Normal serum concentrations of anti-Müllerian hormone in a population of fertile women in their first trimester of pregnancy

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**BACKGROUND:** Anti-Müllerian hormone (AMH) levels are used to evaluate the ovarian reserve. AMH serum concentrations have recently been studied among women attending fertility centers, and among women with regular menstrual cycles but normative values have not been established among fertile women: the objective of this study was to establish those values.

**METHODS:** This prospective cross-sectional study included 340 healthy fertile women attending a single centre, aged between 15 and 50 years. The women were all in the first trimester of pregnancy, had no serious medical history and attended the abortion service of the University Hospital of Nice, France. Serum AMH was measured using a second-generation AMH enzyme-linked immunosorbent assay.

**RESULTS:** Median AMH concentration was 2.42 ng/ml (25–75 percentiles 1.19–4.12). The relationship between AMH concentration and age was best fitted by a polynomial function. Serum AMH values rose until age 29 years and then showed a significant decline ($R^2 = 0.289, P < 0.001$). Normative values for serum AMH were established in different age groups between 15 and 50 years.

**CONCLUSIONS:** We established the normative values for serum AMH in a population of French fertile women in their first trimester of pregnancy.

**Key words:** anti-Müllerian hormone / epidemiology / female infertility / pregnancy / ovarian reserve

**Introduction**

Anti-Müllerian hormone (AMH) is nowadays used widely as a routine test in assisted reproduction technology (ART). AMH is exclusively of ovarian origin (Long et al., 2000; La Marca et al., 2005a), and is considered as a quantitative as well as a qualitative biomarker for ovarian reserve (Ebner et al., 2006; van Disseldorp et al., 2008). Levels of AMH tend to decline with normal aging (de Vet et al., 2002; van Rooij et al., 2005) and become undetectable ~5 years preceding the final menstrual period (Sowers et al., 2008, 2010). AMH represents the best biomarker, when compared with FSH, inhibin B and estradiol, to reflect the age-related decline of reproductive capacity (van Rooij et al., 2005), and allows the early detection of an altered ovarian reserve. AMH is also used as a predictive marker for the ovarian response to gonadotrophins in ART, as it helps to identify poor and hyper-responders (La Marca et al., 2010). AMH serum levels during the menstrual cycle have been studied. Two studies reported variability of the serum AMH levels during a cycle, showing a late follicular peak in AMH levels (Cook et al., 2000; Wunder et al., 2008), while others found no significant change during the menstrual cycle (La Marca et al., 2004, 2006; Hehenkamp et al., 2006; Tsepelidis et al., 2007; Sowers et al., 2010). During pregnancy, serum AMH values in the first trimester are the same as the non-pregnant values, and tend to decrease significantly as the pregnancy progresses (La Marca et al., 2005b, 2009; Nelson et al., 2010). There are actually no normative values available for circulating AMH levels in fertile women whose fertility is well-established at the moment of the analysis. We conducted a prospective study in order to establish those AMH values.
Materials and Methods

Subjects
This prospective cross-sectional study was conducted in a group of Caucasian fertile women aged 15–50 years, attending the abortion service of the University Hospital of Nice, France. Data were collected between August 2009 and June 2010. All women attending the abortion service were considered as being eligible to participate in the study, since they were pregnant and therefore fertile. During their first appointment, all women were informed of the study. They were handed a questionnaire and an informed consent form which they filled in, if they agreed to participate. Women under 18 years of age had to give their consent and also obtain the consent of an adult aged 18 years or more, to enter the study. The questionnaire asked for information about: age, height and weight to calculate the BMI; last menstrual period or conception date and contraception used while or before being pregnant. Patients were also asked to specify whether they had a medical history, which could have interfered with their fertility, such as cancer, and chemotherapy or radiotherapy treatment. Missing data were completed using the patients’ medical records. The gestational age was determined by the last menstrual period or by early ultrasound.

Age groups were defined a priori based on clinical relevance: < 20, 20–24.9, 25–29.9, 30–34.9, 35–39.9, 40 years and above.

A sample of blood was taken for the study at the same time as the usual blood tests carried out for the abortion procedure. The blood sample was then conveyed to the biochemistry laboratory where it was centrifuged and stored at –20°C until assay.

AMH Assay
Serum AMH analysis was performed using the AMH GEN II Beckman Coulter assay, Marseille, France (Kumar et al., 2010). This enzyme-linked immunosorbent assay is an enzymatically amplified two-site immunoassay: calibrators, controls and samples are incubated in microwell plates which have been coated with anti-AMH antibodies. After incubation and washing, anti-AMH detection antibodies labeled with biotin are added to each well. After a second incubation and washing step, streptavidin/horse-radish peroxidase is added to the wells. After a third incubation and washing step, the substrate tetramethylbenzindine is added. Finally, an acidic neutralizer solution is added.

The degree of enzymatic turnover of the substrate is determined by dual wavelength absorbance measurements at 450 nm and between 600 and 630 nm. The absorbance measured is directly proportional to the AMH concentration in the sample or the calibrator. The AMH concentration in the samples can then be calculated from the calibration curve.

Twenty microlitres of serum were necessary for the test. All values were stated in ng/ml. The conversion factor for 1 ng/ml is 7.14 pM.

The antibodies used in the assay bind to the mature AMH molecule, which is more stable against proteolysis than the prohormone. This well characterized dual monoclonal antibody pair is specific to AMH and does not detect inhibin A, activin A, FSH and LH, at twice their physiological concentrations.

The lowest AMH concentration in a sample which could be detected with a 95% probability was 0.08 ng/ml (lower detection limit). The limit of quantification is 0.16 ng/ml with an estimated 20% error margin. The inter-assay and intra-assay coefficients of variation were ≤ 5.6 and 5.4%, respectively. Undetectable AMH serum levels found in our study were set to 0.15 ng/ml for statistical analysis.

Statistical analysis
Collected data were centralized in an anonymous database. Linear variables were log transformed to obtain normal distribution. Correlation tests were performed between AMH concentration and independent variables, such as gestational age at the time of the sampling, BMI and age (Pearson’s correlation coefficient, r). Comparisons of AMH concentration between age groups were performed using one-way analysis of variance and Bonferroni post hoc test on log-transformed data. Regression analysis was performed to determine whether changes in AMH concentrations were best fitted by a linear, exponential or polynomial function of the independent variables. The significant function with the highest R² value was considered the best-fitting model. Multiple linear regression was performed to combine the significant variables. Reference intervals were calculated using non-parametric method with 500 bootstrap iterations and a 90% confidence interval. The Statistical Package for the Social Sciences version 16.0 (SPSS Inc., Chicago, IL, USA) and RefVal 4.11 (Oslo, Norway) (Solberg, 2004) were used for data analysis. A P-value < 0.05 was considered significant.

According to the International Federation of Clinical Chemistry (IFCC) requirements for reference intervals, a minimum of 40 women in each age group was needed to establish the AMH normative values (Poulsen et al., 1997; Solberg, 2004).

Results
The total number of patients included in the study was 340. Table I presents background characteristics for the women overall and by age group. As expected, frequency of nulliparity significantly decreases with age, whereas BMI tends to increase.

Figure 1 shows the distribution of serum AMH concentrations in the whole population. The median AMH concentration was 2.42 ng/ml and the 25–75 percentiles were 1.19 and 4.12. Three women aged 42, 35 and 32 years old had undetectable AMH serum concentrations. The median age was 27 years, ranging from 14 to 50 years. Gestational age varied between 5 and 14 weeks (abortion being illegal in France later than this). No patients had significant medical history. Concerning fertility, 63% of the patients fully answered the questions and had no past infertility history. For the remaining 37%, either the answer was not given or was incomplete.

Log AMH concentrations, according to age group, are represented in Fig. 1 and comparisons among groups showed that AMH concentration was higher for women <30 years of age compared with all women over 30 years (3.63 versus 1.92 ng/ml, P < 0.001).

Correlation between AMH concentration and age was high (R² = 24.9%): 24.9% of the AMH variation linked to age variation (P < 0.001). The relationship between AMH concentration and age was best fitted by a polynomial function: log AMH = −0.05 + 0.05 × age −0.001 × age². R² = 0.289; P < 0.001 (Fig. 2).

There was no correlation between gestational age and serum AMH values (r = −0.015, P = 0.787). No difference was found in mean AMH serum values in the smoking versus non-smoking group (3.04 versus 2.86 ng/ml, P = 0.103).

There was a negative linear correlation between serum AMH concentration and BMI (log AMH = −0.800 × log BMI −1407; R² = 0.021; P = 0.007). However, BMI also increased with age (r = 0.169; P = 0.001). When BMI was included in a multiple regression model controlling for age, with log AMH as a dependent variable, BMI no longer had a significant impact on serum AMH.

The reference intervals for serum AMH concentration were established in the six different age groups, as shown in Table II and
Table I  Background characteristics of the study population.

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Age groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 340</td>
<td>&lt;20 (n = 49)</td>
</tr>
<tr>
<td>Age, years(a)</td>
<td>27.0 (21.0–33.5)</td>
<td>20–24 (n = 86)</td>
</tr>
<tr>
<td>BMI, kg/m(^2) (a)</td>
<td>21.8 (19.7–24.6)</td>
<td>25–29 (n = 73)</td>
</tr>
<tr>
<td>&lt;18.5 kg/m(^2)</td>
<td>40 (11.8)</td>
<td>30–34 (n = 52)</td>
</tr>
<tr>
<td>18.5–24.9 kg/m(^2)</td>
<td>218 (64.1)</td>
<td>35–39 (n = 40)</td>
</tr>
<tr>
<td>≥30 kg/m(^2)</td>
<td>21 (6.2)</td>
<td>≥40 (n = 40)</td>
</tr>
<tr>
<td>Smoking(b)</td>
<td>178 (53.3)</td>
<td>Age groups</td>
</tr>
<tr>
<td>Nulliparous(b)</td>
<td>154 (45.3)</td>
<td>&lt;20 (n = 49)</td>
</tr>
<tr>
<td>Contraception(b)</td>
<td>154 (45.3)</td>
<td>20–24 (n = 86)</td>
</tr>
<tr>
<td>No contraception for 1 year and less</td>
<td>64 (25.1)</td>
<td>25–29 (n = 73)</td>
</tr>
<tr>
<td>No contraception for more than 1 year</td>
<td>12 (4.7)</td>
<td>30–34 (n = 52)</td>
</tr>
<tr>
<td>No contraception (no detail)</td>
<td>39 (15.3)</td>
<td>35–39 (n = 40)</td>
</tr>
<tr>
<td>Missuse of contraception and Pregnancy under contraception</td>
<td>140 (54.9)</td>
<td>≥40 (n = 40)</td>
</tr>
<tr>
<td>Gestational age, weeks(a)</td>
<td>7.3 (6.1–9.1)</td>
<td>Age groups</td>
</tr>
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<td>Gestational age, weeks(a)</td>
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<td>Age groups</td>
</tr>
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\(a\)Median (25–75 percentile).
\(b\)n(%).
\(c\)Analysis of variance for continuous variables and \(\chi^2\) for categorical variables.
Supplementary data. AMH levels tend to diminish as age advances, after having reached a peak in the 25–29.9 year old group.

**Discussion**

Our study is, to our knowledge, the largest prospective study to report AMH serum concentration values in fertile women of reproductive age. A total of 340 patients were included and divided into six age groups in order to determine normal values of AMH concentration. The characteristics of the reference subjects were clearly defined.

**Figure 1**

Anti-Müllerian Hormone (AMH) serum concentrations in all the study population (first box plot) and depending on age groups. In each box plot, hinges at the top and bottom of the box represent the upper and lower quartile; thick black line inside the box indicates the median and horizontal lines above and below the boxes correspond to limits of 99% of the distribution. Log of serum AMH concentration was significantly higher before age 30 (Bonferroni post hoc tests, \( P < 0.001 \)).

**Figure 2**

Log of serum AMH concentration (Log AMH in ng/ml) depending on age of women—mean and 95% confidence intervals of the regression model are shown.

**Table II**

Reference intervals for serum AMH concentration (ng/ml) in women in the first trimester of pregnancy, according to age group (90% confidence intervals).

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Percentile</th>
<th>2.5</th>
<th>5</th>
<th>10</th>
<th>25</th>
<th>50</th>
<th>75</th>
<th>90</th>
<th>95</th>
<th>97.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than 20</td>
<td>0.90 (0.87–1.29)</td>
<td>1.14 (0.97–1.67)</td>
<td>2.20 (1.54–4.02)</td>
<td>3.25 (2.50–4.04)</td>
<td>4.29 (3.26–4.64)</td>
<td>6.34 (5.36–8.77)</td>
<td>7.79 (6.31–8.81)</td>
<td>9.80 (7.85–10.40)</td>
<td>14.40 (8.98–15.94)</td>
<td>13.10 (9.30–15.94)</td>
</tr>
<tr>
<td>20–24.9</td>
<td>0.87 (0.70–1.18)</td>
<td>0.87 (0.86–1.23)</td>
<td>1.24 (1.12–1.61)</td>
<td>2.08 (1.71–2.35)</td>
<td>2.98 (2.57–3.33)</td>
<td>4.21 (3.55–4.86)</td>
<td>5.25 (4.23–6.25)</td>
<td>6.45 (5.50–7.40)</td>
<td>9.61 (8.03–11.17)</td>
<td>9.61 (7.98–11.17)</td>
</tr>
<tr>
<td>25–29.9</td>
<td>0.53 (0.42–0.77)</td>
<td>0.69 (0.53–1.17)</td>
<td>1.16 (0.73–1.57)</td>
<td>2.14 (1.71–2.56)</td>
<td>3.43 (2.74–3.93)</td>
<td>5.02 (3.89–6.15)</td>
<td>6.89 (5.76–9.44)</td>
<td>9.86 (7.19–14.67)</td>
<td>14.40 (8.98–15.94)</td>
<td>13.10 (9.30–15.94)</td>
</tr>
<tr>
<td>30–34.9</td>
<td>0.27 (0.19–0.36)</td>
<td>0.39 (0.25–0.57)</td>
<td>0.51 (0.35–0.77)</td>
<td>0.88 (0.70–1.08)</td>
<td>1.50 (1.14–1.93)</td>
<td>2.08 (1.71–2.56)</td>
<td>2.14 (1.71–2.56)</td>
<td>2.08 (1.71–2.56)</td>
<td>3.43 (2.74–3.93)</td>
<td>5.25 (4.23–6.25)</td>
</tr>
<tr>
<td>35–39.9</td>
<td>0.15 (0.15–0.18)</td>
<td>0.15 (0.15–0.24)</td>
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<td>0.15 (0.15–0.24)</td>
<td>0.15 (0.15–0.24)</td>
</tr>
<tr>
<td>40 and more</td>
<td>0.15 (0.15–0.18)</td>
<td>0.15 (0.15–0.24)</td>
<td>0.15 (0.15–0.24)</td>
<td>0.15 (0.15–0.24)</td>
<td>0.15 (0.15–0.24)</td>
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Log of serum AMH concentration (Log AMH in ng/ml) in women in the first trimester of pregnancy, according to age group (90% confidence intervals).
Normal anti-Müllerian hormone levels in pregnancy

Conclusion

Our study established normal serum AMH values among different age groups in fertile women. These fertile women were represented by a group of pregnant women in the first trimester pregnancy. In agreement with data in the literature, serum AMH values decreased with aging, although there was great variability in AMH values between patients belonging to the same age group. Undetectable to low AMH values were found in our fertile subjects, which should lead practitioners to be cautious when counseling patients presenting with very low or undetectable AMH serum concentrations, because spontaneous pregnancy is still possible in this case. Furthermore, normal serum AMH values in fertile women will be useful for the practitioner to refer to when answering the questions of women consulting in fertility centers. Studies including larger cohorts should be performed among fertile women in order to confirm the values found in our study.

Supplementary data

Supplementary data are available at http://humrep.oxfordjournals.org/.

Authors’ roles

V.M. played a role in the conception, design and acquisition of data; the interpretation of data; drafting the article; the final approval of the version to be published. P.F. took part in the conception, design and acquisition of data; interpretation of data; drafting the article; the final approval of the version to be published. I.B.: was involved in the analysis and interpretation of data; revising the draft critically for important intellectual content; the final approval of the version to be published. J.D. played a role in interpretation of data; revising the draft critically for important intellectual content; the final approval of the version to be published. V.I. contributed to the conception of the study; revised the draft critically for important intellectual content; gave final approval of the version to be published. A.B. made contributions to the conception and design of the study; the critical revision of the draft for important intellectual content; the final approval of the version to be published.

Other studies establishing reference serum AMH values have recently been published, two of which (Seifer et al., 2011; Shebl et al., 2011) are retrospective studies among women attending fertility clinics and therefore do not reflect the normal serum AMH values in a fertile population. La Marca et al. (2010) evaluated AMH normal serum concentrations in women with regular cycles; however, their fertility was not proved at the time of the study. The serum AMH values in our study, however, are generally lower than those found in other studies and this could be explained by the fact that we used a different assay. Another hypothesis is that the other studies included more women with polycystic ovaries. Finally, women aged above 40 years in the study of La Marca et al. (2010) had regular menstrual cycles, whereas in our study we hypothesized that women above 40 years of age attending the abortion service had believed that they could no longer become pregnant, as their menstrual cycles were irregular.

The impact of smoking on AMH serum levels was not significant in our study. We found no difference in serum AMH levels when we considered the whole cohort or when we compared smoking and non-smoking women in each age group (data not shown). These results contradict findings in the literature, which suggest that there is a negative impact of active smoking on AMH serum levels (Plante et al., 2010). Our results might be explained by a selection bias: we only selected fertile women, whose ovarian reserve might not yet have been affected by smoking.

According to our results, there was a significant negative linear correlation between AMH serum concentration and BMI, however this correlation was explained by the fact that aging women tend to have a higher BMI. AMH therefore tends to decline as a result of aging and not owing to the increase in BMI. Serum concentrations were below 0.75 ng/ml for 47 (13.5%) women, and 10 patients (2.8%) had serum concentrations ≤0.4 ng/ml. Serum AMH values were undetectable in three women aged 32, 35 and 42 years, none of whom had a past medical history of infertility, cancer or gonadotoxic treatment. Furthermore, their pregnancies followed contraceptive pill omission for one patient, a single sexual intercourse without contraception for the second and the third patient used no contraception and had had three spontaneous pregnancies in 1992, 1996 and 2000. AMH values varying between 0.3 and 0.75 ng/ml are usually considered as being predictive of a poor response in IVF (La Marca et al., 2010). However, the occurrence of spontaneous and induced pregnancies in the presence of undetectable, and undetectable to low (<0.4 ng/ml), serum AMH concentrations has already been reported (Fraisse et al., 2008; Barad et al., 2009; Tocci et al., 2009; Gleicher et al., 2010). Gleicher et al. (2010) observed a pregnancy rate of 5% per IVF cycle in women whose AMH concentration level ranged from undetectable to 0.4 ng/ml.

according to IFCC requirements for reference intervals. Results obtained to confirm the findings of previous studies showing the decline of AMH serum concentrations with increasing age (de Vet et al., 2002; Wunder et al., 2008; La Marca, 2010).

As the aim of this study was to establish serum AMH values in fertile women, it was conducted exclusively among pregnant women in the first trimester of pregnancy. We based our study on previous findings showing that AMH values were the same during the first trimester of pregnancy as before pregnancy (La Marca et al., 2005b, 2009; Nelson et al., 2007, 2009, 2010). We did not measure the serum AMH values after the abortion because of the high loss to follow up in this population. Indeed, in our experience up to one-third of the patients do not come for their routine check-up exam after the abortion is performed.

Our results show a progressive rise in serum AMH from age <20 years (median AMH concentration 2.20 ng/ml) to age 25–29.9 years, when the median AMH concentration was 2.66 ng/ml. Studies in mice and women have previously shown that mean AMH levels remain at a constant level at a young age (Kevenaar et al., 2006; van Deldendorp et al., 2008), and to explain this finding it was suggested that compensatory mechanisms maintain the number of growing follicles at a constant level. Serum AMH concentrations then shows a progressive decline, according to La Marca et al. (2005a). It has been well established that there is a gradual decline of fertility starting at age 30 years (Broekmans et al., 2009), and our results are concordant with this fact.

Other studies establishing reference serum AMH values have recently been published, two of which (Seifer et al., 2011; Shebl et al., 2011) are retrospective studies among women attending fertility clinics and therefore do not reflect the normal serum AMH values in a fertile population. La Marca et al. (2010) evaluated AMH normal serum concentrations in women with regular cycles; however, their fertility was not proved at the time of the study. The serum AMH values in our study, however, are generally lower than those found in other studies and this could be explained by the fact that we used a different assay. Another hypothesis is that the other studies included more women with polycystic ovaries. Finally, women aged above 40 years in the study of La Marca et al. (2010) had regular menstrual cycles, whereas in our study we hypothesized that women above 40 years of age attending the abortion service had believed that they could no longer become pregnant, as their menstrual cycles were irregular.
Acknowledgement

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