth C, Godehardt E, Frank-Herrmann P, Friol K, Tigges J, Freundl G. Definition and prevalence of subfertility and infertility. *Hum Reprod* 2005;**20**:1144–1147.

Gougeon A, Ecochard R, Thalabard JC. Age-related changes of the population of human ovarian follicles: increase in the disappearance rate of non-growing and early-growing follicles in aging women. *Biol Reprod* 1994;**50**:653–663.

Gruijters MJ, Visser JA, Durlinger AL, Themmen AP. Anti-Mullerian hormone and its role in ovarian function. *Mol Cell Endocrinol* 2003;**211**:85–90.

Hehenkamp WJ, Looman CW, Themmen AP, de Jong FH, te Velde ER, Broekmans FJ. Anti-Mullerian hormone levels in the spontaneous menstrual cycle do not show substantial fluctuation. *J Clin Endocrinol Metab* 2006;**91**:4057–4063.

Kline J, Kinney A, Kelly A, Reuss ML, Levin B. Predictors of antral follicle count during the reproductive years. *Hum Reprod* 2005;**20**:2179–2189.

Klinkert ER, Broekmans FJ, Looman CW, Habbema JD, te Velde ER. Expected poor responders on the basis of an antral follicle count do not benefit from a higher starting dose of gonadotrophins in IVF treatment: a randomized controlled trial. *Hum Reprod* 2005;**20**:611–615.

Knight PG, Glister C. TGF-beta superfamily members and ovarian follicle development. *Reproduction* 2006;**132**:191–206.

La Marca A, Stabile G, Artenisio AC, Volpe A. Serum anti-Mullerian hormone throughout the human menstrual cycle. *Hum Reprod* 2006;**21**:3103–3107.

La Marca A, Giulini S, Tirelli A, Bertucci E, Marsella T, Xella S, Volpe A. Anti-Mullerian hormone measurement on any day of the menstrual cycle strongly predicts ovarian response in assisted reproductive technology. *Hum Reprod* 2007;**22**:766–771.

Lawson R, El Toukhy T, Kassab A, Taylor A, Braude P, Parsons J, Seed P. Poor response to ovulation induction is a stronger predictor of early menopause than elevated basal FSH: a life table analysis. *Hum Reprod* 2003;**18**:527–533.

Meduri G, Massin N, Guibourdenche J, Bachelot A, Fiori O, Kuttenn F, Misrahi M, Touraine P. Serum anti-Mullerian hormone expression in women with premature ovarian failure. *Hum Reprod* 2007;**22**:117–123.

Muttukrishna S, McGarrigle H, Wakim R, Khadum I, Ranieri DM, Serhal P. Antral follicle count, anti-Mullerian hormone and inhibin B: predictors of ovarian response in assisted reproductive technology? *BJOG* 2005;**112**:1384–1390.

Nelson SM, Yates RW, Fleming R. Serum anti-Mullerian hormone and FSH: prediction of live birth and extremes of response in stimulated cycles implications for individualization of therapy. *Hum Reprod* 2007;**22**:2414–2421.

Nikolaou D, Lavery S, Turner C, Margara R, Trew G. Is there a link between an extremely poor response to ovarian hyperstimulation and early ovarian failure? *Hum Reprod* 2002;**17**:1106–1111.

Nikolaou D, Templeton A. Early ovarian ageing: a hypothesis: detection and clinical relevance. *Hum Reprod* 2003;**18**:1137–1139.

Pigny P, Merlen E, Robert Y, Cortet-Rudelli C, Decanter C, Jonard S, Dewailly D. Elevated serum level of anti-mullerian hormone in patients with polycystic ovary syndrome: relationship to the ovarian follicle excess and to the follicular arrest. *J Clin Endocrinol Metab* 2003;**88**:5957–5962.

Pigny P, Jonard S, Robert Y, Dewailly D. Serum anti-Mullerian hormone as a surrogate for antral follicle count for definition of the polycystic ovary syndrome. *J Clin Endocrinol Metab* 2006;**91**:941–945.

Scheffer GJ, Broekmans FJ, Dorland M, Habbema JD, Looman CW, te Velde ER. Antral follicle counts by transvaginal ultrasonography are related to age in women with proven natural fertility. *Fertil Steril* 1999;**72**:845–851.

Scheffer GJ, Broekmans FJM, Looman CWN, Blankenstein M, Fauser BCJM, de Jong FH, te Velde ER. The number of antral follicles in normal women with proven fertility is the best reflection of reproductive age. *Hum Reprod* 2003;**18**:700–706.

Schipper I, de Jong FH, Fauser BC. Lack of correlation between maximum early follicular phase serum follicle stimulating hormone concentrations and menstrual cycle characteristics in women under the age of 35 years. *Hum Reprod* 1998;**13**:1442–1448.

Smeenk JM, Sweep FC, Zielhuis GA, Kremer JA, Thomas CM, Braat DD. Antimullerian hormone predicts ovarian responsiveness, but not embryo quality or pregnancy, after in vitro fertilization or intracyoplasmic sperm injection. *Fertil Steril* 2007;**87**:223–226.

te Velde ER, Pearson PL. The variability of female reproductive ageing. *Hum Reprod Update* 2002;**8**:141–154.

Tremellen KP, Kolo M, Gilmore A, Lekamge DN. Anti-mullerian hormone as a marker of ovarian reserve. *Aust N Z J Obstet Gynaecol* 2005;**45**:20–24.

van Rooij IA, Tonkelaar I, Broekmans FJ, Looman CW, Scheffer GJ, de Jong FH, Themmen AP, te Velde ER. Anti-mullerian hormone is a promising predictor for the occurrence of the menopausal transition. *Menopause* 2004;**11**:601–606.

van Rooij IA, Broekmans FJ, Scheffer GJ, Looman CW, Habbema JD, de Jong FH, Fauser BJ, Themmen AP, te Velde ER. Serum antimullerian hormone levels best reflect the reproductive decline with age in normal women with proven fertility: a longitudinal study. *Fertil Steril* 2005;**83**:979–987.

van Rooij IA, Broekmans FJ, Hunault CC, Scheffer GJ, Eijkemans MJ, de Jong FH, Themmen AP, te Velde ER. Use of ovarian reserve tests for the prediction of ongoing pregnancy in couples with unexplained or mild male infertility. *Reprod Biomed Online* 2006;**12**:182–190.

Visser JA, Themmen AP. Anti-Mullerian hormone and folliculogenesis. *Mol Cell Endocrinol* 2005;**234**:81–86.

Visser JA, de Jong FH, Laven JS, Themmen AP. Anti-Mullerian hormone: a new marker for ovarian function. *Reproduction* 2006;**131**:1–9.

Submitted on August 21, 2007; resubmitted on March 3, 2008; accepted on March 12, 2008