Low concentration of circulating antimüllerian hormone is not predictive of reduced fecundability in young healthy women: a prospective cohort study

Casper P. Hagen, M.D., Sonja Vestergaard, Ph.D., Anders Juul, Dm.S.C., Niels Erik Skakkebæk, Dm.S.C., Anna-Maria Andersson, Ph.D., Katharina M. Main, Ph.D., Niels Henrik Hjollund, Ph.D., Erik Ernst, Ph.D., Jens Peter Bonde, Dm.S.C., Richard A. Anderson, Ph.D., Erik Ernst, Ph.D., Katharina M. Main, Ph.D., Niels Henrik Hjøllund, Ph.D., and Tina Kold Jensen, Ph.D.

Department of Growth and Reproduction, Rigshospitalet, University of Copenhagen, Copenhagen; Department of Environmental Medicine, Institute of Public Health, University of Southern Denmark, Odense; Department of Occupational Medicine, Herning Regional Hospital, Herning; Department of Clinical Epidemiology, Department of Gynecology and Obstetrics, and Department of Occupational Medicine, Aarhus University Hospital, Aarhus; Department of Occupational and Environmental Medicine, Bispebjerg Hospital, Copenhagen, Denmark; and Medical Research Council Centre for Reproductive Health, University of Edinburgh, Edinburgh, United Kingdom

Objective: To evaluate whether circulating levels of antimüllerian hormone (AMH) predict fecundability in young healthy women.

Design: Prospective cohort study.

Setting: General community.

Patient(s): A total of 186 couples who intended to discontinue contraception to become pregnant were followed until pregnancy or for six menstrual cycles.

Intervention(s): None.

Main Outcome Measure(s): Fecundability was evaluated by the monthly probability of conceiving (i.e., fecundability ratio [FR]). In addition, circulating levels of LH, FSH, T, and sex hormone-binding globulin (SHBG) were evaluated in 158 of 186 women.

Result(s): Fifty-nine percent of couples conceived during the study period. Compared to the reference group of women with medium AMH (AMH quintiles 2–4), fecundability did not differ significantly in women with low AMH (AMH quintile 1) (FR 0.81; 95% confidence interval [CI] 0.44–1.40). In contrast, women with high AMH (AMH quintile 5) had reduced fecundability (FR 0.62; 95% CI 0.39–0.99) after adjustment for covariates (woman’s age, body mass index [BMI], smoking, diseases affecting fecundability, and oligozoospermia). Irregular menstrual cycles were more prevalent in women with high AMH compared with women with low or medium AMH levels, and they had higher levels of LH (geometric mean: 8.4 vs. 5.3 IU/L) and LH:FSH ratio (2.4 vs. 1.8). After exclusion of women with irregular cycles, women with high AMH still had reduced fecundability (FR 0.48; 95% CI 0.27–0.85) and elevated LH:FSH ratio (2.4 vs. 1.7).

Conclusion(s): Low AMH in healthy women in their mid-20s did not predict reduced fecundability. Even after exclusion of women with irregular cycles, the probability of conceiving was reduced in women with high AMH. (Fertil Steril 2012;98:1602–8. ©2012 by American Society for Reproductive Medicine.)

Key Words: AMH, MIS, time to pregnancy, fecundity, fecundability, PCOS

Discuss: You can discuss this article with its authors and with other ASRM members at http://fertstertforum.com/hagencp-anti-mullerian-hormone-fecundability/
Antimullerian hormone (AMH) is produced by granulosa cells (GC) surrounding follicles that have undergone recruitment from the primordial follicle pool but have not been selected for dominance (preantral and early antral follicles) \(1, 2\). In adult women, serum AMH concentration is considered to be a predictor of the follicle reserve. High AMH is associated with high antral follicle count \(3\), and with a high number of resting primordial follicles \(4\).

After minor fluctuations during puberty \(5\), AMH levels are stable through adolescence \(6\). Cross-sectional data from multiple studies suggest that AMH peaks at an average age of 25 years \(7\) before declining and becoming undetectable before menopause \(8\). The AMH level varies considerably between individuals, as does the ovarian reserve \(9, 10\). In healthy women in their mid-20s, AMH range from 4–58 pmol/L \(6\). Low AMH level is predictive of ovarian failure in patients with Turner syndrome \(6\), and earlier age of menopause in healthy women \(11–13\). In contrast, women with polycystic ovarian syndrome (PCOS) have elevated concentrations of AMH \(14, 15\).

The main value of AMH in IVF treatment is to predict the ovarian response \(16\), and in some but not all analyses AMH level has been found to positively associated with the chance of conception \(17, 18\). The main predictive value of AMH in ovarian stimulation is therefore quantitative rather than qualitative.

There are very limited data on its relation to natural conception. Time to pregnancy (TTP) is a functional measure of fecundability \(19\), and to our knowledge, only one study has assessed whether circulating levels of AMH in women are associated with fecundability evaluated by TTP. In that study \(n = 100\) of women in their late reproductive life \(30–42\) years) reduced fecundability was found in women with very low AMH levels \(≤0.7\ ng/mL) \(20\).

In this prospective population-based cohort study of healthy Danish couples with no prior knowledge of fecundability, we hypothesized that low AMH might predict reduced fecundability. In addition, we anticipated that women with high AMH levels, a group that would include women with PCOS, would also have reduced fecundability.

**MATERIALS AND METHODS**

**Sample Description**

A total of 430 couples were recruited between 1992 and 1995 after a nationwide mailing of personal letters to 52,255 trade union members (metal workers, office workers, nurses, and day care workers) who were 20–35 years old, lived with a partner, and had no children. Couples with no previous reproductive experience who intended to discontinue contraception to become pregnant were eligible for enrolment. The number of eligible couples in the source population of 52,255 people is unknown. However, assuming that 75% of pregnancies in Denmark are planned, a participation rate of 16% was estimated by using data from union, age, parity, and calendar-specific birth rates obtained from the Danish civil registration system. The couples were enrolled into the study when they discontinued contraception and were followed for six menstrual cycles or until a clinically recognized pregnancy was achieved, if sooner. At enrolment both partners filled in a questionnaire on demographic, medical, reproductive, occupational, and lifestyle factors. The men provided a semen sample and both partners, a blood sample. A detailed description of the study has previously been published \(21, 22\). From the 430 couples enrolled, AMH concentrations were determined in a subgroup of 186 women from one of the two data collecting centers (Sealand) from whom blood samples were available for analyses. None of the couples were lost to follow-up. Due to limited volume of remaining sera, analyses of LH, FSH, sex hormone-binding globulin (SHBG), and T concentrations were possible in 158 of the 186 women included in this study. The glycated hemoglobin \(A1C\) (HbA1C) levels, which have been reported previously \(23\), were available in 135 women.

**Outcome**

To evaluate the fecundability ratio (FR) (i.e., the monthly probability of conceiving) TTP was measured as the number of cycles from cessation of birth control to pregnancy or for a maximum of six cycles. Menstrual cycle log books were updated daily during the period at risk. The method of cycle determination has been described previously \(21, 22\).

In short, the first cycle of follow-up was defined as the cycle in which birth control was discontinued if more than 10 days had elapsed from discontinuation to the next menstrual bleeding. If not, then the next cycle was considered the first. We obtained cycle-specific information about frequency of sexual intercourse and defined a fertile window with at least one act of sexual intercourse between day 11 and day 20 before the first day of the menstrual cycle, as no couples without sexual intercourse in this window conceived. However, cycle-specific information on sexual intercourse was missing in 192 cycles of 789 cycles, and we therefore did not include this information in the main analyses, but performed subanalyses after exclusion of 22 cycles without sexual intercourse in the fertile window.

Pregnancy was determined clinically by a physician, by a urine pregnancy test, or by serum hCG measurement. Subfecundability was defined as TTP more than six cycles.

**Covariates**

Age (in years) was categorized in three groups \(19–24, 25–29,\) and \(30–35\). Body weight (in kilograms) and body height (in meters) was obtained and body mass index \((\text{BMI}, \text{kg/m}^2)\) was calculated and categorized into three groups \(<20, 20–25,\) and \(>25\). Smoking (never, ever, current), cycle length \((20–24, 25–34,\) and \(\geq 35\) days), and cycle regularity (regular, i.e., “almost always regular cycles”; irregular, i.e., “almost always irregular cycles”; regular cycles while on the combined contraceptive pill before enrolment) were determined by questionnaire. Similarly, self-reported diseases related to fecundability (women: salpingitis, ovarian cysts, gonorrhea, perforated appendicitis, Crohn’s disease, history of amenorrhea, hormonal disorder or hormonal treatment; men: epididymitis, adult parotitis, gonorrhea, testicular cancer, varicocele, sperm cyst, and cryptorchidism) were categorized.
into a variable (present or not present) [21]. Based on a fresh semen sample obtained at enrolment, sperm concentration (in million per milliliter) was determined and categorized into normal or oligozoospermia (<20 million/mL).

**Hormone Assays**

Blood samples were collected randomly during the menstrual cycle. All samples were withdrawn from an antecubital vein, clotted, centrifuged, and serum was stored at −20°C until hormone analyses were performed. Analyses were performed after approximately 15 years of storage in the freezer at −20°C. All samples were analyzed blind for study outcomes in the same laboratory.

Serum AMH levels were determined using the Beckman Coulter enzyme immunoassay, generation I (Immuno-Tech, Beckman Coulter Ltd.) with a detection limit of 2.0 pmol/L. The intra-assay coefficients of variation (CVs) were less than 7.8% and 5.4% at 13 and 123 pmol/L, respectively.

Our AMH results were compared to those of other studies using another assay and other units. To compare levels measured on different assays, the following conversion was used: AMH (Beckman Coulter) pmol/L = AMH (Diagnostic Systems Laboratories, Inc.) μg/L × 2.0 × 7.14 pmol/μg [24].

Serum FSH, LH, SHBG, and T were measured by time-resolved immunofluorometric assays (Delfia; PerkinElmer−WallaC Oy) with the following detection limits: FSH, 0.06 IU/L; LH, 0.05 IU/L; SHBG and T, 0.23 nmol/L. Intra-assay and interassay CVs were FSH and LH, <5%; SHBG, <6%; T, <12%. Hyperandrogenemia was defined as T >2.94 nmol/L (25). Free androgen index was calculated as 100 × T (nmol/L) / SHBG (nmol/L). The percentage of HbA1C was determined in capillary whole blood or venous whole blood using an immunoturbidimetric assay (DCA 2000 HbA1C System, Bayer). The validity of this assay has previously been tested [26].

**Statistical Analyses**

To normalize the distribution, hormone levels were log10-transformed. From back-transformed data, we report the geometric mean ± 1.96 SD.

To maintain group sizes large enough for statistical power, data analyses was planned based on dividing the women in AMH quintiles (5 groups) referring to the number of participating women. The three middle AMH quintiles (quintiles 2–4, n = 113) were joined to serve as the reference group to compare with women with low AMH (quintile 1, n = 36) and high AMH (quintile 5, n = 37).

Kaplan-Meier curves were drawn to illustrate the cumulative proportion of women achieving pregnancy by AMH subgroups. The difference between curves was tested by log-rank test. To determine whether variables with possible influence on AMH levels were equally distributed between AMH subgroups, χ² test was used.

Difference in TTP between low, medium, and high AMH subgroups was determined by the FR and analyzed using discrete-time survival models [27, 28] with complementary log-log link in STATA 11.1 (StataCorp). A priori, we decided to include woman’s age, BMI, reproductive organ diseases, smoking, and oligozoospermia in the adjusted models, as these have been reported to affect fecundability [22, 29], although not strictly qualifying to be confounders within the present data set. This information was available in 185 of 186 couples. Proportional hazards assumptions were tested using the log-rank goodness of fit test based on Schoenfeld residuals including all variables in the fully adjusted model. We also took into consideration the issue on fertile window by performing analysis excluding cycles without at least one intercourse between day 11 and day 20 before the first day of the period [30]. Odds of subfecundability (waiting more than 6 months to conceive) were analyzed using logistic regression.

Independent sample t-test was used to compare age, BMI, and hormone levels (LH, FSH, LH/FSH ratio, SHBG, T, free androgen index, and HbA1C) between AMH subgroups. Fisher’s exact test was used to determine whether the prevalence of diagnostic PCOS criteria (irregular menstrual cycles and hyperandrogenemia) were different between AMH subgroups. For all analyses, P<.05 was regarded as indicating statistical significance.

**Ethical Considerations**

The study protocol was approved by the local ethics committee. All participants gave their informed written consent.

**RESULTS**

Women from whom serum AMH levels were measured (n = 186) did not differ from women without AMH assessment (n = 244) in TTP, BMI, or prevalence of smokers. However, the women included in this study were slightly older than the women without AMH assessment (mean age, 26.6 years [95% confidence interval {CI} 21.4–31.8 vs. 25.7 years [95% CI 20.1–31.3]; P<.001). Pregnancy was achieved in six cycles in 110 of the 186 couples (59%).

Serum AMH levels ranged from undetectable to 183 pmol/L. Fifteen women had AMH levels <10 pmol/L. The AMH levels in quintile 1 (low AMH) were <14 pmol/L; the reference group of quintiles 2–4 (medium AMH) ranged from 14–39 pmol/L; and women in quintile 5 (high AMH) had AMH levels >39 pmol/L (Table 1). These groups comprised 36, 113, and 37 women, respectively.

Except for menstrual cycle regularity and cycle length, baseline characteristics were not associated with AMH levels (Table 1). Women with high AMH had more irregular menstrual cycles and their cycles lasted longer than in women with low and medium AMH (P<.001).

The cumulative proportions of women in the low, medium, and high AMH groups achieving pregnancy during the six cycles are illustrated in Figure 1. Compared with the reference group of women with medium AMH levels, the unadjusted odds ratios of not becoming pregnant within the first six cycles for those with low AMH and high AMH were 1.35 (95% CI 0.63–2.89) and 1.60 (0.76–3.39), respectively (Fig. 1 and Table 2). Compared with women with medium AMH, the monthly probabilities of conceiving (FR) for those with low and high AMH were 0.87 (95% CI 0.51–1.46) and
did not have intercourse between day 11 and day 20 before menstrual bleeding (22 cycles) did not change the estimates (FR 0.60 [95% CI 0.38–0.97]). To evaluate whether reduced fecundability in the women with high AMH level were caused by women with PCOS, we reanalyzed the data after exclusion of women with irregular cycles (n = 14). The reduced FR in women with high AMH levels and regular cycles was maintained (unadjusted FR 0.55 [95% CI 0.30–0.98] and adjusted FR 0.48 [95% CI 0.27–0.85]). The prevalence of irregular menstrual cycles and hyperandrogenemia was higher in women with high AMH (3 of 30) compared to women with medium AMH (0 of 96), P = .012.

Women with high AMH levels had higher levels of LH (geometric mean, 8.4 vs. 5.3 IU/L; P = .017) and LH:FSH ratio (2.4 vs. 1.8; P = .016) than the reference group of women with medium AMH (Supplemental Fig. 1). There was a trend toward elevated levels of T (2.0 vs. 1.7 nmol/L; P = .069) and free androgen index (3.0 vs. 2.4; P = .102) in women with high AMH compared to women with medium AMH (Supplemental Fig. 2). Levels of HbA1c (4.8% vs. 4.8%; P = .585, data not shown) and BMI (21.7 vs. 22.1 kg/m²; P = .481, data not shown) were not different in women with high AMH levels compared to the reference group of women with medium AMH. After exclusion of women with irregular cycles, the LH:FSH ratio remained elevated (2.4 vs. 1.7; P = .031) and LH tended to be higher (7.8 vs. 5.2 IU/L; P = .065) in women with high AMH levels.

The AMH levels were evenly scattered among the 19 women with high AMH achieving pregnancy (range, 40–98 pmol/L). Thus, it was not possible to establish a cutoff level of AMH with a positive predictive value of subfecundability.

### DISCUSSION

To our knowledge, this is the first prospective study evaluating AMH as a marker of fecundability in healthy young women with no prior knowledge of their fecundity. Although very low AMH levels have been reported to be associated with reduced fecundability in one study, which included older women (20), in the present study low AMH levels were not associated with reduced fecundability. However, we had limited power in the lowest AMH range, as few women had very low AMH levels. Conversely, the probability of conceiving was reduced approximately 40% in women with high AMH levels, and this persisted after exclusion of women with irregular cycles.

In IVF settings, low AMH levels predict poor ovarian response (17), and high levels predict ovarian hyper-response syndrome (31). Very low AMH levels (≤0.7 ng/mL, equal to ≤10 pmol/L) have been associated with subfecundability in healthy women aged 30–42 years (20), and we have previously found AMH <8 pmol/L to be a sensitive and specific marker of premature ovarian failure (POF) in adolescents with Turner syndrome (6). Thus, we expected low AMH levels to be a predictor of prolonged time to pregnancy. However, in the present study, women with low AMH (lowest quintile) and very low AMH (<10 pmol/L) did not have reduced fecundability compared with the reference group of women with medium AMH levels. This study does not have the power to determine the effect of even lower AMH levels. It is difficult

### TABLE 1

Baseline characteristics by subgroups of low, medium, and high AMH levels.

<table>
<thead>
<tr>
<th>AMH levels</th>
<th>Low</th>
<th>Medium</th>
<th>High</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>36</td>
<td>113</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td>Median (pmol/L)</td>
<td>10</td>
<td>22</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Range (pmol/L)</td>
<td>0–13</td>
<td>14–39</td>
<td>40–183</td>
<td></td>
</tr>
<tr>
<td>25th–75th percentile (pmol/L)</td>
<td>7–12</td>
<td>18–30</td>
<td>43–73</td>
<td></td>
</tr>
</tbody>
</table>

Female characteristics

- Age groups (y)
  - 19–24: 17, 30, 30
  - 25–29: 75, 61, 62
  - 30–35: 8, 9, 9
- Smoking
  - Never: 42, 45, 51
  - Ever: 28, 23, 11
- Current: 30, 32, 38
- Diseases affecting fecundability
  - No: 89, 88, 86
  - Yes: 11, 12, 14
- BMI (kg/m²)
  - <20: 19, 19, 22
  - 20–25: 56, 62, 61
  - >25: 25, 19, 17
- Maternal smoking during pregnancy
  - No: 52, 66, 50
  - Yes: 48, 34, 50
- Cycle regularity
  - Regular cycles: 53, 68, 60
  - Irregular cycles: 0, 5, 24
  - Regular on combination OC: 47, 27, 16
  - Cycle length (d)
    - 20–24: 3, 0, 5
    - 25–34: 97, 89, 46
    - >35: 0, 11, 49
- Male characteristics
  - Diseases affecting fecundability
    - No: 89, 92, 89
    - Yes: 11, 8, 11
  - Male oligozoospermia
    - No: 92, 86, 92
    - Yes: 8, 14, 8

Note: AMH levels listed in percentages. Group differences tested by χ² test. AMH = anti-mullerian hormone; BMI = body mass index; OC = oral contraceptive.


0.67 [95% CI 0.42–1.08], respectively (Table 2, unadjusted data in model 1).

In the low AMH group, the adjusted FR was not different to the reference group, 0.81 (95% CI 0.44–1.40). Performing the analysis while applying the same cutoff point as used in the study by Steiner et al. (20) (AMH ≤0.7 ng/mL, corresponding to 10 pmol/L by the assay system used herein) and then comparing these women with the reference group with medium AMH, resulted in a FR of 0.88 (95% CI 0.48–1.61). The ratio did not change after adjusting for covariates. In women with low AMH, FSH (4.3 vs. 3.0 IU/L; P = .027) and SHBG (88 vs. 71 nmol/L; P = .031) were elevated compared with the reference group of women with medium AMH (Supplemental Figs. 1 and 2, available online).

After adjustment for covariates, women with high AMH level had significantly reduced FR compared with the reference group with medium AMH (FR 0.62 [95% CI 0.39–0.99]) (Table 2, model 2). Excluding the cycles in which the couple
to compare the present study findings with that of Steiner et al. (20) as their cohort of women were much older, it was more heterogeneous (37% had previous pregnancies), and male confounders were not evaluated. Corresponding to the higher age, the prevalence of very low AMH (<0.7 ng/mL, equal to <10 pmol/L) was higher in that study compared to this one (18% vs. 11%). The present findings suggest that AMH in the lowest part of the normal range (4–58 pmol/L) among young women is not a predictor of reduced fecundability. Therefore, assessment of circulating AMH as a clinical marker of reduced fecundability and as a tool in epidemiological studies of female fecundability must be interpreted with caution (32). Nevertheless, in clinical counseling of individual reproductive capacity, low AMH level may be associated with early age of menopause (11–13). Two women with highly elevated FSH concentrations (and regular menstrual cycles) were largely responsible for the significantly higher FSH levels in women in the low AMH group, indicating a low ovarian reserve and what has been termed occult ovarian insufficiency (33). One achieved pregnancy during follow up. In the IVF-related literature, low AMH level is more useful as a marker of follicle quantity than oocyte quality. This study demonstrates that in the context of natural conception in young women, where only one oocyte is involved, low AMH level also does not reflect oocyte quality.

Steiner et al. (20) did not report whether women with the highest AMH levels had reduced fecundability. We found that apparently healthy women with the highest levels of AMH had significantly reduced fecundability compared with women with medium levels. Inclusion of women with a phenotype including part of the heterogeneous PCOS seems to be the most likely explanation to our findings. The features of PCOS include subfecundability, menstrual cycle irregularity, insulin resistance, overweight, clinical and biochemical hyperandrogenism, elevated LH, and elevated LH:FSH ratio (34). According to the Rotterdam criteria, PCOS is diagnosed if two of the following three criteria are fulfilled: polycystic ovarian morphology, clinical or biochemical hyperandrogenism, elevated LH, and elevated LH:FSH ratio (35). Ovarian ultrasound was not performed, and information on clinical signs of hirsutism was not obtained as the questionnaire in this study was not designed to address whether the women had PCOS. Retrospectively, PCOS could therefore only be diagnosed with confidence in three women with high AMH levels, irregular menstrual cycles, and hyperandrogenemia, with another six women of this group of 37 having irregular cycles. The hormone profile in women with high AMH levels also showed

![FIGURE 1](A) Kaplan-Meier curves showing cumulative proportion of pregnancy by serum level of antimüllerian hormone (AMH). Low AMH (quintile 1) orange line, medium AMH (quintiles 2–4) black line, high AMH (quintile 5) red line. P value describes difference between curves (log-rank test); P = 0.289. (B) Antimüllerian hormone level as a function of age in 186 participating women. Colors correspond to subgroups of AMH levels: low (orange), medium (black), and high (red).

features of PCOS compared with the reference group of women with medium AMH (i.e., elevated LH, LH:FSH ratio, and a trend toward hyperandrogenemia). To exclude women with likely PCOS, the data were reanalyzed after excluding those women with irregular cycles; reduced fecundability was still apparent in the high AMH group. We acknowledge that some of the women with regular cycles on combined contraceptive pill may have concealed irregular cycles and PCOS, but their presence in the medium AMH group would tend to underestimate the association with fecundability between the medium and high AMH groups. Although having regular cycles does not exclude the diagnosis of PCOS, the persistence of reduced fecundability in the high AMH group after exclusion of women with irregular cycles suggests that inclusion of women with clearly diagnosable PCOS is not the sole reason for the finding of reduced fecundability in the high AMH group. These findings, therefore, identify a group of women with regular cycles but high follicular activity (reflected in their high AMH concentrations) who have a PCOS-like ovarian phenotype and reduced fecundability. These women may be similar to the group often termed ovulatory PCOS (36). Limited data concerning fecundity in women with ovulatory PCOS exist; however, reduced fecundability has been suggested (37). The BMI and HbA1C levels indicate that the phenotype does not include obesity or elevated long-term glucose levels in this study population of relatively lean women. In support of our findings, the prevalence of nonobese Danish women having polycystic ovaries (PCO) on ultrasound examination is high (38). Furthermore, Danish women with high AMH levels have elevated circulating levels of T and longer menstrual cycles compared with women with medium and low AMH levels (39).

Antimüllerian hormone has been proposed as a diagnostic test in PCOS (40). We were not able to establish an AMH cutoff level with a useful positive predictive value of subfecundability; however, future larger studies may be able to do this.

This is a large prospective study based on the general population including information on lifestyle and menstrual cycle characteristics. The participants in the study are likely to be selected, but as women are unaware of their AMH levels and fecundability, this is unlikely to have affected their motivation to participate. Thus, we believe we have achieved an accurate estimate of AMH as a predictor of fecundability in healthy young women. However, couples with unplanned pregnancies are excluded in this type of study, and those women may have different AMH levels. The main limitation of the study is limited statistical power, which does not allow for analyses in subgroups.

Individual AMH levels are relatively unaffected by phase of the menstrual cycle (24), and in peripubertal girls and adolescents AMH levels show only minor fluctuations over time (5). Thus, a randomly sampled AMH seems to be representative, whereas individual levels of other reproductive hormones may be affected by the nonstandardized drawing of samples during the menstrual cycle. However, the risk of measuring peaking gonadotropins due to ovulation was decreased in women with long and irregular menstrual cycles, indicating that this was unlikely to be the cause of the higher LH concentrations and LH:FSH ratio in women with high AMH levels.

Given the long-term storage of serum samples (approximately 15 years) it is possible that integrity of the serum samples for hormone analysis is reduced. Unfortunately, to our knowledge, there are no studies on the degradation of AMH in long-term stored frozen serum samples. However, in our hands, AMH has proven to be extremely stable during repeated freeze-thaw cycles (6), and all samples were handled identically.

In the adjusted models, we included covariates previously reported to affect fecundability. Women with subfecundability due to low AMH levels or a PCOS-like phenotype (high AMH) would most likely present with menstrual cycle irregularity. Therefore, we did not include this variable in the standard adjusted model, as this is probably an intermediate factor between AMH and fecundability.

In conclusion, low serum AMH levels in young healthy women do not seem to be a predictor of reduced fecundability. This is consistent with high oocyte quality in these young women, despite a reduced ovarian reserve. Conversely, the probability of conceiving was reduced approximately 40% in women with high AMH levels, and this persisted after exclusion of women with irregular cycles. This may be caused by an intermediate phenotype between healthy women and those with PCOS.

### TABLE 2

<table>
<thead>
<tr>
<th>Pregnancies (n)/ subfecundity (%)</th>
<th>Odds of subfecundability (OR; 95% CI)</th>
<th>Fecundability ratio (FR; 95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1 (n = 186)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>20 (44)</td>
<td>1.35 (0.63–2.89)</td>
</tr>
<tr>
<td>Medium</td>
<td>71 (37)</td>
<td>1</td>
</tr>
<tr>
<td>High</td>
<td>19 (49)</td>
<td>1.60 (0.76–3.39)</td>
</tr>
<tr>
<td>Model 2 (n = 185)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>20 (44)</td>
<td>1.55 (0.69–3.47)</td>
</tr>
<tr>
<td>Medium</td>
<td>71 (37)</td>
<td>1</td>
</tr>
<tr>
<td>High</td>
<td>19 (49)</td>
<td>1.88 (0.83–4.25)</td>
</tr>
</tbody>
</table>

Note: Fecundability ratio <1 describes a poorer chance of attaining pregnancy. Women with medium AMH levels serve as the reference group. Model 1: unadjusted. Model 2: adjusted for female age, BMI, smoking, diseases affecting fecundability, and oligozoospermia. BMI = body mass index; CI = confidence interval; Fecundability ratio = monthly probability of conceiving; Odds of subfecundability = waiting >6 cycles to conceive; OR = odds ratio. Hagen. Low AMH predicts normal fecundability. Fertil Steril 2012.
REFERENCES


SUPPLEMENTAL FIGURE 1

(A) LH (IU/L), (B) FSH (IU/L), and (C) LH:FSH ratio in women with low antimüllerian hormone (AMH) (quintile 1, n = 32), medium AMH (quintiles 2–4, n = 96), and high AMH (quintile 5, n = 30). Left, hormone levels as a function of age. Orange triangles, low AMH levels; black triangles, medium AMH levels; red triangles, high AMH levels; black lines, geometric mean ± 1.96 SD, previously published (41). Right, boxplot of log_{10}-transformed hormone levels indicating median (fat line), 25th–75th percentile (box) and 2.5th–97.5th percentile (hinges). Arrow indicates a serum value below the chosen scale of y-axis. *P* values indicate level of significance (independent sample t-tests, low vs. medium and medium vs. high, LH, FSH, and LH:FSH ratio: *P* = .409, *P* = .017, *P* = .027, *P* = .328, *P* = .114, *P* = .016, respectively).

SUPPLEMENTAL FIGURE 2

(A) T (nmol/L), (B) sex hormone-binding globulin (SHBG; nmol/L), and (C) free androgen index in women with low antimüllerian hormone (AMH) (quintile 1, n = 32), medium AMH (quintiles 2–4, n = 96), and high AMH (quintile 5, n = 30). Left, hormone levels as a function of age. Orange triangles, low AMH levels; black triangles, medium AMH levels; red triangles, high AMH levels. Right, boxplot of log10-transformed hormone levels indicating median (fat line), 25th–75th percentile (box), and 2.5th–97.5th percentile (hinges). Arrow indicates a serum value above the chosen scale of y-axis. P values indicate level of significance (independent sample t-tests, low vs. medium, and medium vs. high, T, SHBG, and free androgen index: P = .230, P = .069; P = .031, P = .558; P = .013, P = .102, respectively).