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Antimüllerian Hormone as a Predictor of Natural Fecundability in Women Aged 30–42 Years

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Abstract

Objective—To generate estimates of the association between markers of ovarian aging and natural fertility in a community sample at risk for ovarian aging.

Methods—Women aged 30–44 years with no history of infertility who had been trying to conceive for less than 3 months provided early-follicular phase serum and urine (N=100). Subsequently, these women kept a diary to record menstrual bleeding and intercourse and conducted standardized pregnancy testing for up to 6 months. Serum was analyzed for estradiol, follicle-stimulating hormone (FSH), antimüllerian hormone, and inhibin B. Urine was analyzed for FSH and estrone 3-glucuronide ($E_1$3G). Diary data on menstrual cycle day and patterns of intercourse were used to calculate day-specific fecundability ratios.

Results—Sixty-three percent of subjects conceived within 6 months. After adjusting for age, 18 women (18%) with serum antimüllerian hormone levels of 0.7 ng/ml or less had significantly reduced fecundability given intercourse on a fertile day compared to women with higher antimüllerian hormone levels (fecundability ratio 0.38, 95% CI:0.08–0.91). The day-specific fecundability for women with early-follicular phase serum FSH values greater than 10 mIU/ml compared to women with lower FSH levels was also reduced, though nonsignificantly (11% of women affected; fecundability ratio 0.44, 95% CI: 0.08, 1.10). The association with urinary FSH was weaker (27% women affected; fecundability ratio 0.61, 95% CI: 0.26, 1.26), and the associations for the other markers were weaker still.

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Conclusions—Early-follicular phase antimüllerian hormone appears to be associated with natural fertility in the general population.

Introduction

Societal and behavioral shifts in recent years have resulted in a trend toward delayed childbearing. Women are choosing to delay attempts to conceive until they reach their thirties and forties, ages associated with a lower probability of conceiving (1). The observed decline in fecundability is due to reproductive aging, a natural progression through stages of puberty, fertility, subfertility, the menopause transition, and finally, menopause (2). The rate of movement through these stages varies among individuals; women of the same chronologic age clearly differ in their reproductive potential, just as age at menopause varies between individuals. Despite variability in the age of onset of subfertility, no validated biomarkers exist to monitor an individual woman's fertility.

Ovarian aging is associated with a decline in oocyte number reflected by a decline in circulating levels of antimüllerian hormone (AMH) and inhibin B and a rise in early follicular phase follicle stimulating hormone (FSH) and estradiol levels in blood (3–8). While these markers are physiologically associated with changes in ovarian function, their ability to predict fertility in the general population has never been rigorously examined. Previous research included only infertile subjects, measured outcomes only following treatment with assisted reproductive technology (ART), or used menopause as the outcome. Despite limited supporting evidence, FSH levels are frequently measured at doctors' offices and in commercial home kits marketed in the U.S. as a method of assessing one's fertility. Commercial, dipstick kits measure FSH in urine. A positive test is noted when urinary values exceed the cut-off value equivalent to a serum value of 10mIU/ml. These unproven tests have the potential to lead to unnecessary treatment or false reassurance, induce anxiety, and result in inappropriate costs and inconvenience to consumers if the tests are not valid (9).

Fertility of individuals can be compared a number of ways. Relative fecundability, the difference in fecundability (probability of conceiving in a menstrual cycle) of one group compared to another, is estimated by analyzing data on time-to-pregnancy, the number of menstrual cycles required to conceive. Because retrospective assessments of time to pregnancy are subject to strong bias effects (10), prospective studies are preferable. If information on menstrual bleeding and intercourse is also collected, time-to-pregnancy studies can be used to compare day-specific probabilities of pregnancy for affected and unaffected individuals or the relative probability of pregnancy given an act of intercourse on a fertile day.

Since specific endocrinologic changes appear to reflect ovarian aging, which is the presumed major cause of decline in fecundability, we sought to estimate the association between endocrine indices of ovarian aging (early follicular phase FSH, estradiol, AMH, and inhibin B in serum, as well as FSH and the estrogen metabolite, estrone 3-glucuronide ($E_1$3G) in urine and fecundability in a community sample at risk for ovarian aging.

Materials and Methods

Time to Conceive, a time-to-pregnancy study, was approved by the institutional review board of the University of North Carolina. English-speaking women between 30 and 44 years of age, who were attempting to conceive for 3 months or less or were about to start trying to conceive, were eligible for participation in the study. Women with a history of infertility, polycystic ovarian disease, pelvic inflammatory disease, endometriosis, pelvic radiation, or with a partner with a history of infertility were excluded from participation.

Eligible women were enrolled and provided informed consent at their study visit, which was scheduled for the 2nd, 3rd, or 4th menstrual cycle day (1st day defined as the 1st day of menses) following determination of eligibility. Women, who were determined to be eligible while contracepting, were enrolled in the menstrual cycle immediately following cessation of birth control.

At the study visit, women provided a urine and blood sample. To prevent breakdown of FSH that otherwise occurs in frozen samples, urine (5 ml) was transferred to a polypropylene vial containing glycerol (7% final glycerol dilution) (11). The serum and urine samples were stored frozen at −80° C until analyses. At the study visit participants were also provided and instructed on the use of the study diary, which was designed to collect daily information on vaginal bleeding, intercourse, pregnancy test results, and medication use. Subjects were asked to complete the diary daily until pregnancy was detected or 3 menstrual cycles had passed. In addition, women were provided with free home pregnancy tests (sensitivity = 20 mIU hCG/ml) and instructed to use them at the time of missed menses.

Women were instructed to inform study staff of a positive pregnancy test. Women were provided a free pregnancy ultrasound to encourage notification of pregnancy results. Women who did not report a positive pregnancy test were contacted at 3 and 6 months after the study visit. Women were followed until a positive pregnancy test or until 6 months of attempt following the study visit.

Urine was shipped frozen to the NIOSH Reproductive Endocrinology Laboratory, where the samples were assayed for FSH, $E_1$3G, and creatinine. Urinary FSH concentrations were assayed in duplicate using a non-competitive, two-site time-resolved immunofluorometric assay (12). Urinary $E_1$3G concentrations were measured in triplicate using competitive double- antibody time-resolved fluoroimmunoassay developed and characterized in the NIOSH laboratory (13). Urinary creatinine (cr) concentrations were measured using a Vitros 250 Chemistry Analyzer (Ortho-Clinical Diagnostics) that employs a slide composed of a dry, multilayered analytical element coated on a polyester support. Urinary endocrine values (FSH and $E_1$3G) were divided by the respective sample's creatinine concentration to adjust for urine flow rate. All samples were measured in one assay per analyte. Intraassay coefficients of variation were 3.5% for FSH, 16.1% for $E_1$3G, and 1.1 % for creatinine.

Serum was shipped frozen to the University of Southern California Reproductive Endocrinology Laboratory, where the samples were assayed by sensitive and specific assays for FSH, estradiol, AMH, and inhibin B. FSH was measured by a direct immunochemiluminometric assay using the automated Immulite system (Siemens Medical Solutions Diagnostics, Malvern, PA). Estradiol was measured by radioimmunoassay following an organic solvent extraction step. AMH and inhibin B assays used a monoclonal two site ELISA (Beckman-Coulter Diagnostic System Laboratories, Webster, TX). Inter-assay coefficients of variation ranged from 7–11%.

Statistical Analysis

Initially Pearson's correlation coefficients and p-values were calculated to estimate correlation between hormone levels and between the hormone levels and age. Subsequently we evaluated the associations between the hormone levels of interest and day-specific probabilities of pregnancy. Pregnancy was defined by the report of a positive home pregnancy test. Because of the relatively small sample size, maternal age was dichotomized at a cut-point of 35 years of age. All hormone levels were dichotomized at standard levels used in clinical practice. When clinical cut-off values were not available (as with serum estradiol and inhibin B and urinary $E_1$3G) cut-points were based on quartiles of the data (14). Specifically, we dichotomized serum FSH at a cut-point of 10 mIU/ml, AMH at 0.7 ng/

ml, inhibin B at the 25th percentile (21.9 pg/ml), and estradiol at the 75th percentile (54.6 pg/ml). Creatinine-corrected urinary FSH was dichotomized at a cut-point of 11.5 mIU/mg creatinine (equivalent to serum value of 10 mIU/ml). Creatinine-corrected urinary $E_1$3G was dichotomized at the 75th percentile (13.5 ng/mg creatinine). In a sensitivity analysis, urinary cut-points for FSH of 10 and 13 mIU/mg creatinine were also analyzed.

Information from the diary on days of menstrual bleeding, days of intercourse, and results of pregnancy tests were used to estimate day-specific probabilities of pregnancy (probability of pregnancy given an act of intercourse on a fertile day). Ovulation was assumed to have occurred 14 days prior to first day of menses or first positive home pregnancy test, with the fertile window designated as extending from 6 days before to 5 days after day of ovulation based on the standard days method (15). The day specific-probabilities model by Scarpa and Dunson (16) was used to generate day-specific fecundability ratios (FR). An FR less than 1.0 suggests reduced fecundability. The model accommodates for multiple acts of intercourse during the fertile window. The model assumes independence between acts of intercourse. In the model, the first observed menstrual cycle is entered at the appropriate attempt time (cycle 1 for those who enrolled when they started trying to conceive, cycle 2–4 for those who enrolled later in their attempt). Prospectively observed cycles were included in the model for up to 3 observed cycles or until pregnancy was detected. There were 224 cycles available for analysis. We fit both unadjusted models and age-adjusted models for each hormone of interest. Data from cycles without diary information were used to provide descriptive information, e.g. the percentage of women achieving pregnancy within 6 months.

Results

A total of 100 women were enrolled in the study, providing blood and urine. Follow-up was available for 98 women. Thirty percent of women were 35 years of age or older and 5% were 40 years old or older. Participants tended to be nulliparous (63%), White (86%), highly educated (61% with graduate or professional degrees), and normal body mass index (62% between 18.5 and 24.9 kg/m^2). Twelve percent of subjects were obese (over 30kg/m^2). Median male partner age was 33.5 years (interquartile range (IQR) = 31–37, range 27–56). Women reported approximately 2 acts of intercourse per week (IQR=1.5–3.0, range 0.2–6). Thirty-eight percent of women enrolled during their first cycle of attempt to become pregnant. By 6 months of enrollment, 63.6% of women had conceived. Eighty percent of women completed at least one cycle of daily fertility diaries.

Median serum values were 7.1 FSH mIU/ml (IQR=6.0–8.7, range 3–51)), 1.7 AMH ng/ml (IQR=0.8–3.0, range <0.06–8.3), 27.9 inhibin B pg/ml (IQR=14.9–46.6, range <10–120), and 42.8 estradiol pg/ml (IQR=35.2–54.6, range 15–437). AMH and inhibin B values below the limit of detection (n=3 and 15, respectively) were imputed as the limit of detection (0.06 ng/ml and 10 pg/ml, respectively) divided by square root of 2, following standard practice (17). Median creatinine-corrected urinary FSH and $E_1$3G were 9.1 mIU/mg cr (IQR=6.7–11.7, range 4.1–14.0) and 9.9 ng/mg cr (IQR=7.4–13.5, 2.3–77.3), respectively. Urinary and serum FSH were highly correlated (r=0.85, p<0.0001). Regression analysis revealed that the creatinine-corrected urinary FSH value comparable to a serum FSH value of 10mIU/ml was 11.5 mIU/mg cr. Eleven percent of women had serum FSH values over 10 mIU/mL, and 27% of women had urinary FSH values over 11.5 mIU/mg cr. Serum estradiol and urinary $E_1$3G were also highly correlated (r=0.78, p<0.0001). Correlation statistics between all marker values are presented in Table 1.

Age was a strong predictor of the day-specific probability of pregnancy; women who were aged 35 or older had significantly reduced fecundability compared to younger women given an act of intercourse on a fertile day (FR=0.42, 95% CI: 0.15, 0.85). After adjustment for

serum hormone measures, the age effect was even more pronounced with a corresponding FR of 0.32 (95% CI: 0.01, 0.75). AMH was strongly associated with day-specific fecundability (Table 2). Women with AMH levels of 0.7 ng/ml or less had significantly lower fecundability compared to their counterparts with higher AMH levels (FR=0.36, 95% CI: 0.01, 0.84) given an act of intercourse on a fertile day; this effect remained after adjustment for age (FR=0.38; 95% CI:0.08, 0.91). After adjusting for age, women with early follicular phase serum FSH values greater than 10 mIU/ml had estimated an FR of 0.44 (95% CI: 0.08, 1.10) compared to women with lower values. Women with urinary values of FSH over a serum equivalent of 10 mIU/ml (11.5 mIU FSH/mg cr), exhibited 40% reduced day-specific fecundability compared to women with normal urinary values. A higher cut-off value (13 mIU/mg creatinine) resulted in fewer affected women (N=16) but did not strengthen the association (FR 0.61, 95% CI: 0.21, 1.37). The estimated FRs for the remaining endocrine markers (serum inhibin B, serum estradiol, and urinary $E_1$3G) were also not statistically significant, and confidence intervals were even broader than for urinary FSH.

Discussion

In this study we found that serum AMH levels were strongly and significantly associated with natural fertility as measured by day-specific probabilities of pregnancy. Of the remaining markers, early follicular phase serum FSH values over 10 mIU/ml were most closely associated with reduced fertility; however, the confidence interval was broad, and the result not statistically significant. With urinary FSH cut-off values equivalent to a serum cut-off of 10mIU/ml, we did not see a significant association between elevated urinary FSH and reduced fertility. Serum analytes (estradiol and FSH) were significantly and strongly correlated with their urinary analytes; however, correlation between different hormones was low (generally less than 0.3).

Of all the serum and urinary markers of ovarian aging that were tested, serum AMH levels were most strongly associated with fecundability. Previous studies have shown that AMH declines with age (18), predicts stages of the menopause transition (19), and is associated with the probability of conceiving following in vitro fertilization (20). Measurement of AMH may have value in future epidemiologic studies as a measure of diminished fertility due to ovarian aging or diminished oocyte number (ovarian reserve). AMH serum values are relatively unaffected by cycle day (21) and appear to have value as an ovarian index independent of the woman's age. We are not aware of a urinary assay for AMH; however, dried blood spots have been a mechanism for measuring other reproductive hormones in population studies and may be useful for AMH measurements. (22).

Elevated serum FSH (>10 mIU/ml -a commonly used cut-off value) was seen infrequently in our cohort. Women in this group also tended to have reduced day-specific fecundability; however, in our small cohort the association was not statistically significant. Due to the relatively small sample size, the predictive value AMH and FSH (or other markers) could not be compared with formal statistical analyses. While early follicular phase FSH has been used for years as a marker for staging the menopause transition (23) and as a predictor of pregnancy following in vitro fertilization (24), it has only recently been evaluated as a marker of natural fertility (25), albeit among subfertile women. Our study is unique in that it enrolled women without a history of subfertility. One other study examined pregnancy in relation to FSH in women over 30 without a history of subfertility, but was designed to compare FSH levels for women with early pregnancy loss and women with ongoing pregnancies. (26) Though no formal analysis of time to pregnancy was conducted, they found no significant differences in FSH between women who conceived and those who did not.

Commercial urinary fertility kits employ a cut-off level equivalent to 10 mIU/mL serum FSH (27). Thus for this study, we choose a urinary FSH cut-off value of 11.5 mIU/mg creatinine as a cut-off, which was our equivalent to a serum FSH value of 10 mIU/ml based on regression analysis. While serum and urinary FSH levels are highly correlated (r=0.85), an elevated urinary FSH (>11.5 mIU/mg creatinine) was only weakly and non-significantly associated with fecundability. Selecting an even higher urinary FSH cut-off value (13mIU/mg), did not strengthen the association. Our findings raise concern about the commercial use of urinary FSH test kits to predict fertility. Urinary FSH should be tested further to better understand its relationship to fecundability in the general population.

Given their age, this cohort was at higher risk for ovarian aging than the general population trying to conceive. Still, the majority of participants were in their early thirties, and only 11–18% of the women had abnormal measures of ovarian aging, as measured by early follicular phase serum FSH levels over 10 mIU/ml or serum AMH levels under 0.7 ng/ml—commonly accepted clinical cut-off values. We also noted, that although statistically significant, the correlation between the markers was surprisingly low (typically less than 0.3), indicating that these markers are not redundant to each other. Finally, adjustment for age did not significantly alter the measures of association between the various endocrine measures and day-specific probabilities of pregnancy. It is possible that these markers reflect biologic phenomena associated with reproductive aging, independent of chronologic age. If so, a combination of indices including age may have greater potential than any single biologic marker or age alone for predicting a decline in fecundability due to reproductive aging.

The primary limitation of this study is its relatively small sample size. This limited our ability to adjust for a broad range of potential covariates (including male factors) and to explore in detail the relationship among the markers of ovarian aging. Women with partners who had known fertility problems were excluded from the study, but no semen analyses were performed. Such a study requirement reduces participation in community studies, potentially affecting generalizability. As many women conceive in their first 3 cycles of attempt, our sample, which included some women who failed to conceive in these first few cycles, can be assumed to be less fertile than the general population attempting pregnancy. Our time-to-pregnancy analysis did allow for variation of estimates by time of enrollment. In addition, we screened out women with strong risk factors for infertility, creating a more homogeneous cohort to study the specific effect of ovarian aging. The homogeneity of the cohort, a largely White and highly educated group strengthens the internal validity of our findings, but may reduce generalizability. However, measures of ovarian aging have not been consistently shown to differ by ethnic group or socioeconomic status. The small sample of women in their 40s also may limit generalizability to this age group.

This study did use daily diaries to collect information on bleeding and intercourse patterns, allowing us to calculate day-specific probabilities of pregnancy, which adjusts for intercourse patterns. Adjustment for intercourse frequency is important, as frequency of intercourse is reported to decline with age (28). A larger study is needed to estimate if the strength of these associations differ by age or parity. In addition, precision of day-specific probability estimates are strengthened with the use of ovulation predictors (either cervical mucus or urinary kits), which this study did not use.

The prospective design of our study is vital for an analysis of the relationship between markers of ovarian aging and fertility because women, who may never conceive are included. A retrospective study of time-to pregnancy within a pregnancy cohort, a commonly used design, would miss women who do not conceive. Such women represent a large percentage of the population in older age groups. In this study women were provided free home pregnancy tests and instructions as to when to test for pregnancy. Therefore,

differential detection of early pregnancy is unlikely. In addition, this study evaluated multiple endocrine markers, both in urine and in serum, as indices of ovarian aging and used models to adjust for age and intercourse patterns.

Urinary home kits generally call for first morning urine but do not adjust for urine flow rates. In this study, first morning urine was not collected; however, we adjusted for urine flow rates using urinary creatinine concentrations in the samples. As in previous studies, (29) our creatinine-corrected FSH values were highly correlated with serum FSH values. Creatinine corrected gonadotropin values have been shown to improve measurement precision (30) and have been used in previous epidemiologic studies (31, 32).

In summary, serum AMH appears to be a predictor of age-related reductions in fecundability in the general population. Urinary FSH, the marker used in commercially-available kits for women's self-assessment, may only be weakly predictive. Larger studies are needed to confirm these findings and to explore the way the different endocrine markers interact as potential joint predictors of fertility.

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Table 1

Estimated correlation between markers of ovarian aging

		Serum Markers				Urinary Markers (creatinine-adjusted)	
	Age	**FSH**	**AMH**	**Inhibin B**	**Estradiol**	**FSH**	**$E_1$3G**
Age	1.00	0.13	−0.21 *	−0.04	0.01	0.21 *	−0.02
Serum Markers							
FSH		1.00	−0.28 **	−0.08	0.07	0.85 **	−0.06
AMH			1.00	0.23 *	−0.08	−0.36 **	−0.04
Inhibin B				1.00	0.08	−0.16	−0.01
Estradiol					1.00	0.10	0.78 **
Urinary Markers (creatinine-adjusted)							
FSH						1.00	−0.06
$E_1$3G							1.00

Pearson's correlation coefficients presented.

* $p<0.05$;

** $p<0.01$.

Table 2

Estimated associations of endocrine markers with day-specific probability of pregnancy

	Number (%)of affected subjects	Day-specific fecundability ratio* (95% confidence interval)	
		Unadjusted	Age-adjusted
Serum Measures			
FSH>10mIU/ml	11 (11%)	0.47 (0.08, 1.15)	0.44 (0.08, 1.10)
AMH≤0.7ng/ml	18 (18%)	0.36 (0.01, 0.84)	0.38 (0.08, 0.91)
Inhibin B≤21.9 pg/ml	20 (20%)	0.82 (0.35, 1.55)	0.83 (0.35, 1.55)
Estradiol≥54.6 pg/ml	23 (23%)	0.74 (0.27, 1.47)	0.71 (0.25, 1.42)
Urinary Measures			
FSH≥11.5 mIU/mg creatinine	26 (27%)	0.59 (0.25, 1.22)	0.61 (0.26, 1.26)
$E_1$3G≥13.5 ng/mg creatinine	24 (24%)	0.61 (0.27, 1.15)	0.62 (0.27, 1.16)

* Interpreted as relative probability of pregnancy given an act of intercourse on a fertile day. A fecundability ratio less than 1.0 suggests reduced fecundability. Analyzed 221 cycles from 98 subjects for urinary hormones; 184 cycles from 78 subjects for serum hormones.