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Anti-Müllerian hormone measurement on any day of the menstrual cycle strongly predicts ovarian response in assisted reproductive technology

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BACKGROUND: Recently, a new marker, the anti-Müllerian hormone (AMH), has been evaluated as a marker of ovarian response. Serum AMH levels have been measured at frequent time-points during the menstrual cycle, suggesting the complete absence of fluctuation. The aim of this study was to evaluate whether serum AMH measurement on any day of the menstrual cycle could predict ovarian response in women undergoing assisted reproductive technology (ART). METHODS: This study included 48 women attending the IVF/ICSI programme. Blood withdrawal for AMH measurement was performed in all the patients independently of the day of the menstrual cycle. RESULTS: Women in the lowest AMH quartile (<0.4 ng/ml) were older and required a higher dose of recombinant FSH than women in the highest quartile (>7 ng/ml). All the cancelled cycles due to absent response were in the group of the lowest AMH quartile, whereas the cancelled cycles due to risk of ovarian hyperstimulation syndrome (OHSS) were in the group of the highest AMH quartile. This study demonstrated a strong correlation between serum AMH levels and ovarian response to gonadotrophin stimulation. CONCLUSION: For the first time, clinicians may have a reliable serum marker of ovarian response that can be measured independently of the day of the menstrual cycle.

Key words: AMH/IVF or ICSI/ovarian stimulation/poor response/OHSS

Introduction

Optimal evaluation of women and proper treatment are essential for successful outcome of assisted reproductive technology (ART). To obtain good results, it is necessary to assess ovarian reserve before planning treatment. The identification of both low and high responders before treatment may decrease cycle cancellation rate and side-effects such as ovarian hyperstimulation syndrome (OHSS).

Age, day 3 FSH, inhibin B, antral follicle count, ovarian volume and several dynamic tests have been demonstrated to be correlated with ovarian response in ART. However, their predictive value remains somewhat controversial and disappointing (Navot *et al.*, 1987; Muasher *et al.*, 1988; Fanchin *et al.*, 1994; Faddy and Gosden, 1996; Lass *et al.*, 1997; Tomas *et al.*, 1997; Hall *et al.*, 1999; Ravhon *et al.*, 2000; Bancsi *et al.*, 2002).

Recently, a new endocrine marker, anti-Müllerian hormone (AMH), has been evaluated by several groups as a marker of ovarian response (Seifer *et al.*, 2002; Van Rooij *et al.*, 2002; Fanchin *et al.*, 2003; Hazout *et al.*, 2004; Muttukrishna *et al.*, 2004; Eldar-Geva *et al.*, 2005; Penarrubia *et al.*, 2005; Tremellen *et al.*, 2005; Ficicioglu *et al.*, 2006).

AMH is a dimeric glycoprotein belonging to the transforming growth factor-beta (TGF-β) superfamily, which acts on tissue growth and differentiation (Cate *et al.*, 1986; Josso, 1990; Josso *et al.*, 2001).

AMH is expressed in granulosa cells from pre-antral and small antral follicles (Durlinger *et al.*, 2002) and continues to be expressed in the growing follicles in the ovary until they have reached the size and differentiation state at which they are to be selected for dominance. In the human, this occurs at the antral stage when the follicle size is 4–6 mm (Rajpert-De Meyts *et al.*, 1999; Weenen *et al.*, 2004).

AMH is generally considered as a negative regulator of the early stages of follicular development. Homozygous AMH-knockout female mice have more growing pre-antral and small antral follicles than wild-type mice (Durlinger *et al.*, 2002). It has been showed that their stock of primordial follicles is depleted earlier in life.

Serum AMH levels from women are lower than those in men throughout life. In women, serum AMH levels can be almost undetectable at birth (Rajpert-De Meyts *et al.*, 1999) with a subtle increase within the first 2 or 4 years of age; after that, AMH seems to be stable until adulthood but found to decreases as a sign of follicular reserve exhaustion (Lee *et al.*, 1996; La Marca *et al.*, 2005a).

Serum AMH levels have been recently measured at frequent time-points during the menstrual cycle, with the results

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suggesting the complete absence of fluctuation (La Marca *et al.*, 2006; Hehenkamp *et al.*, 2006). The absence of variations in AMH serum levels may be consistent with the continuous non-cyclic growth of small follicles throughout the cycle (La Marca *et al*., 2006a).

With respect to other known markers of ovarian reserve (FSH, inhibin B and antral follicle count (AFC)), AMH seems to better reflect the continuous decline of the follicle pool with age (van Rooij *et al.*, 2005). The decrease in AMH with advancing age may be present before changes in currently known ageing-related variables, indicating that AMH in serum levels may be the best marker for ovarian ageing (Van Rooij *et al.*, 2004).

As AMH is produced by the growing antral follicles (4–6 mm) in the human ovary up to the selection stage (Weenen *et al.*, 2004), it may serve as a serum marker of ovarian reserve for women undergoing IVF.

Accordingly, serum AMH levels have been shown to be significantly lower in poor responders than in normal responders (Seifer *et al.*, 2002; Van Rooij *et al.*, 2002). Moreover, serum AMH levels are highly correlated with the number of antral follicles before treatment and the number of oocytes retrieved upon ovarian stimulation (Van Rooij *et al.*, 2002). Interestingly, logistic regression analysis for the prediction of poor response has shown that serum AMH levels have a better prediction value than FSH or inhibin B.

A relevant characteristic of AMH is the complete absence of fluctuation throughout the menstrual cycle (La Marca *et al.*, 2006a; Hehenkamp *et al.*, 2006). The stability of AMH levels supports the concept that AMH could be easily used as a marker for ovarian response to controlled ovarian stimulation independently of the day of the cycle in which the blood sample is obtained.

The aim of this study was to evaluate whether serum AMH measurement on any day of the menstrual cycle could predict ovarian response in women undergoing ART.

Materials and methods

This study included 48 women attending the IVF/ICSI programme of the Mother–Infant Department of University Hospital Modena for the first time from March 2005 to January 2006. Each patient gave informed consent authorizing the examination, and the Institutional Review Board (IRB) approval was obtained.

The criteria for inclusion were as follows: (i) age 18–43 years, (ii) first IVF or ICSI attempt, (iii) no evidence of endocrinological disorders, (iv) no evidence of polycystic ovary syndrome (PCOS) and (v) regular menstrual cycles. All the patients were managed based on accepted principles of infertility practice.

The blood sample for AMH determination was taken on the day on which it was decided to introduce the couple to the IVF/ICSI procedure, independently of the last menstrual cycle. The IVF/ICSI procedure was performed in the next month or two after the blood sampling.

The blood sample was obtained by venipuncture at approximately 9:00–12:00 a.m. The samples were obtained from 28 women during follicular phase and from 20 women during luteal phase. Only seven patients were in days 2–5 when the blood samples were obtained. The blood was centrifuged at 3500 cycles/min for 10 min, and the serum was stored in 1.5-ml polypropylene tubes at −80°C.

Serum AMH was measured by enzyme-linked immunosorbent assay (ELISA) using the AMH/MIS ELISA kit (Immunotech-Beckman, Marseilles, France). The detection limit of the assay was 0.1 ng/ml (0.7 pmol/l); intra- and interassay coefficients of variation were 5.3 and 8.7%, respectively, for a serum AMH concentration of 5 ng/ml and 4.9 and 7.8% for a serum AMH concentration of 157 ng/ml. No cross-reaction was observed with TGF-β.

Long-protocol GnRH analogue was used for ovarian suppression. Treatment with recombinant FSH 150–300 IU/day was started when down-regulation was reached. The starting dose was chosen on the basis of age and body mass index (BMI). Step-up or step-down protocols were decided according to subsequent ultrasound controls. When three or more follicles reached >18 mm, 10 000 IU of hCG was administrated i.m., and 36 h later, follicles were aspirated under patient sedation. Insemination was performed by standard IVF or ICSI. According to the new Italian law regulating ART, only three oocytes were fertilized at one time. Light microscopic evaluation established fertilization 14–18 h later. We performed cleavage-stage embryo transfers on day 2 or 3. At transfer, we used ultrasound to guide embryo placement to the mid-uterine cavity. A serum hCG pregnancy test was performed 14 days after retrieval and repeated 2 days later if positive. Ongoing pregnancy was established by at least one ultrasonographically confirmed viable fetus within the uterus 6 weeks after embryo transfer.

The main outcome measures were the number of retrieved oocytes and the cycle cancellation rate. Poor ovarian response was defined as a fewer than four oocytes or cancellation due to impaired or absent follicular growth in response to ovarian stimulation. A normal ovarian response was defined as a collection of four to eight oocytes. A good ovarian response was defined as a collection of 9–16 oocytes. Patients were considered high responders when >16 oocytes were collected at ovum retrieval or when the cycle was cancelled because of exaggerated response.

The implantation rates (IRs) and pregnancy rates (PRs) have been reported but were not considered as outcome measures because of the absence of homogeneity in the couples included in the study. Indeed, in this study, couples with tubal factor, male factor and idiopathic infertility were included. Fertilization rate has not been reported, because according to the new Italian law regulating ART, no more than three oocytes can be fertilized at one time. Hence, the fertilization rate calculated in this study is not informative.

Ongoing clinical PR was calculated as the number of viable fetuses detected at a 6-week post-retrieval ultrasound divided by the number of embryo transfers performed. IR was calculated as the number of sacs on a 4-week post-retrieval ultrasound divided by the number of embryos transferred.

Values are presented as mean ± SD. To compare poor responders with normal, good and high responders, we performed the Mann–Whitney or χ^2 test whenever appropriate. The correlation between different parameters was expressed as a Spearman's correlation coefficient.

The follicular sensitivity was calculated as the ratio between units of FSH administered and the number of follicles >16 mm on the day of hCG. Statistical analysis was performed with the Statsoft software. $P < 0.05$ was considered significant.

Results

Of the 48 patients included in the study, six patients did not undergo oocyte retrieval. Four cycles were cancelled because of absent ovarian response. Two cycles were cancelled because of hyper-response. However, all these cancelled cycles were considered for statistical analysis, as the main outcome measure was the ovarian response to ovarian stimulation.

The causes of infertility were male factor (26 couples), tubal factor (9 couples) and idiopathic (13 couples).

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The age of the female patients ranged between 25 and 43 years. The infertility period was between 16 and 80 months. The mean BMI of women was in the normal range.

Hormonal and IVF/ICSI cycle characteristics of the patients divided into poor, normal, good and high responders are summarized in the Table I. The poor responders were somewhat older than high responders. Serum AMH levels were significantly lower and higher in poor and high responders, respectively, than in normal and good responders.

The mean duration of ovarian stimulation was similar in all the groups, whereas the poor responders had a mean total dose of 4243 ± 1018 IU of FSH, which was significantly higher than the other groups. The number of follicles >16 mm on the day of hCG was significantly lower in poor responders than in normal, good and high responders. Not surprisingly, this figure was significantly higher in the high responders than in the other groups.

The number of retrieved oocytes in the different groups is also summarized in Table I. Although two patients in the high-responder group had their cycle cancelled because of the risk of OHSS, no ovum retrieval took place in four poor responders because of insufficient follicular growth.

The mean number of oocytes was significantly lower and higher in poor and high responders, respectively, than in the other groups. No significant differences were observed in the percentage of mature oocytes retrieved in the different groups.

Follicle sensitivity (indicating the ratio between the number of gonadotrophin units and the number of follicles on the day of hCG) was significantly higher in poor-responder patients than in normal and good responders. High responders exhibited significant lower follicle sensitivity than the other groups.

IRs and PRs were significantly higher in hyper-responders than in poor responders (16 versus 6% and 40 versus 12.5%, respectively, $P < 0.05$).

Patients were further subdivided into four groups using the 25th, 50th and 75th percentiles of AMH values (Table II).

Consequently, the four new groups of patients (with very low, low, high and very high AMH) were each composed of 12 patients. Table II summarizes their IVF/ICSI cycle characteristics.

Women in the lowest AMH quartile (<0.4 ng/ml) were older and required higher dose of recombinant FSH than women in the highest quartile (>7 ng/ml).

The number of follicles on the day of hCG was significantly lower in patients in the lowest quartile than the other groups. This figure was significantly increased in patients in the highest quartile.

The follicle sensitivity was significantly higher in patients with the lowest serum AMH levels than the remaining groups. Women in the highest AMH quartile exhibited a significantly lower follicle sensitivity than the other groups.

The four cancelled cycles due to absent response were in the group of the lowest AMH quartile, whereas the two cycles cancelled due to the risk of OHSS were in the group of the highest AMH quartile.

IRs and PRs were significantly higher in the group with the highest serum AMH levels than in the group with the lowest levels (14.3 versus 6.5% and 30 versus 12.5%, respectively; $P < 0.05$).

As summarized in Table III, AMH significantly correlated with age ($r = –0.44$, $P = 0.02$), total FSH dose ($r = –0.52$, $P = 0.01$), number of follicles >16 mm on the day of hCG ($r = 0.76$, $P = 0.001$), follicle sensitivity ($r = 0.72$, $p = 0.001$) and the number of oocytes retrieved ($r = 0.73$, $P = 0.0001$). No significant correlations were found between AMH and the duration of FSH stimulation.

Serum AMH levels with a threshold of 0.75 ng/ml had a sensitivity of 80% and specificity of 93% in predicting poor

Table I. Hormonal and IVF/ICSI cycle characteristics of the patients

	A (<4 oocytes, poor responders)	B (4–8 oocytes, normal responders)	C (9–16 oocytes, good responders)	D (>16 oocytes, hyper-responders)	*P*
N	12	16	13	7	
Age (years)	38.2 ± 3.55	35.5 ± 2.93	35.8 ± 3.3	32.5 ± 3.5	A versus D, $P < 0.05$
AMH (ng/ml)	0.947 ± 2	5.98 ± 3.2	6.87 ± 3.15	10.13 ± 1.2	A versus B, C and D, $P < 0.001$; D versus B and C, $P < 0.001$
Duration of stimulation (days)	12.3 ± 2.3	11.9 ± 2.17	11.5 ± 1.27	11.5 ± 0.7	NS
Total FSH dose (IU)	4243 ± 1018	2982 ± 864	3000 ± 590	2712 ± 123	A versus B, C and D, $P < 0.001$
Follicles on day of hCG					
16–18 mm	0.8 ± 0.9	4.1 ± 3.2	5.1 ± 3.6	12 ± 3	A versus B, C and D, $P < 0.01$; D versus B and C, $P < 0.01$
>18 mm	1.4 ± 1	3.5 ± 2.9	3.1 ± 2.7	3 ± 1.4	A versus B, C and D, $P < 0.05$
Total follicles >16 mm	2.2 ± 1.9	7.7 ± 4.1	8.2 ± 4.2	15 ± 6	A versus B, C and D, $P < 0.01$; D versus B and C, $P < 0.01$
Retrieved oocytes (*n*)	2.1 ± 1.44	6.5 ± 2.5	13.8 ± 6.5	19.5 ± 5.7	A versus B, C and D, $P < 0.01$; D versus B and C, $P < 0.05$
Mature oocytes (*n*)	1.7 ± 1.3	5 ± 2	10.4 ± 3.9	13.5 ± 6.3	A versus B, C and D, $P < 0.01$; D versus B, $P < 0.05$
Mature oocytes (%)	82.2 ± 26.9	77 ± 14.2	78.2 ± 14.9%	61 ± 7	NS
Follicle sensitivity (IU/follicle)	2269 ± 1443	458 ± 632	448 ± 218	306 ± 62	A versus B, C and D, $P < 0.01$; D versus B and C, $P < 0.05$
Implantation rate (%)	6.2	12.5	10	16	D versus A, $P < 0.05$
Pregnancy rate (%)	12.5	25	23	40	D versus A, $P < 0.05$
Cancelled cycles for absent response (*n*)	4	0	0	0	$P < 0.01$ ($\chi 2$)
Cancelled cycles for risk of OHSS (*n*)	0	0	0	2	$P < 0.05$ ($\chi 2$)

AMH, anti-Müllerian hormone; OHSS, ovarian hyperstimulation syndrome.

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Table II. Hormonal and cycles characteristics according to the anti-Müllerian hormone (AMH) quartiles

	A (<25th percentile, *n* = 12)	B (25–50th percentile, *n* = 12)	C (50–75th percentile, *n* = 12)	D (>75th percentile, *n* = 12)	*P*
AMH range (ng/ml)	0–0.4	0.5–2.5	2.6–6.9	7–11	
AMH (ng/ml)	0.066 ± 0.16	0.9 ± 0.79	4.59 ± 1.64	8.98 ± 1.13	A versus B versus C versus D, $P < 0.001$
Age	38.6 ± 4.4	37.3 ± 3.8	37.5 ± 1.37	34.3 ± 3.2	A versus D, $P < 0.05$
Duration of stimulation (days)	13 ± 1.8	13.3 ± 3.2	11.1 ± 1.4	11.3 ± 0.8	NS
Total FSH dose (IU)	4762 ± 540	4041 ± 1545	3145 ± 829	2691 ± 717	A versus C and D, $P < 0.05$; D versus A and B, $P < 0.05$
Follicles on the day of hCG					
16–18 mm	0.75 ± 0.5	1.5 ± 1.6	2 ± 0.89	6.8 ± 2.6	A versus D, $P < 0.05$
>18 mm	1.25 ± 0.5	2 ± 1.2	3 ± 0.26	4.5 ± 3.2	A versus D, $P < 0.05$
Total	2 ± 0.8	3.5 ± 1.6	5 ± 2.2	11.3 ± 4.3	A versus D, $P < 0.01$
Follicle sensitivity (IU/follicle)	2847 ± 1638	1568 ± 1009	700 ± 255	241 ± 69	A versus C and D, $P < 0.05$; D versus B and C, $P < 0.05$
Retrieved oocytes (*n*)	2.25 ± 1.25	4.3 ± 2.7	6.83 ± 2.6	18 ± 5.4	A versus C and D, $P < 0.05$; D versus B and C, $P < 0.05$
Implantation rate (%)	6.5	7	13.3	14.3	D versus A, $P < 0.05$
Pregnancy rate (%)	12.5	16.6	25	30	D versus A, $P < 0.05$
Cancelled cycles for absent response (*n*)	4	0	0	0	$P < 0.05$ ($\chi 2$)
Cancelled cycles for risk of OHSS (*n*)	0	0	0	2	$P < 0.05$ ($\chi 2$)

OHSS, ovarian hyperstimulation syndrome.

Table III. Correlation among anti-Müllerian hormone (AMH) serum levels, age and cycle characteristics

Variables (AMH)	*r*	*P*
Age	–0.44	0.02
Total FSH dose	–0.52	0.01
No follicles >16 mm	0.76	0.001
Follicle sensitivity	0.72	0.001
Retrieved oocytes	0.73	0.0001

Table IV. Performance of anti-Müllerian hormone (AMH) serum levels in predicting poor ovarian response

AMH threshold (ng/ml)	Sensitivity (%)	Specificity (%)	Correctly classified (%)
0.5	85	82.3	81.2
0.75	80	93	87.5

ovarian response. Using a threshold of 0.5 ng/ml, the sensitivity was 85% and specificity was 82.3% in predicting poor response (Table IV).

Discussion

This study has observed a strong correlation between serum AMH levels and ovarian response to gonadotrophin stimulation during IVF/ICSI treatments. The innovation in this study is that AMH measurement has been performed independently of the day of the menstrual cycle.

The excellent correlation between serum AMH levels on day 3 and ovarian response to FSH in ART cycles has been clearly demonstrated in previous studies (Table V).

In a cohort of women undergoing IVF treatment, the use of AMH serum levels as a measure of ovarian reserve was tested (Van Rooij *et al.*, 2002). Hormonal parameters and AFC were determined by transvaginal ultrasonography on the third day of the menstrual cycle in 119 IVF patients, not more than 3 months before IVF treatment. The parameters measured were analysed after the division of the patients into two groups on the basis of the number of oocytes retrieved after IVF treatment: normal responders (four or more retrieved oocytes) and poor responders (less oocytes and cancellations).

AMH serum levels were lower in the poor responders than in the normal responding women. Serum AMH levels correlated strongly with the AFC, the number follicles retrieved, age, inhibin B and FSH. In addition, logistic regression analysis in predictive models of poor or normal response showed that both AFC and AMH serum levels were equally important for prediction.

Similar results were found in a recent study that confirmed the relevance of AMH measurements in predicting ovarian response to ovarian stimulation (Seifer *et al.*, 2002). Serum AMH levels on day 3 have been reported to have greater prognostic value than age, serum FSH, inhibin B or estradiol (Hazout *et al.*, 2004). In this retrospective study including 109 women undergoing IVF, basal serum AMH levels <1.1 ng/ml were associated with IVF failure. Moreover, regression analysis showed that AMH explained 26% of the variance for success or failure with IVF, whereas FSH, age and inhibin B explained 7, 6 and 0.5%, respectively.

This finding has been confirmed in a large prospective study conducted on 238 women undergoing IVF. Using a threshold value of 1.13 ng/ml, AMH assessment was shown to predict ovarian reserve with a sensitivity of 80% and a specificity of 85% (Tremellen *et al.*, 2005).

AMH levels have also been shown to be 10-fold lower in the cancelled cycles compared with patients who had a complete IVF cycle. In ~75% of cancelled cycles, AMH levels were below the detection limit (0.098 ng/ml) (Muttukrishna *et al.*, 2004). The authors concluded that AMH seems to be a better marker for predicting a cancelled cycle compared with FSH or

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Table V. Anti-Müllerian hormone (AMH) in the prediction of ovarian response to COH

Reference	*n*	Study design	Day of AMH measurement	Prediction of poor response						
				Correlation with retrieved oocytes (*n*)	ROC_{AUC}	Threshold (ng/ml)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Van Rooij *et al*. (2002)	130	Prospective	3	0.57	0.86					
Seifer *et al*. (2002)	107	Retrospective	3	0.522						
Hazout *et al*. (2004)	109	Retrospective	3	0.38		<1.1				
Muttukrishna *et al*. (2004)	69	Prospective	5 to 6	0.69		<0.1	87.5	72.2		
Eldar-Geva *et al*. (2005)	56	Prospective	3 to 4	0.48	0.75[a]					
Tremellen *et al*. (2005)	238	Prospective	3 to 5			<1.1	80	85	67	92
Muttukrishna *et al.* (2005)	108	Retrospective	3	0.509		<0.2	87	64		
Penarrubia *et al*. (2005)	80	Prospective	3		0.67		40	91.7		
Ficicioglu *et al*. (2006)	50	Prospective	3	0.564	0.92	<0.25	90.9	90.9	96.8	76.9

NPV, negative predictive value; PPV, positive predictive value.
[a]ROC_{AUC} for prediction of pregnancy.

inhibin B. Using a threshold of 0.1 ng/ml, AMH had a sensitivity of 87.5% and a specificity of 72.2% in the prediction of cancellation.

In all the previous studies, the performance of AMH in identifying poor responders was very similar to that of AFC (Van Rooij *et al.*, 2002; Eldar-Geva *et al.*, 2005; Ficicioglu *et al.*, 2006). This finding is not surprising, as AMH levels are believed to be a reflection of the number of growing follicles, which is also related to the number of small antral follicles.

Our data strongly support all the previous published studies dealing with the prognostic value of the AMH on cycle cancellation and ovarian response to FSH.

In addition, this is the first article that could demonstrate the clear possibility of using the serum AMH level measurement independent of the day of menstrual cycle. This possibility is clearly secondary to the absence of fluctuation in AMH serum levels throughout the menstrual cycle (La Marca *et al.*, 2006a; Hehenkamp *et al.*, 2006).

The absence of modifications in serum AMH levels throughout the menstrual cycle is a clear demonstration that AMH expression is not regulated by gonadotrophins. Accordingly, it has been observed that conditions associated with very low or suppressed FSH are not associated with significant modifications in serum AMH levels. These conditions include hypogonadotrophic hypogonadism (La Marca *et al.*, 2006) and pregnancy (La Marca *et al.*, 2005b).

In this study, cycle cancellation rate was strongly correlated with AMH levels. Using a cut-off value of 0.75 or 0.5 ng/ml, the sensitivity was 80 and 85%, respectively, in predicting poor ovarian response.

In our study, all the couples with the IVF/ICSI cycles cancelled due to absent ovarian response were in the group of patients in the lowest AMH quartile (<0.4 ng/ml). Conversely, the IVF/ICSI cycles cancelled due to the risk of OHSS were in the group in the highest AMH quartile (>7 ng/ml).

The ability to predict poor ovarian response may be a valuable tool for adjusting the doses of hormones used for ovarian stimulation as well as for patient counselling as poor responders have a lower probability of pregnancy.

The IRs and PRs have been reported but were not considered as outcome measures because of the absence of homogeneity in the couples included in the study. Indeed, IRs and PRs depend on ovarian reserve and on any other factors such as sperm parameters. Therefore, in a small series, factors found to predict oocyte number may not predict the probability of pregnancy.

However, we found that high serum AMH levels correlated with high IRs and PRs. These results confirm those found by Silberstein and colleagues (2006). The authors reported a significant correlation between serum AMH levels and embryo morphology score. They concluded that AMH >2.7 ng/ml indicated good oocyte quality as reflected by a higher IR and a trend towards a better clinical PR.

In conclusion, our results demonstrated a strong association between AMH and ovarian response to gonadotrophins. Serum AMH seems to result from the follicular pool, and its production is independent of the gonadotrophin-dependent indicators of ovarian reserve. This makes AMH unique in providing a perspective, which is not possible with current serum markers (La Marca and Volpe 2006). Moreover, for the first time, clinicians may have a reliable serum marker of ovarian response that can be measured independently of the day of the menstrual cycle.

The stability of AMH during the cycle and its predictive power make it the most discriminating hormonal prognostic marker of ovarian response in ART.

References

Bancsi LF, Broekmans FJ, Eijkemans MJ, de Jong FH, Habbema JD and te Velde ER (2002) Predictors of poor ovarian response in in vitro fertilization: a prospective study comparing basal markers of ovarian reserve. Fertil Steril 77,328–336.

Cate RL, Mattaliano RJ, Hession C, Tizard R, Farber NM, Cheung A, Ninfa EG, Frey AZ, Gash DJ, Chow EP *et al*. (1986) Isolation of the bovine and human genes for Mullerian inhibiting substance and expression of the human gene in animal cells. Cell 45,685–698.

Durlinger AL, Gruijters MJ, Kramer P, Karels B, Ingraham HA, Nachtigal MW, Uilenbroek JT, Grootegoed JA and Themmen AP (2002) Anti-Mullerian hormone inhibits initiation of primordial follicle growth in the mouse ovary. Endocrinology 143,1076–1084.

Eldar-Geva T, Ben-Chetrit A, Spitz IM, Rabinowitz R, Markowitz E, Mimoni T, Gal M, Zylber-Haran E and Margalioth EJ (2005) Dynamic assays of inhibin B, anti-Mullerian hormone and estradiol following FSH stimulation and ovarian ultrasonography as predictors of IVF outcome. Hum Reprod 20,3178–3183.

Faddy MJ and Gosden RG (1996) A model conforming the decline in follicle numbers to the age of menopause in women. Hum Reprod 11,1484–1486.

Fanchin R, de Ziegler D, Olivennes F, Taieb J, Dzik A and Frydman R (1994) Exogenous follicle stimulating hormone ovarian reserve test (EFORT): a

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simple and reliable screening test for detecting 'poor responders' in in-vitro fertilization. Hum Reprod 9,1607–1611.

Fanchin R, Schonauer LM, Righini C, Guibourdenche J, Frydman R and Taieb J (2003) Serum anti-Mullerian hormone is more strongly related to ovarian follicular status than serum inhibin B, estradiol, FSH and LH on day 3. Hum Reprod 18,323–327.

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

Hall JE, Welt CK and Cramer DW (1999) Inhibin A and inhibin B reflect ovarian function in assisted reproduction but are less useful at predicting outcome. Hum Reprod 14,409–415.

Hazout A, Bouchard P, Seifer DB, Aussage P, Junca AM and Cohen-Bacrie P (2004) Serum antimullerian hormone/mullerian-inhibiting substance appears to be a more discriminatory marker of assisted reproductive technology outcome than follicle-stimulating hormone, inhibin B, or estradiol. Fertil Steril 82,1323–1329.

Hehenkamp WJ, Looman CW, Themmen AP, de Jong FH, Te Velde ER and Broekmans FJ (2006) Anti-Mullerian Hormone levels in the spontaneous menstrual cycle do not show substantial fluctuation. J Clin Endocrinol Metab 91,4057–4063.

Josso N (1990) Anti-mullerian hormone: hormone or growth factor? Prog Growth Factor Res 2,169–179.

Josso N, di Clemente N and Gouedard L (2001) Anti-Mullerian hormone and its receptors. Mol Cell Endocrinol 179,25–32.

La Marca A and Volpe A (2006c) Anti-Mullerian hormone (AMH) in female reproduction: is measurement of circulating AMH a useful tool? Clin Endocrinol (Oxf) 64,603–610.

La Marca A, De Leo V, Giulini S, Orvieto R, Malmusi S, Giannella L and Volpe A (2005a) Anti-Mullerian hormone in premenopausal women and after spontaneous or surgically induced menopause. J Soc Gynecol Invest 12,545–548.

La Marca A, Giulini S, Orvieto R, De Leo V and Volpe A (2005b) Anti-Mullerian hormone concentrations in maternal serum during pregnancy. Hum Reprod 20,1569–1572.

La Marca A, Stabile G, Carducci Artenisio A and Volpe A (2006a) Serum anti-Mullerian hormone throughout the human menstrual cycle. Hum Reprod, in press.

La Marca A, Pati M, Orvieto R, Stabile G, Carducci Artenisio A and Volpe A (2006b) Serum anti-Mullerian hormone levels in women with secondary amenorrhea. Fertil Steril 85,1547–1549.

Lass A, Skull J, McVeigh E, Margara R and Winston RM (1997) Measurement of ovarian volume by transvaginal sonography before ovulation induction with human menopausal gonadotrophin for in-vitro fertilization can predict poor response. Hum Reprod 12,294–297.

Lee MM, Donahoe PK, Hasegawa T, Silverman B, Crist GB, Best S, Hasegawa Y, Noto RA, Schoenfeld D and MacLaughlin DT (1996) Mullerian inhibiting substance in humans: normal levels from infancy to adulthood. J Clin Endocrinol Metab 81,571–576.

Muasher SJ, Oehninger S, Simonetti S, Matta J, Ellis LM, Liu HC, Jones GS and Rosenwaks Z (1988) The value of basal and/or stimulated serum gonadotropin levels in prediction of stimulation response and in vitro fertilization outcome. Fertil Steril 50,298–307.

Muttukrishna S, Suharjono H, McGarrigle H and Sathanandan M (2004) Inhibin B and anti-Mullerian hormone: markers of ovarian response in IVF/ICSI patients? BJOG 111,1248–1253.

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

Navot D, Margalioth EJ, Laufer N, Brzezinski A, Birkenfeld A and Schenker JG (1987) Periovulatory 17 beta-estradiol pattern in conceptional and non-conceptional cycles during menotropin treatment of anovulatory infertility. Fertil Steril 47,234–237.

Penarrubia J, Fabregues F, Manau D, Creus M, Casals G, Casamitjana R, Carmona F, Vanrell JA and Balasch J (2005) Basal and stimulation day 5 anti-Mullerian hormone serum concentrations as predictors of ovarian response and pregnancy in assisted reproductive technology cycles stimulated with gonadotropin-releasing hormone agonist – gonadotropin treatment. Hum Reprod 20,915–922.

Rajpert-De Meyts E, Jorgensen N, Graem N, Muller J, Cate RL and Skakkebaek NE (1999) Expression of anti-Mullerian hormone during normal and pathological gonadal development: association with differentiation of sertoli and granulosa cells. J Clin Endocrinol Metab 84,3836–3844.

Ravhon A, Lavery S, Michael S, Donaldson M, Margara R, Trew G and Winston R (2000) Dynamic assays of inhibin B and oestradiol following buserelin acetate administration as predictors of ovarian response in IVF. Hum Reprod 15,2297–2301.

Seifer DB, MacLaughlin DT, Christian BP, Feng B and Shelden RM (2002) Early follicular serum mullerian-inhibiting substance levels are associated with ovarian response during assisted reproductive technology cycles. Fertil Steril 77,468–471.

Silberstein T, MacLaughlin DT, Shai I, Trimarchi JR, Lambert-Messerlian G, Seifer DB, Keefe DL and Blazar AS (2006) Mullerian inhibiting substance levels at the time of HCG administration in IVF cycles predict both ovarian reserve and embryo morphology. Hum Reprod 21,159–163.

Tomas C, Nuojua-Huttunen S and Martikainen H (1997) Pretreatment transvaginal ultrasound examination predicts ovarian responsiveness to gonadotrophins in in-vitro fertilization. Hum Reprod 12,220–223.

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

Van Rooij IA, Broekmans FJ, te Velde ER, Fauser BC, Bancsi LF, Jong FH and Themmen AP (2002) Serum anti-Mullerian hormone levels: a novel measure of ovarian reserve. Hum Reprod 17,3065–3071.

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

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

Weenen C, Laven JS, Von Bergh AR, Cranfield M, Groome NP, Visser JA, Kramer P, Fauser BC and Themmen AP (2004) Anti-Mullerian hormone expression pattern in the human ovary: potential implications for initial and cyclic follicle recruitment. Mol Hum Reprod 10,77–83.

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