Antimüllerian hormone as predictor of reproductive outcome in subfertile women with elevated basal follicle-stimulating hormone levels: a follow-up study

Felicia Yarde, M.D.,[a] Marlies Voorhuis, M.D.,[a] Madeleine Dólleman, M.D.,[a] Erik A. H. Knauff, M.D., Ph.D.,[a] Marinus J. C. Eijkemans, M.Sc.,[b] and Frank J. M. Broekmans, M.D., Ph.D.[a]

[a] Department of Reproductive Medicine and Gynaecology; and [b] Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht, the Netherlands

Objective: To investigate the role of serum antimüllerian hormone (AMH) as a predictor of live birth and reproductive stage in subfertile women with elevated basal FSH levels.
Design: A prospective observational cohort study conducted between February 2005 and June 2009.
Setting: Tertiary fertility center.
Patient(s): Subfertile women with [1] a regular menstrual cycle (mean cycle length 25–35 days); [2] basal FSH concentrations $\geq$ 12.3 IU/L; and [3] younger than 40 years (n = 96).
Intervention(s): None.
Main Outcome Measure(s): Live birth and reproductive stage according to the Stages of Reproductive Aging Workshop.
Result(s): A cumulative live birth rate of 63.5% was observed during a median follow-up of 3.3 years (n = 85). The AMH level was significantly associated with live birth. There was evidence of a nonlinear prediction pattern, with an increase in chances of live birth until an AMH level of 1 μg/L. Other ovarian reserve tests and chronological age appeared of limited value in predicting live birth. In addition, AMH was significantly associated with the timing of reproductive stages (n = 68) (i.e., the occurrence of menopausal transition or menopause during follow-up).
Conclusion(s): The present findings suggest applicability of AMH determination as a marker for actual fertility in subfertile women with elevated basal FSH levels. (Fertil Steril® 2013;100:831–8. ©2013 by American Society for Reproductive Medicine.)
Key Words: Subfertility, elevated basal FSH, antimüllerian hormone, prediction, live birth

Discuss: You can discuss this article with its authors and with other ASRM members at http://fertstertforum.com/yardef-amh-subfertility-elevated-basal-fsh/

Use your smartphone to scan this QR code and connect to the discussion forum for this article now.*

* Download a free QR code scanner by searching for "QR scanner" in your smartphone's app store or app marketplace.

Female reproductive aging is a process dominated by the gradual decline of both oocyte quantity and quality (1). With increasing chronological age, female fecundity decreases (2). The progressive follicle decline is accompanied by notable changes in menstrual cycle regularity with menopause as the final step in the ovarian aging process (3–5). Before cycle irregularity marks the onset of the perimenopausal transition, an increase in early follicular FSH level occurs, a clinical condition referred to as late reproductive aging (stage -3a) according to the Stages of Reproductive Aging Workshop (STRAW) classification (6). By definition this is the period before the onset of the menopausal transition, characterized by the presence of a regular menstrual cycle and elevated basal FSH levels, in women with a high probability of being infertile (7).

Received February 1, 2013; revised May 7, 2013; accepted May 8, 2013; published online June 10, 2013.
F.Y. has nothing to disclose. M.V. has nothing to disclose. M.D. has nothing to disclose. E.A.H.K. has nothing to disclose. M.J.C.E. has nothing to disclose. F.J.M.B. has received fees and grant support from the following companies (in alphabetical order): Ferring, Gedeon Richter, Merck Serono, Merck Sharp and Dome, and Roche.
Presented at the 28th annual meeting of the European Society of Human Reproduction and Embryology, Istanbul, Turkey, July 1–4, 2012.
Reprint requests: Felicia Yarde, M.D., Department of Reproductive Medicine and Gynaecology, University Medical Center Utrecht. P.O. Box 85500, 3508 GA Utrecht, the Netherlands (E-mail: F.Yarde@umcutrecht.nl).

Fertility and Sterility® Vol. 100, No. 3, September 2013 0015-0282/$36.00

http://dx.doi.org/10.1016/j.fertnstert.2013.05.009

Historically, FSH was the first tool to be identified for assessing ovarian reserve and, as a result, it is often routinely measured in the early follicular phase in the diagnostic workup of infertile couples. An elevation in FSH levels is generally thought to imply lower chances of pregnancy (8). However, alternate explanations for elevated basal FSH levels exist, including physiological causes where prolonged quiescence of the hypothalamic-pituitary-ovarian axis, such as during lactational amenorrhea (9) or after oral contraceptive (OC) use (10) elicits an overshoot secretion of FSH at resumption of the menstrual cycle. In addition, in mothers with familial dizygotic twins, elevated FSH levels are associated with an increase in the secretory drive of FSH instead of inadequate gonadal feedback (11). Another possible reason for slightly elevated FSH levels is the FSH receptor variant, where higher FSH levels are required to compensate for a less active receptor to obtain normal function, but these adjusted FSH levels are usually around the upper limit of the normal range (12–14). Therefore, in most women with regular cycles, elevated early follicular FSH levels will either be based on reduced ovarian reserve or increased secretory drive.

An ongoing debate exists about the value of an elevated basal FSH level in clinical practice. Is expectative management with regard to pregnancy prospects justified or should these women be advised to start infertility treatment immediately? Also, the long-term outcome in hypergonadotrophic women with regard to fertility remains unknown.

Antimüllerian hormone (AMH) is a novel method to reflect a woman's ovarian reserve. Recent studies suggest this dimeric glycoprotein to be superior and more reliable in comparison with FSH in predicting ovarian reserve (15–20). Synthesis and release from the later antral follicle stages will allow the build-up of serum levels, in a cycle independent fashion (18, 21–24). Because of the gradual loss of primordial follicles from the ovaries, which in turn affects the number of antral follicles at any given time, serum AMH level shows a consistent decline with increasing female age (24–27). Some small studies have suggested that in young hypergonadotropic women the combined information of age and AMH level could identify a subset of couples with still reasonable pregnancy prospects (28, 29).

In this context the question arises whether measuring AMH levels could identify a subgroup of women with manifest advanced ovarian aging and as such could be useful in the clinical management of subfertile hypergonadotrophic women. The aim of this study therefore is to investigate the role of AMH as a predictor of live birth and reproductive stage according to STRAW in subfertile, regularly cycling women with elevated basal FSH levels.

MATERIALS AND METHODS

Study Design

The proposed study, focusing on the role of AMH in predicting live birth and reproductive stage according to STRAW in subfertile women with elevated basal FSH levels, was designed as an observational follow-up cohort study.

Participants

Women under treatment at the Reproductive Medicine Unit of the University Medical Centre Utrecht with infertility and elevated FSH levels ($\geq$12.3 IU/L) at initial ovarian reserve screening were subjected to the so-called COLA (Cycle disorders, OLigo- and Amenorrhea, World Health Organization III) screening. All consecutively screened women were registered in the COLA database. We selected those women with a regular menstrual cycle (i.e., an average cycle length between 25 and 35 days), who were younger than 40 years of age, had serum FSH concentrations exceeding 12.3 IU/L in the early follicular phase (cycle days 2–5), and who were screened between February 2005 and June 2009. Exclusion criteria were poor ovarian response in a previous IVF cycle (<5 oocytes at retrieval) or cycle cancellation (<3 developing follicles of at least 12 mm in size), endocrine disease, and use of sex steroid medication (Supplemental Fig. 1, available online).

In the COLA screening procedure (t = 0) data on the medical history, obstetric and gynecologic history, as well as smoking status and use of medication were recorded. Physical examination was performed, including body height and weight. Transvaginal ultrasound was performed to assess the antral follicle count using the 7.5-MHz transvaginal probe on a Aloka SSD-4000 (Hitachi-Aloka Medical). Antral follicle count was calculated by adding the follicles with a diameter of 2–5 mm from both ovaries as these sizes of antral follicles show the strongest correlation with ovarian reserve status (30). For the counting procedure a standard systematic approach was used by the operators (31).

Fasting serum samples were drawn for endocrine markers. The FSH concentrations were measured using a chemoluminescence-based immunometric assay (ADVIA Centaur/Bayer) up until December 2006. Interassay and intra-assay coefficients of variation (CV) for this assay system were less than 3.9% and 2.9%. From January 2007 onward, there was an in-house change of immunoassay (Unicel DXI 800 Beckman Coulter). The 95% interassay and intra-assay CV in this assay were less than 4.3% and 3.4%. In-house correlation was performed in our laboratory, resulting in the following formula, which was consistent across the whole range of assay results: [FSH measured by DXI 800 BC] = 1.16 x [FSH measured by ADVIA Centaur] + 0.46 IU/L. The FSH levels measured by ADVIA Centaur were converted to the DXI Assay. Increased baseline FSH was then defined as a level of serum FSH more than 12.3 IU/L, which corresponds to 10.2 IU/L measured with the older ADVIA Centaur assay. The FSH cutoff of 10.2 IU/L that we used in this study was based on what the ADVIA Centaur assay considered to be the upper 95% reference value of the normal range. These references values were based on measures of FSH in the early follicular phase of healthy women without fertility problems.

Stored serum samples were used for measuring AMH levels using the sandwich ELISA (AMH Gen II ELISA, A79765, Beckman Coulter) in one complete batch. The detection limit of the assay was 0.20 μg/L, interassay CVs were 8.5% and 5.5% at 0.5 and 7.7 μg/L, respectively. None of the women had used any hormonal medication for at least 12 months before screening. Approval was obtained from the Institutional Review Board of the University Medical Center Utrecht.

Outcome: Live Birth

Outcome parameters were obtained after a follow-up of at least 12 months (1.1–5.2 years), when women filled out a standardized questionnaire concerning reproductive outcome. Women were asked whether they had been pregnant after the COLA visit and whether they made use of assisted reproductive technology (ART), including IUI, IVF, intracytoplasmic sperm injection (ICSI), or conceived spontaneously. Number of pregnancies and pregnancy outcomes were recorded, including fetal loss before 12 weeks or live birth.

Outcome: Reproductive Stage

Reproductive stage was assessed at the same time as reproductive outcome and was categorized according to the STRAW classification (6) into [1] regular menstrual cycles: average cycle length between 25 and 35 days; [2] menopausal transition: transformation to irregular cycles (> 35 days) or not able to predict the next menstrual bleed within 7 days precision, two or more skipped cycles, or at least one intermenstrual interval of ≥60 days; and [3] menopause: 12 consecutive months of amenorrhoea. Women were categorized as unknown if data concerning menstrual cycle were missing, due to current pregnancy or use of sex steroid medication. Reproductive stage refers to the presence or absence of having a regular menstrual cycle at follow-up.

Statistical Analysis

Descriptive parameters and patient characteristics were reported as mean ± SD or median (range) depending on the distribution. The Kaplan-Meier method was used to estimate the cumulative probability of live birth, with the period between COLA screening visit and live birth as the time variable. Women who did not achieve a pregnancy or had conceptions that resulted in a fetal loss before 12 weeks were censored at the date of filling out the questionnaire (t = 1). The predictive value of patient characteristics and ovarian reserve tests (ORTs) was analyzed using a Cox proportional hazard model of the time to live birth. Results were expressed as a hazard ratio. The log-rank test was used; $P<.05$ was considered statistically significant. Subsequently, a multivariable Cox proportional hazard analysis was performed, using a forward stepwise selection method on all prognostic factors and female age. In case of an undetectable AMH level (<0.20 μg/L), AMH values were arbitrarily assigned the level of 0.10 μg/L. To detect a possible nonlinear relationship between the predictive variables and outcome, a restricted cubic spline was used. For the second outcome, reproductive stage at follow-up, univariate and multivariate Cox proportional hazard models were used to investigate the predictive value of patient characteristics and ORTs.

Statistical analysis was performed using SPSS for Windows, version 20.0 and R version 2.15.1 (http://www.r-project.org).

RESULTS

A total of 138 eligible subfertile women with elevated basal FSH levels were identified from the COLA World Health Organization III database and were sent the questionnaire (see flow chart in Supplemental Fig. 1). Forty-two women did not respond after repeated effort by telephone or e-mail, resulting in 96 women who completed the questionnaire (response rate, 70%). The mean age at COLA screening was 35.0 (± 3.2) years (Table 1). In 11 women (11.5%), AMH levels in serum was undetectable. In case of a detectable AMH level, the median was 0.97 μg/L (range, 0.20–4.50 μg/L). Antral follicle count ranged from 0–20 with a median count of 5 follicles. Responders and nonresponders to the questionnaire did not differ in any of the baseline characteristics (data not shown). The median duration of follow-up was 3.3 years (range, 1.1–5.2 years), with a mean age at end of follow-up of 38.3 ± 3.2 years.

Outcome: Live Birth

The statistical analysis of reproductive outcome was performed only in those women who wished to conceive after the COLA visit and did not start an oocyte donation program. This resulted in 85 women eligible for analysis. After the COLA visit for infertility screening, 57 women (67.1%) became pregnant. Thirty-six women (63.2%) became pregnant using ART (20 IVF, 8 IUI, 8 ICSI) and 21 women (36.8%) conceived spontaneously. The choice for ART or expectant management was made by the clinician and based on applying a prediction protocol for spontaneous pregnancy (32), the preference of the couple, and additional factors that would affect the couple's fertility such as semen quality and tubal function. Three pregnancies resulted in a fetal loss before 12 weeks: two spontaneous conceptions and one ART pregnancy. In total 63.5% of the women carried a pregnancy to a live birth. The cumulative live birth rate, estimated by the Kaplan Meier method, is shown in Figure 1. After a follow-up of 1 year, 22.4% of the women reported a live birth of a baby. The cumulative live birth rate after 2 years of follow-up increased to 50.6%. The median time to reach a live birth was 14 months. No pregnancies occurred in case of an undetectable AMH level.

The results of the Cox proportional hazard models for the relationship between patient characteristics and ORTs on the

TABLE 1

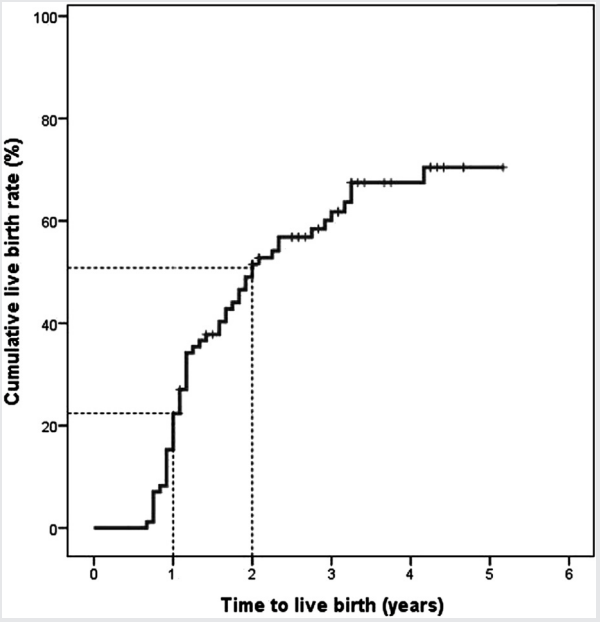
Baseline characteristics of subfertile, regularly cycling women with elevated basal FSH levels (n = 96).

Characteristic	Median	Range
Age at COLA screening (y)	35.4	24.5–39.6
Menstrual cycle minimum length (d)	26.0	21–30
Menstrual cycle maximum length (d)	29.0	21–35
Early follicular FSH (IU/L)	15.3	11–56
Undetectable AMH levels, n (%)	11.0	11.5%
AMH (μg/L) if detectable	0.97	0.20–4.50
Antral follicle count (2–5 mm)	5.0	0–20
Age at menarche (y)	13.0	9–17
Parity	0	0–2
Body mass index (kg/m^2)	22.0	17–40
Current smokers, n (%)	17.0	17.7%
Pack years if smoking	8.5	1–25
Duration infertility (y)	2.5	1.0–7.8

Note: Results in presented as median and range, except for undetectable AMH levels and smoking prevalence. AMH = antimüllerian hormone; COLA = Cycle disorders, OLigo- and Amenorrhea.

Yarde. Value of AMH in women with high FSH. Fertil Steril 2013.

FIGURE 1



Cumulative live birth rate in subfertile women with elevated basal FSH levels, calculated by the Kaplan-Meier method. Time to live birth includes the duration of the pregnancy. The dotted lines demonstrate the live birth rate after 1 and 2 years of follow-up.

Yarde. Value of AMH in women with high FSH. Fertil Steril 2013.

one hand and live birth on the other are shown in Table 2. Univariate analysis showed serum AMH and FSH levels to be significant predictors of live birth, within the total follow-up period of 5.2 years. In the multivariable analysis, including age at initial COLA screening, AMH and FSH level, only AMH level remained significant. Per unit (in micrograms per liter) increase of AMH the probability of a live birth increased by 31% (hazard ratio 1.31, 95% confidence interval 1.05–1.63). In addition, there was evidence from the spline analysis for a nonlinear pattern of AMH levels with an increase in chances of live birth until an AMH serum level of 1 μg/L (Supplemental Fig. 2A). Up to a 1 μg/L increase in AMH level was associated with higher live birth rates. More than 1 μg/L, however, further increases in AMH levels no longer resulted in significantly increased pregnancy rates (PRs) resulting in a live birth. This nonlinear effect was statistically significant ($P=.04$). This is illustrated in Supplemental Figure 2B where AMH is divided into two categories: AMH level <1 μg/L (n = 45) and AMH levels of ≥ 1 μg/L (n = 40). Supplemental Figure 2B demonstrates the cumulative live birth rates for both AMH categories.

Outcome: Reproductive Stage

At follow-up, 58 of the women (60.4%) still had regular menstrual cycles. Eight women (8.3%) developed irregular menses during follow-up and shifted toward menopausal transition, and two women (2.1%) reached (premature) menopause at the age of 37.5 and 38.4 years. Due to missing data, current pregnancy or hormone therapy at follow-up, reproductive stage remained unknown in 29.2% of the women. Predictors of reproductive stage are listed in Table 3. Sixty-eight women with a defined reproductive stage at follow-up were available for analysis: 58 regularly cycling women, 8 women who entered the menopausal transition, and 2 postmenopausal women. Univariate Cox proportional hazard analysis showed a significant influence of FSH and AMH levels on reproductive stage (Table 3). In the multivariate analysis, including age at initial COLA screening, FSH and AMH levels, only early follicular FSH level remained a significant predictor of reproductive stage, with a hazard ratio of 1.08 and 95% confidence interval of 1.03–1.14.

DISCUSSION

This study demonstrates that serum AMH level is an independent predictor of pregnancy resulting in live birth in subfertile women with elevated basal FSH levels. Also, the present cohort of women with elevated basal FSH levels did not have a strikingly poor pregnancy prognosis, as 67.1% became pregnant, of which 36.8% were spontaneous conceptions. These results suggest a limited predictive value of elevated

TABLE 2

Cox proportional hazard analysis for predictors of live birth in subfertile, regularly cycling women with elevated basal FSH levels (n = 85).

	Pregnancy resulting in live birth			Univariate hazard ratio
	Yes (n = 54)	No (n = 31)	*P* value	(95% CI)
Age at COLA visit (y)	34.3 ± 3.2	35.8 ± 3.4	.27	0.96 (0.90–1.03)
Duration of infertility (y)	2.8 ± 1.6	3.0 ± 1.6	.42	0.93 (0.78–1.11)
Early follicular FSH (IU/L)	15.5 ± 3.9	19.3 ± 8.4	.04	0.94 (0.88–0.996)
Undetectable AMH levels (n)	0 (0)	8.0 (25.8)	.07	0.04 (0.001–1.24)
AMH (μg/L)	1.50 ± 1.09	0.80 ± 0.87	.02	1.31 (1.05–1.63)
Antral follicle count 2–5 mm	6.0 ± 3.0	6.0 ± 4.0	.39	1.03 (0.96–1.10)
Menopausal transition or menopause (n)	1.0 (2.9)	4.0 (16.7)	.17	0.25 (0.03–1.81)
Pack years smoking (y)	3.3 ± 6.3	3.5 ± 5.1	.87	1.00 (0.95–1.05)
BMI (kg/m^2)	22.9 ± 4.0	22.9 ± 4.5	.99	1.00 (0.94–1.06)

Note: Hazard ratios for live birth with 95% confidence interval (CI). *P* values were determined using the log-rank test. AMH = antimüllerian hormone; BMI = body mass index; COLA = Cycle disorders, OLigo- and Amenorrhea.

Yarde. Value of AMH in women with high FSH. Fertil Steril 2013.

TABLE 3

Cox proportional hazard analysis for predictors of reproductive stage in subfertile, regularly cycling women with elevated basal FSH levels (n = 68).

	Menopausal transition or menopause at follow-up			Univariate hazard ratio
	No (n = 58)	Yes (n = 10)	*P* value	(95% CI)
Age at COLA visit (y)	35.0 ± 3.6	35.1 ± 3.1	.62	1.05 (0.88–1.25)
Early follicular FSH (IU/L)	16.3 ± 4.4	26.6 ± 14.3	.002	1.08 (1.03–1.14)
Undetectable AMH levels (n)	5.0 (8.6)	3.0 (30.0)	.02	5.20 (1.26–21.53)
AMH (μg/L)	1.19 ± 0.93	0.45 ± 0.51	.02	0.09 (0.01–0.72)
Antral follicle count 2–5 mm	6.0 ± 4.0	4.0 ± 3.0	.39	0.90 (0.70–1.15)
Pack years smoking (y)	2.6 ± 4.7	4.1 ± 6.0	.21	1.07 (0.96–1.20)
BMI (kg/m^2)	22.9 ± 3.8	21.2 ± 2.6	.56	0.93 (0.72–1.19)
Age at follow-up (y)	38.2 ± 3.5	38.7 ± 3.3	.93	0.99 (0.85–1.17)

Note: Hazard ratios for the occurrence of menopausal transition or menopause at follow-up (reproductive stage according to STRAW [Stages of Reproductive Aging Workshop]) with 95% confidence interval (CI). *P* values were determined using the log-rank test. AMH = antimüllerian hormone; BMI = body mass index; COLA = Cycle disorders, OLigo- and Amenorrhea.

Yarde. Value of AMH in women with high FSH. Fertil Steril 2013.

basal FSH level in young women with regular menstrual cycles during infertility evaluation. To our knowledge this is the first study to demonstrate serum AMH level as a predictor of live birth in subfertile women with elevated early follicular FSH levels in a prospective study with the outcome live birth after both ART and spontaneous conceptions.

The reported pregnancy prospects are in line with previous publications concerning hypergonadotropic, regularly cycling, subfertile women (33–35). Van Rooij et al. (35) found an ongoing PR of 39% in regularly cycling, subfertile women with basal FSH levels between 15 and 20 IU/L. A prospective cohort study from the same group demonstrated an ongoing PR per ET of 40% in women with elevated basal FSH levels (34). In another population of young, regularly cycling, subfertile women with a FSH level >10 IU/L a live birth rate of 42% was observed (33). These findings are in contrast to the well-known suggestions of basal FSH levels being a strong predictor of nonsuccess in ART (36). However, in the studies on IVF cutoff levels for FSH were often much higher and exposure to pregnancy was established in a single treatment cycle. It is therefore not unexpected that various studies have demonstrated much lower accuracy for basal FSH level in predicting outcome pregnancy after IVF (37), especially if cumulative cycles were considered (38).

The present results do suggest that both AMH and FSH forecast the advent of menopausal transition and menopause at follow-up. Assessment of both of these ORTs in a multivariate model reveals that FSH may be a stronger predictor for timing future cycle status than AMH in women with already elevated basal FSH levels. These results should be interpreted with caution as only a subgroup of 68 women was available for the analysis of reproductive stage at follow-up. In addition, from the available subgroup only two women reached menopause and eight women entered the menopausal transition.

In line with the endocrine changes accompanying the menopausal transition, FSH level becomes increasingly elevated, whereas AMH levels may become very low or undetectable quite soon after cycle irregularity has become established (39). With this in mind it can be theorized that in this specific group of women, FSH is a better marker of the short-term event menopausal transition, whereas AMH functions better as a long-term predictor of the reproductive event menopause (as AMH levels start to decline before FSH elevations become evident).

Surprisingly, no statistically significant effect for female age was found in predicting live birth in subfertile women with elevated basal FSH levels. A possible explanation for this finding could be the relatively small size of this cohort. Also, the predictive capacity of female age may be flawed by an asymmetrical distribution of age across the cohort. However, the age at baseline ranged between 25 and 39 years, which is not an inadequate distribution. Also, there was no evidence for a nonlinear distribution of age as the spline function analysis of age in relation to hazard of having a live birth was not significant (P=.09). Aside from methodological explanations, such as age distribution and sample size, it may be hypothesized that female age is not predictive in this specific phenotype of diminished ovarian reserve. Perhaps the limited reserve is generally reflected by the elevated FSH level and the true quantity of the remaining follicles is better represented by serum AMH level. Because AMH levels decrease before there is a substantial increase in basal FSH level, one would expect AMH levels to be very low once FSH is elevated. However, our findings demonstrate that there is a subgroup of women who have relatively high AMH levels (≥1 μg/L), despite elevated FSH levels, with better pregnancy prospects than women with low AMH levels. The latter is supported by the observation that no pregnancies occurred in women with undetectable AMH levels trying to conceive after the COLA visit (n = 8). Taken together the expression of quantity is clearly better from AMH than basal FSH levels, a finding that corresponds to ovarian response studies in ART, where FSH has demonstrated to be less well related to outcome categories such as poor or excessive response (40). The observation that no pregnancies occurred in women with undetectable AMH levels is not surprising, as our cohort represents women with already unfavorable pregnancy prospects due to an elevated FSH level. However, based on the relatively small amount of women with undetectable AMH levels, this observation does not fully exclude the possibility of a pregnancy occurring. The discrepancies with other studies, where pregnancies have

been reported in women with undetectable AMH levels (41, 42) may stem from various sources such as AMH assay failures or variation in storing and handling of the samples (43). Also, truly undetectable AMH levels may spuriously indicate a poor ovarian reserve if the sample has been taken during OC usage (44).

Ovarian reserve is currently defined as an interplay between the quantity and quality of the follicles left in the ovary and several proxy variables for pool size are well described in the literature. However, whether current ORTs can predict pregnancy, which is often used as a proxy for oocyte quality, is still a matter of debate (36, 45, 46). Oocyte quality, however, is thought to be predominantly affected by female age. An explanation for our finding that AMH level can predict live birth in this cohort could be the fact that higher AMH values are associated with a higher oocyte yield in IVF treatment. This higher oocyte yield consequently results in higher chances of pregnancy. This notion has received support from recent reports (25, 47). Because a large proportion of our cohort tried to conceive with ART, fecundity is highly dependent on the number of available follicles and this could be the driver of this phenomenon. The current follow-up study of subfertile women with elevated basal FSH levels was not designed to compare treatment methods, leaving this question unanswered. However, with regard to the time to live birth after the COLA screening, no differences were observed between the women who conceived naturally and those who underwent ART (1.6 ± 0.99 years vs. 1.5 ± 0.69 years). In addition, in the comparison of women who conceived quickly (live birth within 1 year of follow-up, $n = 13$) and those who took longer to reach a live birth (≥ 2 years, $n = 13$), the proportion of women who conceived naturally was similar to those who underwent ART. Also, no differences were observed in patient characteristics or ORTs in women who conceived quickly or those who took longer to conceive.

The association between serum AMH level and oocyte yield in IVF treatment could also explain the observation that an AMH level >1 μg/L no longer resulted in significantly increased live birth rates. Previous studies (48–51) have demonstrated that there is an optimal range of oocyte numbers for achieving pregnancy. Only below a certain oocyte number, pregnancy prospects are clearly affected. Our demonstrated cutoff of 1 μg/L might be the lower limit of this optimum and therefore women with an AMH >1 μg/L will have chances of pregnancy irrespective of the specific level of AMH. This has also been demonstrated in ART studies where AMH and female age were used to model prognosis categories (52).

The strength of this study lies in the fact that this is a well-defined cohort of subfertile women with elevated early follicular FSH levels. We used strict inclusion criteria to ensure all women were regularly cycling and younger than 40 years of age with a basal FSH of 12.3 IU/L or higher. Within our well-defined cohort we observed a wide variation in the ovarian reserve parameters, suggesting that women with elevated FSH levels constitute a heterogeneous group.

To prevent selection bias, women with a poor response in IVF treatment before the COLA screening were excluded, as these women represent a group with already unfavorable pregnancy prospects, expressed by their poor response to ovarian hyperstimulation. In fact, measuring basal FSH and additionally AMH levels, for prognosis assessment and possible adjustment of the treatment, preferably takes place before starting ART. By adding poor responders with identification of elevated FSH level post hoc this study group would become (too) heterogeneous. However, additional analysis including those women with a poor response to IVF treatment revealed that the effect of AMH on prediction of live birth remained the same. It should also be noted that a single measurement of early follicular FSH was used. Temporary normalization of FSH levels is known to occur (53, 54). However, it has been shown that subfertile women with elevated basal FSH levels will always demonstrate some degree of diminished ovarian reserve, even if repeated measurements will yield normal FSH levels (55). A limitation of this study is that some information bias may have occurred. Women who failed to achieve a pregnancy or with unfavorable pregnancy outcomes might have been less inclined to respond to the questionnaires leading to an under-representation of unfavorable pregnancy outcomes and overoptimistic PRs. However, even when a more pessimistic scenario is applied by assuming that all women who did not respond to our questionnaire did not become pregnant, a PR of 41.3%, instead of the observed PR of 67.1%, would still be calculated. With regard to other factors that may influence PRs, the responders and nonresponders did not differ in any of the baseline characteristics. Finally, AMH measurements were carried out on samples that had been stored at -20°C. Currently, the debate on the effect of freezing and thawing on AMH measurements has not been fully completed (56). The fact that this procedure has been applied in all women in the cohort excludes the possibility of creating a large source of bias.

The cutoff value for FSH of 12.3 IU/L should also be considered. Different studies, using different outcomes, have used different FSH cutoff levels (37). Our cutoff of 12.3 IU/L is based on the conversion of the upper limit (10.2 IU/L) of the normal range of the assay used at that time. As noted by STRAW in 2001, most clinicians use a FSH of 10 IU/L as cutoff value (57).

The value of the present findings for clinical practice may be that subfertile women with elevated basal FSH levels may still have reasonable pregnancy prospects. Denial of treatment in these women therefore does not seem to be justified in most cases. Further assessment using AMH level or the response in a trial cycle of IVF may be the way to sort out those women with still reasonable prognosis, and those who may better be referred to egg donation programs. Both early follicular FSH and serum AMH levels could be used as a guide to advise subfertile couples on their pregnancy prospects. However, serum AMH levels provide a more robust cutoff, as our study demonstrated that women with undetectable serum AMH levels were a subgroup with pregnancy prospects close to zero. The conclusion regarding the prediction of reproductive stage at follow-up are based on small numbers and should therefore not be extrapolated to clinical practice.

In summary, this is the first study to suggest AMH as a single predictor for live birth in subfertile women with

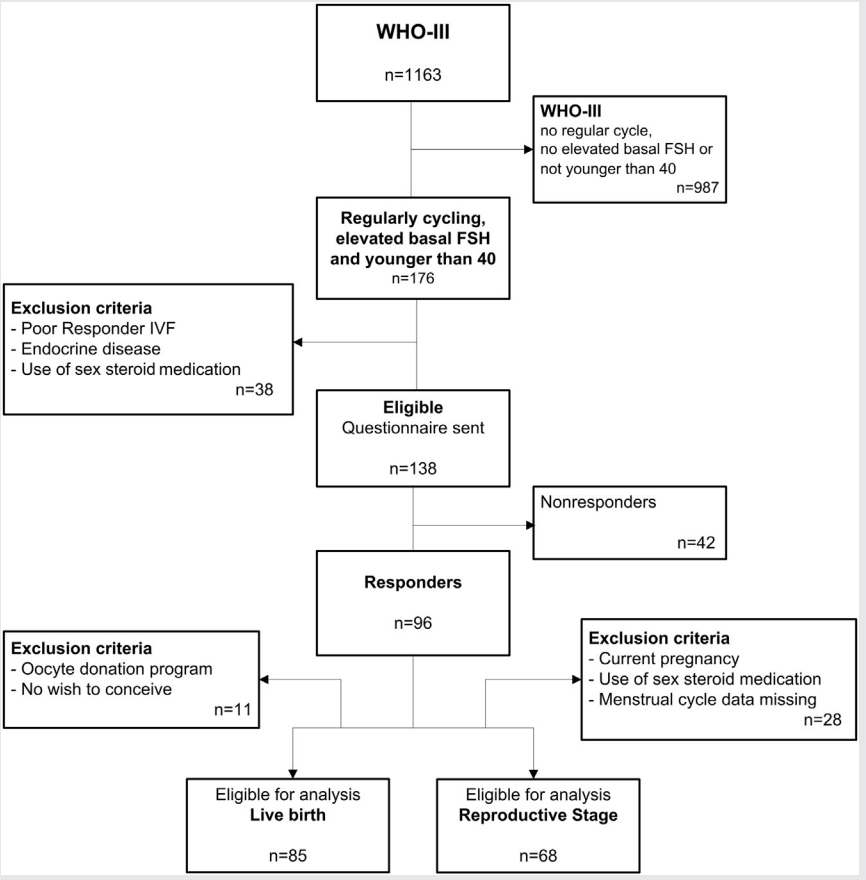
elevated basal FSH levels. These findings suggest that AMH may be applied to identify those women with very poor pregnancy prospects. Also, this study indicates that both AMH and FSH are predictors of the timing of reproductive stages to develop in this specific group of women with elevated FSH levels and subfertility.

REFERENCES

1. Te Velde ER, Pearson PL. The variability of female reproductive ageing. Hum Reprod Update 2002;8:141–54.
2. Broekmans FJ, Soules MR, Fauser BC. Ovarian aging: mechanisms and clinical consequences. Endocrinol Rev 2009;30:465–93.
3. Faddy MJ, Gosden RG, Gougeon A, Richardson SJ, Nelson JF. Accelerated disappearance of ovarian follicles in mid-life: implications for forecasting menopause. Hum Reprod 1992;7:1342–6.
4. Hansen KR, Knowlton NS, Thyer AC, Charleston JS, Soules MR, Klein NA. A new model of reproductive aging: the decline in ovarian non-growing follicle number from birth to menopause. Hum Reprod 2008;23:699–708.
5. Wallace WH, Kelsey TW. Ovarian reserve and reproductive age may be determined from measurement of ovarian volume by transvaginal sonography. Hum Reprod 2004;19:1612–7.
6. Harlow SD, Gass M, Hall JE, Lobo R, Maki P, Rebar RW, et al. Executive summary of the Stages of Reproductive Aging Workshop + 10: addressing the unfinished agenda of staging reproductive aging. J Clin Endocrinol Metab 2012;97:1159–68.
7. Sherman BM, Korenman SG. Hormonal characteristics of the human menstrual cycle throughout reproductive life. J Clin Invest 1975;55:699–706.
8. Scott RT Jr, Hofmann GE. Prognostic assessment of ovarian reserve. Fertil Steril 1995;63:1–11.
9. Tay CC, Glasier AF, McNeilly AS. Effect of antagonists of dopamine and opiates on the basal and GnRH-induced secretion of luteinizing hormone, follicle stimulating hormone and prolactin during lactational amenorrhoea in breastfeeding women. Hum Reprod 1993;8:532–9.
10. Jernstrom H, Knutsson M, Olsson H. Temporary increase of FSH levels in healthy, nulliparous, young women after cessation of low-dose oral contraceptive use. Contraception 1995;52:51–6.
11. Lambalk CB, Boomsma DI, de Boer L, de Koning CH, Schoute E, Popp-Snijders C, et al. Increased levels and pulsatility of follicle-stimulating hormone in mothers of hereditary dizygotic twins. J Clin Endocrinol Metab 1998;83:481–6.
12. De Koning CH, Benjamins T, Harms P, Homburg R, van Montfrans JM, Gromoll J, et al. The distribution of FSH receptor isoforms is related to basal FSH levels in subfertile women with normal menstrual cycles. Hum Reprod 2006;21:443–6.
13. Perez MM, Gromoll J, Behre HM, Gassner C, Nieschlag E, Simoni M. Ovarian response to follicle-stimulating hormone (FSH) stimulation depends on the FSH receptor genotype. J Clin Endocrinol Metab 2000;85:3365–9.
14. Sudo S, Kudo M, Wada S, Sato O, Hsueh AJ, Fujimoto S. Genetic and functional analyses of polymorphisms in the human FSH receptor gene. Mol Hum Reprod 2002;8:893–9.
15. Knauff EA, Eijkemans MJ, Lambalk CB, ten Kate-Booij MJ, Hoek A, Beerendonk CC, et al. Anti-Mullerian hormone, inhibin B, and antral follicle count in young women with ovarian failure. J Clin Endocrinol Metab 2009;94:786–92.
16. Broer SL, Mol B, Dolleman M, Fauser BC, Broekmans FJ. The role of anti-Mullerian hormone assessment in assisted reproductive technology outcome. Curr Opin Obstet Gynecol 2010;22:193–201.
17. 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;21:2022–6.
18. Hazout A, Bouchard P, Seifer DB, Aussage P, Junca AM, Cohen-Bacrie P. 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 2004;82:1323–9.
19. 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;22:2414–21.
20. Riggs RM, Duran EH, Baker MW, Kimble TD, Hobeika E, Yin L, et al. Assessment of ovarian reserve with anti-Mullerian hormone: a comparison of the predictive value of anti-Mullerian hormone, follicle-stimulating hormone, inhibin B, and age. Am J Obstet Gynecol 2008;199:202–8.
21. 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;91:4057–63.
22. Kwee J, Schats R, McDonnell J, Themmen A, de Jong F, Lambalk C. Evaluation of anti-Mullerian hormone as a test for the prediction of ovarian reserve. Fertil Steril 2008;90:737–43.
23. La Marca A, Stabile G, Artenisio AC, Volpe A. Serum anti-Mullerian hormone throughout the human menstrual cycle. Hum Reprod 2006;21:3103–7.
24. Van Rooij I, Broekmans FJ, Scheffer GJ, Looman CW, Habbema JD, de Jong FH, et al. Serum antimullerian hormone levels best reflect the reproductive decline with age in normal women with proven fertility: a longitudinal study. Fertil Steril 2005;83:979–87.
25. 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;77:357–62.
26. La Marca A, Broekmans FJ, Volpe A, Fauser BC, Macklon NS. Anti-Mullerian hormone (AMH): what do we still need to know? Hum Reprod 2009;24:2264–75.
27. Tremellen KP, Kolo M, Gilmore A, Lekamge DN. Anti-mullerian hormone as a marker of ovarian reserve. Aust N Z J Obstet Gynaecol 2005;45:20–4.
28. Buyuk E, Seifer DB, Younger J, Grazi RV, Lieman H. Random anti-Mullerian hormone (AMH) is a predictor of ovarian response in women with elevated baseline early follicular follicle-stimulating hormone levels. Fertil Steril 2011;95:2369–72.
29. Gleicher N, Weghofer A, Barad DH. Anti-Mullerian hormone (AMH) defines, independent of age, low versus good live-birth chances in women with severely diminished ovarian reserve. Fertil Steril 2010;94:2824–7.
30. Haadsma ML, Bukman A, Groen H, Roeloffzen EM, Groenewoud ER, Heineman MJ, et al. The number of small antral follicles (2-6 mm) determines the outcome of endocrine ovarian reserve tests in a subfertile population. Hum Reprod 2007;22:1925–31.
31. Broekmans FJ, de Ziegler D, Howles CM, Gougeon A, Trew G, Olivennes F. The antral follicle count: practical recommendations for better standardization. Fertil Steril 2010;94:1044–51.
32. Van der Steeg JW, Steures P, Eijkemans MJ, Habbema JD, Hompes PG, Broekmans FJ, et al. Pregnancy is predictable: a large-scale prospective external validation of the prediction of spontaneous pregnancy in subfertile couples. Hum Reprod 2007;22:536–42.
33. Van Montfrans JM, Hoek A, van Hooff MH, de Koning CH, Tonch N, Lambalk CB. Predictive value of basal follicle-stimulating hormone concentrations in a general subfertility population. Fertil Steril 2000;74:97–103.
34. Van Rooij I, Bancsi LF, Broekmans FJ, Looman CW, Habbema JD, te Velde ER. Women older than 40 years of age and those with elevated follicle-stimulating hormone levels differ in poor response rate and embryo quality in in vitro fertilization. Fertil Steril 2003;79:482–8.
35. Van Rooij I, de Jong E, Broekmans FJ, Looman CW, Habbema JD, te Velde ER. High follicle-stimulating hormone levels should not necessarily lead to the exclusion of subfertile patients from treatment. Fertil Steril 2004;81:1478–85.
36. 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.
37. Bancsi LF, Broekmans FJ, Mol BW, Habbema JD, te Velde ER. Performance of basal follicle-stimulating hormone in the prediction of poor ovarian response and failure to become pregnant after in vitro fertilization: a meta-analysis. Fertil Steril 2003;79:1091–100.
38. Hendriks DJ, te Velde ER, Looman CW, Bancsi LF, Broekmans FJ. Expected poor ovarian response in predicting cumulative pregnancy rates: a powerful tool. Reprod Biomed Online 2008;17:727–36.

39. Sowers MR, Eyvazzadeh AD, McConnell D, Yosef M, Jannausch ML, Zhang D, et al. Anti-mullerian hormone and inhibin B in the definition of ovarian aging and the menopause transition. J Clin Endocrinol Metab 2008;93:3478–83.
40. Broer SL, van Disseldorp J, Broeze KA, Dolleman M, Opmeer BC, Bossuyt P, et al. Added value of ovarian reserve testing on patient characteristics in the prediction of ovarian response and ongoing pregnancy: an individual patient data approach. Hum Reprod Update 2013;19:26–36.
41. Weghofer A, Dietrich W, Barad DH, Gleicher N. Live birth chances in women with extremely low-serum anti-Mullerian hormone levels. Hum Reprod 2011;26:1905–9.
42. Fraisse T, Ibecheole V, Streuli I, Bischof P, de Ziegler D. Undetectable serum anti-Mullerian hormone levels and occurrence of ongoing pregnancy. Fertil Steril 2008;89:723–11.
43. Rustamov O, Smith A, Roberts SA, Yates AP, Fitzgerald C, Krishnan M, et al. Anti-Mullerian hormone: poor assay reproducibility in a large cohort of subjects suggests sample instability. Hum Reprod 2012;27:3085–91.
44. Dólleman M, Verschuren WMM, Eijkemans MJC, Dollé MET, Jansen EHJM, Broekmans FJM, et al. Reproductive and lifestyle determinants of anti-Müllerian hormone in a large population-based study. J Clin Endocrinol Metab 2013;98:2106–15.
45. Broer SL, Mol BW, Hendriks D, Broekmans FJ. The role of antimullerian hormone in prediction of outcome after IVF: comparison with the antral follicle count. Fertil Steril 2009;91:705–14.
46. Silberstein T, MacLaughlin DT, Shai I, Trimarchi JR, Lambert-Messerlian G, Seifer DB, et al. Mullerian inhibiting substance levels at the time of HCG administration in IVF cycles predict both ovarian reserve and embryo morphology. Hum Reprod 2006;21:159–63.
47. Van Rooij I, Broekmans FJ, te Velde ER, Fauser BC, Bancsi LF, de Jong FH, et al. Serum anti-Mullerian hormone levels: a novel measure of ovarian reserve. Hum Reprod 2002;17:3065–71.
48. Sunkara SK, Rittenberg V, Raine-Fenning N, Bhattacharya S, Zamora J, Coomarasamy A. Association between the number of eggs and live birth in IVF treatment: an analysis of 400 135 treatment cycles. Hum Reprod 2011;26:1768–74.
49. Timeva T, Milachich T, Antonova I, Arabaji T, Shterev A, Omar HA. Correlation between number of retrieved oocytes and pregnancy rate after in vitro fertilization/intracytoplasmic sperm infection. ScientificWorldJournal 2006;6:686–90.
50. Van der Gaast MH, Eijkemans MJ, van der Net JB, de Boer EJ, Burger CW, van Leeuwen FE, et al. Optimum number of oocytes for a successful first IVF treatment cycle. Reprod Biomed Online 2006;13:476–80.
51. Yih MC, Spandorfer SD, Rosenwaks Z. Egg production predicts a doubling of in vitro fertilization pregnancy rates even within defined age and ovarian reserve categories. Fertil Steril 2005;83:24–9.
52. La Marca A, Nelson SM, Sighinolfi G, Manno M, Baraldi E, Roli L, et al. Anti-Mullerian hormone-based prediction model for a live birth in assisted reproduction. Reprod Biomed Online 2011;22:341–9.
53. Brown JR, Liu HC, Sewitch KF, Rosenwaks Z, Berkeley AS. Variability of day 3 follicle-stimulating hormone levels in eumenorrheic women. J Reprod Med 1995;40:620–4.
54. Jain T, Klein NA, Lee DM, Sluss PM, Soules MR. Endocrine assessment of relative reproductive age in normal eumenorrheic younger and older women across multiple cycles. Am J Obstet Gynecol 2003;189:1080–4.
55. De Koning CH, McDonnell J, Themmen AP, de Jong FH, Homburg R, Lambalk CB. The endocrine and follicular growth dynamics throughout the menstrual cycle in women with consistently or variably elevated early follicular phase FSH compared with controls. Hum Reprod 2008;23:1416–23.
56. Kumar A, Kalra B, Patel A, McDavid L, Roudebush WE. Development of a second generation anti-Mullerian hormone (AMH) ELISA. J Immunol Methods 2010;362:51–9.
57. Soules MR, Sherman S, Parrott E, Rebar R, Santoro N, Utian W, et al. Stages of Reproductive Aging Workshop (STRAW). J Womens Health Gend Based Med 2001;10:843–8.

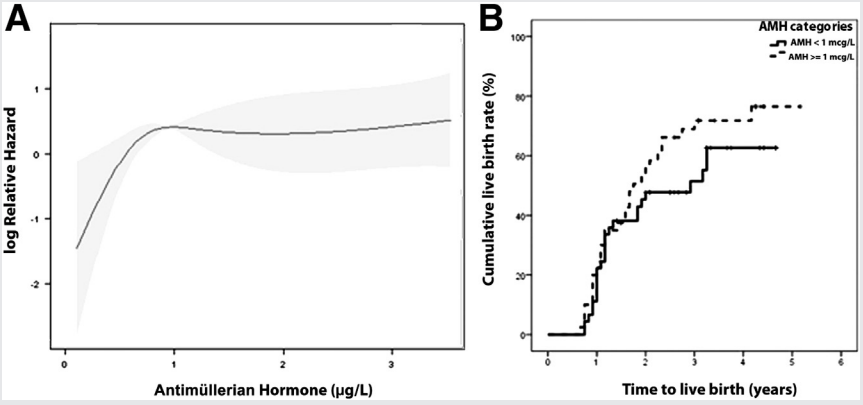
SUPPLEMENTAL FIGURE 1



Search and selection of eligible subfertile women with elevated basal FSH levels from the COLA World Health Organization (WHO) III database of the University Medical Centre Utrecht until June 2009.

Yarde. Value of AMH in women with high FSH. Fertil Steril 2013.

SUPPLEMENTAL FIGURE 2



(A) Nonlinear spline analysis between serum antimüllerian hormone (AMH) level in micrograms per liter and live birth rate. An increase in AMH was associated with higher live birth rates up to 1 μg/L. More than 1 μg/L further increases in AMH no longer resulted in significantly increased live birth rates. (B) Kaplan-Meier curve demonstrating the cumulative live birth rate (%) in subfertile women with elevated basal FSH levels by category of AMH (AMH <1 μg/L [n $=$ 45] vs. AMH ≥ 1 μg/L [n $=$ 40]).

Yarde. Value of AMH in women with high FSH. Fertil Steril 2013.