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Association Between Biomarkers of Ovarian Reserve and Infertility Among Older Women of Reproductive Age

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IMPORTANCE Despite lack of evidence of their utility, biomarkers of ovarian reserve are being promoted as potential markers of reproductive potential.

OBJECTIVE To determine the associations between biomarkers of ovarian reserve and reproductive potential among women of late reproductive age.

DESIGN, SETTING, AND PARTICIPANTS Prospective time-to-pregnancy cohort study (2008 to date of last follow-up in March 2016) of women (N = 981) aged 30 to 44 years without a history of infertility who had been trying to conceive for 3 months or less, recruited from the community in the Raleigh-Durham, North Carolina, area.

EXPOSURES Early-follicular-phase serum level of antimüllerian hormone (AMH), follicle-stimulating hormone (FSH), and inhibin B and urinary level of FSH.

MAIN OUTCOMES AND MEASURES The primary outcomes were the cumulative probability of conception by 6 and 12 cycles of attempt and relative fecundability (probability of conception in a given menstrual cycle). Conception was defined as a positive pregnancy test result.

RESULTS A total of 750 women (mean age, 33.3 [SD, 3.2] years; 77% white; 36% overweight or obese) provided a blood and urine sample and were included in the analysis. After adjusting for age, body mass index, race, current smoking status, and recent hormonal contraceptive use, women with low AMH values (<0.7 ng/mL [n = 84]) did not have a significantly different predicted probability of conceiving by 6 cycles of attempt (65%; 95% CI, 50%-75%) compared with women (n = 579) with normal values (62%; 95% CI, 57%-66%) or by 12 cycles of attempt (84% [95% CI, 70%-91%] vs 75% [95% CI, 70%-79%], respectively). Women with high serum FSH values (>10 mIU/mL [n = 83]) did not have a significantly different predicted probability of conceiving after 6 cycles of attempt (63%; 95% CI, 50%-73%) compared with women (n = 654) with normal values (62%; 95% CI, 57%-66%) or after 12 cycles of attempt (82% [95% CI, 70%-89%] vs 75% [95% CI, 70%-78%], respectively). Women with high urinary FSH values (>11.5 mIU/mg creatinine [n = 69]) did not have a significantly different predicted probability of conceiving after 6 cycles of attempt (61%; 95% CI, 46%-74%) compared with women (n = 660) with normal values (62%; 95% CI, 58%-66%) or after 12 cycles of attempt (70% [95% CI, 54%-80%] vs 76% [95% CI, 72%-80%], respectively). Inhibin B levels (n = 737) were not associated with the probability of conceiving in a given cycle (hazard ratio per 1-pg/mL increase, 0.999; 95% CI, 0.997-1.001).

CONCLUSIONS AND RELEVANCE Among women aged 30 to 44 years without a history of infertility who had been trying to conceive for 3 months or less, biomarkers indicating diminished ovarian reserve compared with normal ovarian reserve were not associated with reduced fertility. These findings do not support the use of urinary or blood follicle-stimulating hormone tests or antimüllerian hormone levels to assess natural fertility for women with these characteristics.

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Women are delaying their attempts to conceive until older ages.[1] As a woman ages, her oocyte and follicular pool declines.[2] As the oocyte and follicular pool declines, granulosa cells secrete less inhibin B and antimüllerian hormone (AMH).[3,4] Lower inhibin B levels lead to an earlier and more rapid increase in follicle-stimulating hormone (FSH) during the follicular phase.[5] Collectively, AMH, early-follicular-phase FSH, and inhibin B have been referred to as biomarkers of ovarian reserve.

The ability of these biomarkers to predict reproductive potential is uncertain. Antimüllerian hormone has been associated with time to menopause in a number of cohorts.[6,7] Among women with infertility undergoing controlled ovarian hyperstimulation for in vitro fertilization, AMH is an excellent predictor of oocyte yield.[8] Studies on the ability of these biomarkers to predict which women will conceive with in vitro fertilization have had inconsistent findings.[9-11] Despite lack of evidence of their utility, biomarkers of ovarian reserve are being used as markers of reproductive potential or fertility tests. Home fertility tests based on day 3 urinary FSH levels are commercially available. Additionally, clinicians use these tests when counseling about elective oocyte cryopreservation.

The objective of this study was to determine the extent to which biomarkers of ovarian reserve (early-follicular-phase serum AMH, serum FSH, serum inhibin B, and urinary FSH) were associated with reproductive potential, as measured by the probability of conceiving naturally, in a cohort of women of older reproductive age recruited from the community. It was hypothesized that women with biomarker values suggesting diminished ovarian reserve would have a lower probability of conceiving in a given cycle (fecundability) by 6 cycles and by 12 cycles of trying to conceive.

Key Points

Question Is diminished ovarian reserve, as measured by low antimüllerian hormone (AMH), associated with infertility among women of late reproductive age?

Findings In this time-to-pregnancy cohort study of women aged 30 to 44 years without a history of infertility, women with a low AMH value had an 84% predicted cumulative probability of conception by 12 cycles of pregnancy attempt compared with 75% in women with a normal AMH value, a nonsignificant difference.

Meaning Among women attempting to conceive naturally, diminished ovarian reserve was not associated with infertility; women should be cautioned against using AMH levels to assess their current fertility.

Methods

The Time to Conceive study, a prospective time-to-pregnancy cohort study, was conducted from April 2008 to March 2016 (date of last follow-up). Women were eligible to participate if they were between 30 and 44 years of age, had been attempting to conceive for 3 months or less, and were cohabitating with a male partner. Women were excluded if they had known fertility problems (history of sterilization, diagnosis of polycystic ovarian syndrome, previous or current use of fertility treatments, known tubal blockage, surgically diagnosed endometriosis) or a partner with a history of infertility. Women who were currently breastfeeding or had used injectable hormonal contraception in the preceding year were also excluded. This study was approved by the institutional review board of the University of North Carolina; all participants provided written informed consent.

Women were recruited through flyers in the community, radio and print ads, informational letters, and mass emails. They were screened for eligibility by telephone using a standardized questionnaire. Women who met eligibility criteria completed a questionnaire including demographics and information on factors potentially related to fertility. To characterize the study population, this questionnaire included a question about race. Women self-selected their race from categories provided. Women were instructed to contact the study coordinator with their subsequent menses. They were scheduled for a study visit on cycle day 2, 3, or 4 of their menstrual cycle. Women were mailed a urine collection kit and instructed to collect a first-morning urine sample on the day of their study visit. At that visit, a blood sample and urine sample (if not collected at home) were obtained from the participant. Women were provided with home urine pregnancy tests (sensitivity: 20 mIU/mL human chorionic gonadotropin). For the first 3 years of the study, women were instructed to perform the pregnancy test with missed menses; subsequently, women were instructed to test starting on menstrual cycle day 28 and every 3 days thereafter.

While attempting to conceive, women completed a daily diary in which they recorded bleeding, intercourse, medications, and results of pregnancy tests. Women completed these diaries for up to 4 months and then subsequently completed monthly questionnaires. Initial versions of the questionnaires were on paper and later versions were web-based. Women were instructed to contact study personnel if they tested positive for pregnancy. They were provided a free pregnancy ultrasound between 6 and 8 weeks' gestation to encourage communication of results. Women were initially followed up for up to 6 months, but the protocol was subsequently modified in March 2010 to follow up with all women for up to 12 months of pregnancy attempt. Women were withdrawn from the study at initiation of fertility medication, on request (most commonly because they were moving or stopped trying to conceive), or when lost to follow-up.

Serum Analysis

Serum samples were stored at −30°C until analysis. Samples were shipped frozen in a single batch to the University of Southern California Reproductive Endocrinology Laboratory. There, they were assayed using sensitive and specific assays for FSH (Immulite analyzer, Siemens), inhibin B (enzyme-linked immunosorbent assay [ELISA], Ansh Laboratories), and AMH (ultrasensitive AMH ELISA, Ansh Laboratories; lower limit of detection, 0.078 ng/mL). Interassay coefficients of variation ranged from 4% to 5% for FSH, 5% to 8% for inhibin B, and 9% to 11% for AMH.

Urine Analysis

Urine samples were stored and shipped frozen to the National Institute for Occupational Safety and Health Reproductive Endocrinology Laboratory, Cincinnati, Ohio. There, they were assayed for FSH and creatinine as described previously.[12] To adjust for urine flow rate, urinary FSH values were divided by the respective creatinine concentration. Results are presented as milli–international units of FSH per milligram of creatinine. Intra-assay coefficients of variation were 3.5% for FSH and 1.5% for creatinine.

Statistical Analysis

The primary outcome measures for the study were the cumulative probability of conception by 6 menstrual cycles and by 12 menstrual cycles and relative fecundability. There were no secondary outcomes, but planned exploratory analyses examined associations between levels of AMH and the primary outcomes among age subgroups and between parity subgroups.

The biomarkers of ovarian reserve were considered as categorical variables where informed choices for cut points were available. It was hypothesized that the relationship between AMH and fertility would be nonlinear. After exploring clinical AMH cutoff values of 0.4 ng/mL, 0.7 ng/mL, and 1.0 ng/mL, the middle cutoff value of 0.7 ng/mL was selected based on previous research.[13] The 90th percentile was selected as the upper-level AMH cutoff value (8.5 ng/mL). The clinical value of 10 mIU/mL was selected a priori as the serum FSH cutoff value.[14] For urine, the corresponding FSH value is 11.5 mIU/mg creatinine, as documented previously.[13] Inhibin B was modeled as a continuous variable because no clinical cutoff values were available.

Nonparametric bivariable analyses were used to compare median biomarker levels by participant characteristics. Because women did not all enter at the same point during their attempts to conceive and some women withdrew, started fertility medications, or were lost to follow-up, the cohort was analyzed using a discrete-time Cox proportional hazards model. Time was menstrual cycles at risk of pregnancy (pregnancy attempt cycle). Pregnancy attempt cycle was determined from the time a woman started trying to conceive, not from the time of enrollment. Attempt cycle at enrollment was defined by the pregnancy attempt cycle (usually cycle 1, 2, or 3) in which the woman began participation (completed diaries or baseline questionnaire). Women were censored at the time they withdrew, started fertility medications, or were lost to follow-up. Thus, cycles from enrollment to censoring were included in the analysis. Because time in these models is measured by menstrual cycles (and not chronologic time) the hazard ratios (HRs) are referred to as fecundability ratios, which are the relative probability of pregnancy in a given cycle for the exposed group relative to the reference group.[15] In such models an HR of less than 1 suggests reduced fecundability in the exposed (or nonreferent) group.

The Cox proportional hazard models were then used to calculate the cumulative probability of conceiving (with 95% confidence intervals) at 6 and 12 cycles of attempt for each biomarker level. All models were adjusted for age (3 categories: <35, 35-37, or 38-44 years),[16] body mass index (4 categories: <18.5, 18.5-24.9, 25-29.9, or ≥30; calculated as weight in kilograms divided by height in meters squared),[17] race (white or nonwhite), current smoking status (yes or no), and hormonal contraceptive use in the preceding year (yes or no). Adjusted Kaplan-Meier curves with 95% confidence intervals were also constructed. The predicted probabilities and Kaplan-Meier curves were calculated by setting all of the covariates to the mean of the cohort. Planned subgroup analyses were conducted by age and parity. To test for interaction by age and parity, a likelihood ratio test was used to compare the fit for the model without the interaction term with that of the model including the interaction term. In addition, post hoc sensitivity analyses were conducted by creating additional Cox models to assess different cutoff values and to evaluate potential biases.

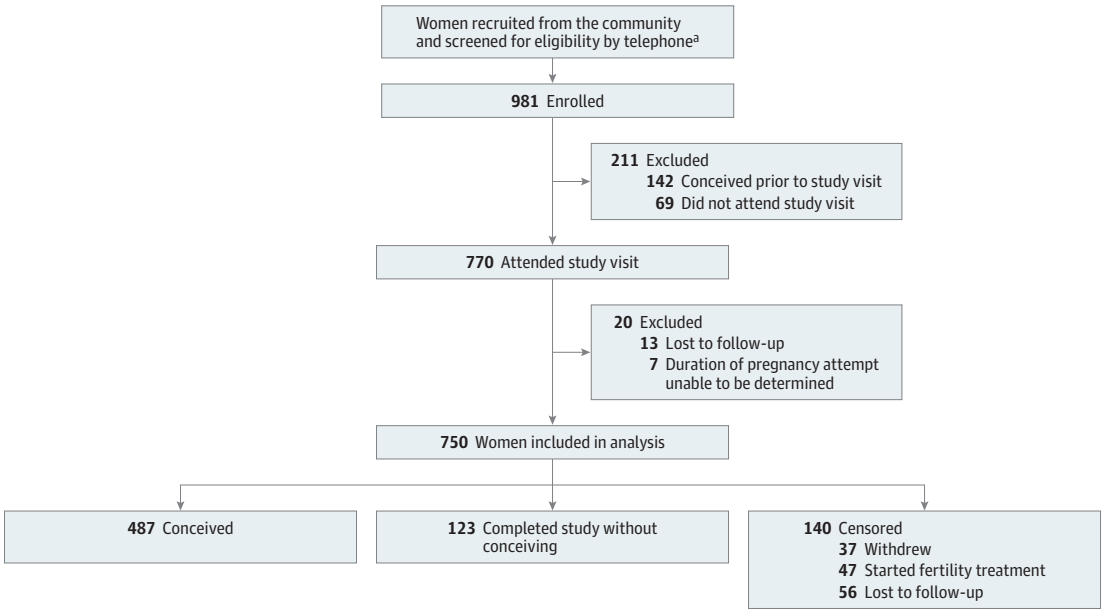
A sample size of 750 women was selected based on an a priori power analysis. A 10% loss to follow-up, 70% pregnancy rate in the control group, 57% pregnancy rate by 6 months in the diminished ovarian reserve group, and 80% power at a type I error rate of .05 was conservatively presumed based on the pilot study.[13] SAS version 9.3 (SAS Institute Inc) and R version 3.3.0 (R Project) were used for statistical analysis. All testing was 2-sided. $P<.05$ was considered statistically significant; there was no adjustment for multiple comparisons.

Results

Study flow is presented in **Figure 1**. Nine hundred eighty-one women were enrolled; 770 of these women had a study visit; 750 women were ultimately included in the analysis. Of these, 37 (5%) withdrew, 47 (6%) started fertility medications, 56 (7%) were lost to follow-up, 487 (65%) conceived, and 123 (17%) completed the study but did not conceive. Of the analyzed cohort, 69% of participants were aged 30 to 34 years, 19% were aged 35 to 37 years, and 12% were aged 38 years or older. Most participants were white (77%) and highly educated (62% with a graduate degree). The majority of women had a normal body mass index (62%), while 3% were underweight and 36% were overweight or obese. Cox analysis showed that the probability of conception was 65% by 6 cycles of attempt and 77% by 12 cycles of attempt. Fecundability over each attempt cycle is presented in **Table 1**.

The distributional statistics for the observed biomarkers of ovarian reserve are as follows. Serum AMH, inhibin B, and FSH values were missing for 13 study participants (2%), who were excluded from AMH, inhibin B, and FSH analyses accordingly. Urinary FSH values were missing for 21 participants (3%), who were excluded from the analyses of urinary FSH. Each participant had at least 1 biomarker value recorded. Eleven percent of women had an AMH value of 0.7 ng/mL or less; by design, 10% had an AMH value of 8.5 ng/mL or higher. Eleven percent had a serum FSH value of 10 mIU/mL or higher; 9% had a urine creatinine-corrected FSH value of 11.5 mIU/mg creatinine or higher. The median value for inhibin B was 70 (interquartile range, 38-102) pg/mL.

Figure 1. Flow of Participants Through the Time to Conceive Cohort Study



[a] Data on number of patients screened and number of and reasons for exclusions are not available.

Table 1. Pregnancy Attempts and Conception by Cycle

Cycle of Pregnancy Attempt	No. of Participants Entered in Study	No. of Women at Risk[a]	No. (%) Conceived	No. Censored in This Cycle
1	388	388	54 (14)	9
2	191	516	84 (16)	10
3	105	527	103 (20)	22
4	39	441	77 (17)	15
5	12	361	49 (14)	24
6	4	292	47 (16)	21
7	1	225	25 (11)	29
8	0	171	16 (9)	24
9	2	133	12 (9)	17
10	0	104	3 (3)	16
11	0	85	5 (6)	9
12	1	72	2 (3)	12

[a] Includes all women who entered the study during this attempt cycle or who entered in a preceding cycle and did not conceive and were not censored for starting a fertility medication, withdrawal, or loss to follow-up. Women at risk in cycle$_x$ = number entered study at cycle$_x$ + number at risk in cycle$_{x-1}$ – (number conceived$_{x-1}$ + number censored$_{x-1}$). For example, number at risk in cycle 3 = 105 + 516 – (84 + 10) = 527.

Table 2 presents the unadjusted median values of each biomarker (with interquartile ranges) by participant characteristics. As expected, AMH levels decreased and urinary FSH values increased with age. Compared with nonobese women, obese women had lower AMH (P = .007) and inhibin B (P = .005) values. Biomarker values did not significantly differ by education level, race, smoking status, hormonal contraceptive use in the preceding year, or cycle of pregnancy attempt in which the study participant was enrolled. Women who had previously been pregnant had significantly lower AMH values and higher urinary FSH values in this unadjusted analysis.

Antimüllerian hormone values were not statistically different across years of sample collection (Kruksal-Wallis test: P = .83), suggesting that AMH levels as measured using the Ansh assay are stable over prolonged storage at −30°C. A subset of samples (n = 99) were analyzed for AMH using the Diagnostic Systems Laboratory assay, Gen II assay (Beckman Coulter), and Ansh assay. There was high pairwise correlation between assay values (Pearson r = 0.96-0.97; P < .001).

Primary Outcomes

The predicted probability of conceiving by 6 cycles or 12 cycles of attempt, as calculated from the Cox models, was not lower for women with low AMH or high FSH, as had been hypothesized (**Table 3**). Women with low AMH values (<0.7 ng/mL) did not have a significantly different cumulative probability of conceiving by 6 cycles of attempt (65%; 95% CI, 50%-75%) compared with women with normal values (62%; 95% CI, 57%-66%) or by 12 cycles of attempt (84% [95% CI, 70%-91%] vs 75% [95% CI, 70%-79%], respectively). Women with high serum FSH values (>10 mIU/mL) did not have a significantly different

Table 2. Median Values for Each Biomarker by Participant Characteristics in the Time to Conceive Cohort

Characteristics	No. (%) (n = 750)	Median (Interquartile Range) Antimüllerian Hormone, ng/mL	Serum Follicle-Stimulating Hormone, mIU/mL	Urinary Follicle-Stimulating Hormone, mIU/mg Creatinine	Inhibin B, ng/mL
Age, y					
30-34	517 (69)	3.27 (1.7-5.8)	6.57 (5.2-8.0)	5.44 (3.6-7.8)	71.7 (41.5-102.2)
35-37	141 (19)	1.90 (0.9-3.7)	6.88 (5.4-9.1)	6.29 (3.9-9.0)	67.7 (32.2-108.3)
38-44	92 (12)	1.27 (0.6-2.8)	6.60 (5.5-9.1)	6.55 (4.6-10.1)	62.1 (28.2-91.0)
P value[a]		<.001	.12	.001	.18
Education					
Less than college degree	56 (7)	3.12 (1.6-5.3)	6.51 (5.1-7.5)	5.66 (4.2-8.7)	64.3 (34.9-105.6)
College degree	157 (21)	2.16 (1.3-4.6)	6.84 (5.3-8.0)	5.62 (4.3-7.7)	67.1 (38.9-98.5)
Some graduate work	70 (10)	3.04 (1.6-6.1)	6.50 (5.7-8.0)	5.44 (3.5-8.4)	71.7 (51.0-109.2)
Graduate degree	467 (62)	2.81 (1.4-5.2)	6.64 (5.2-8.4)	5.85 (3.8-8.2)	72.1 (36.7-102.2)
P value[a]		.18	.73	.88	.48
Body mass index[b]					
<18.5 (underweight)	19 (3)	2.83 (1.7-5.1)	6.08 (5.5-6.8)	7.04 (4.5-8.4)	82.7 (44.2-117.5)
18.5-24.9 (normal)	461 (61)	2.85 (1.5-5.5)	6.72 (5.3-8.4)	5.90 (3.7-8.2)	74.3 (39.6-104.8)
25-29.9 (overweight)	155 (21)	2.92 (1.4-4.8)	6.39 (5.2-7.8)	5.44 (4.1-7.8)	70.2 (43.0-101.5)
≥30 (obese)	114 (15)	2.20 (0.9-4.0)	6.98 (5.2-8.4)	5.35 (3.6-8.4)	57.4 (30.3-84.1)
Missing data	1 (<1)				
P value[a]		.06	.19	.66	.005
Race					
White	576 (77)	2.78 (1.4-5.2)	6.64 (5.2-8.2)	5.55 (3.7-8.0)	70.3 (38.9-101.6)
Nonwhite	174 (23)	2.70 (1.6-5.2)	6.57 (5.7-8.1)	6.13 (4.1-8.6)	68.6 (33.3-103.2)
P value[a]		.72	.60	.08	.56
Current smoker					
Yes	13 (2)	1.45 (1.0-2.3)	6.11 (4.8-9.9)	5.08 (3.6-7.5)	58.9 (22.1-78.3)
No	737 (98)	2.78 (1.4-5.2)	6.63 (5.3-8.2)	5.69 (3.8-8.2)	70.2 (38.0-102.0)
P value[a]		.12	.79	.71	.35
History of pregnancy					
Yes	383 (51)	2.42 (1.1-4.3)	6.63 (5.2-8.2)	6.03 (4.1-8.9)	70.0 (37.2-102.3)
No	367 (49)	3.24 (1.6-5.9)	6.62 (5.4-8.2)	5.44 (3.7-7.8)	70.3 (39.5-101.5)
P value		<.001	.77	.003	.83
Hormonal contraceptive use in preceding year					
Yes	336 (45)	2.77 (1.5-5.7)	6.67 (5.3-8.2)	5.44 (3.7-8.0)	70.3 (36.9-103.5)
No	414 (55)	2.78 (1.3-4.7)	6.60 (5.3-8.2)	5.99 (3.9-8.5)	70.0 (38.5-100.0)
P value[a]		.18	.29	.05	.72
Cycle of pregnancy attempt when enrolled					
1	388 (52)	2.85 (1.4-5.3)	6.50 (5.2-8.2)	5.48 (3.6-7.9)	71.3 (36.8-102.8)
2	191 (25)	2.78 (1.5-5.4)	6.55 (5.5-7.9)	5.54 (3.8-8.0)	66.5 (38.0-95.8)
3	105 (14)	2.43 (1.2-4.7)	7.15 (5.2-9.4)	6.46 (4.3-9.8)	70.2 (35.4-99.0)
4-6	55 (7)	2.61 (1.1-4.9)	6.71 (5.5-8.3)	5.71 (4.1-7.7)	81.2 (51.8-106.9)
>6	11 (2)	1.83 (0.9-2.6)	7.24 (5.7-8.7)	6.05 (5.2-7.3)	66.4 (41.0-108.6)
P value[a]		.41	.31	.20	.56
Partner age, y					
<35	424 (56)	3.15 (1.7-5.7)	6.66 (5.2-8.1)	5.69 (3.8-8.0)	70.1 (39.4-101.6)
35-44	298 (40)	2.27 (1.1-4.4)	6.64 (5.4-8.6)	5.68 (3.8-8.1)	70.3 (38.0-102.5)
≥45	27 (4)	2.33 (0.9-3.9)	5.91 (5.2-7.5)	6.92 (4.0-9.2)	62.9 (24.4-102.3)
Missing data	1 (<1)				
P value[a]		<.001	.49	.55	.62

(continued)

Table 2. Median Values for Each Biomarker by Participant Characteristics in the Time to Conceive Cohort (continued)

Characteristics	No. (%) (n = 750)	Median (Interquartile Range) Antimüllerian Hormone, ng/mL	Serum Follicle-Stimulating Hormone, mIU/mL	Urinary Follicle-Stimulating Hormone, mIU/mg Creatinine	Inhibin B, ng/mL
Partner body mass index[b]					
<18.5 (underweight)	2 (<1)	2.15 (1.7-2.6)	6.91 (5.8-8.0)	6.44 (4.5-8.4)	41.0 (23.7-58.3)
18.5-24.9 (normal)	316 (42)	2.77 (1.5-5.6)	6.62 (5.4-8.2)	5.85 (3.7-8.0)	73.5 (38.7-106.7)
25-29.9 (overweight)	297 (40)	2.78 (1.4-5.1)	6.62 (5.2-8.5)	5.95 (4.1-8.7)	70.6 (37.4-98.3)
≥30 (obese)	135 (18)	2.74 (1.3-4.6)	6.70 (5.3-7.8)	5.18 (3.8-7.5)	62.9 (36.5-89.8)
P value[a]		.82	.88	.34	.09

[a] Kruskal-Wallis rank sum test was used to compare biomarker values within each category.

[b] Body mass index was calculated as weight in kilograms divided by height in meters squared.

Table 3. Association Between Biomarkers of Ovarian Reserve and Predicted Probability of Conceiving in the Time to Conceive Cohort Study

Biomarker	No. of Participants	Conceived During Study, No. (%)[a]	Cumulative Probability of Conception, % (95% CI)[b] By 6 Cycles	By 12 Cycles	Hazard Ratio (95% CI)[c] Unadjusted	Adjusted[b]
Antimüllerian hormone, ng/mL						
<0.7	84	53 (63)	65 (50-75)	84 (70-91)	0.96 (0.72-1.28)	1.19 (0.88-1.61)
0.7-8.4	579	381 (66)	62 (57-66)	75 (70-79)	1 [Reference]	1 [Reference]
≥8.5	74	44 (59)	59 (45-69)	66 (57-77)	0.97 (0.71-1.33)	0.88 (0.64-1.21)
Serum FSH, mIU/mL						
<10	654	420 (64)	62 (57-66)	75 (70-78)	1 [Reference]	1 [Reference]
≥10	83	58 (70)	63 (50-73)	82 (70-89)	1.09 (0.83-1.44)	1.22 (0.92-1.62)
Urine FSH, mIU/mg creatinine						
<11.5	660	432 (65)	62 (58-66)	76 (72-80)	1 [Reference]	1 [Reference]
≥11.5	69	41 (59)	61 (46-74)	70 (54-80)	0.94 (0.68-1.30)	1.07 (0.77-1.49)
Inhibin B, pg/mL	737	478 (65)			0.9996 (0.998-1.002)	0.999 (0.997-1.001)[d]

Abbreviation: FSH, follicle-stimulating hormone.

[a] Women were followed up for up to 12 months in the study (independent of the number of menstrual cycles of attempt at or during enrollment). This includes any pregnancies conceived while enrolled in the study.

[b] Predicted from Cox models, which adjusted for age, body mass index, race, current smoking status, and recent hormonal contraceptive use by setting them to the mean of the cohort.

[c] A hazard ratio less than 1 suggests a lower probability of conception in the exposed group compared with the reference group in a given attempt cycle. A hazard ratio greater than 1 suggests a higher probability of conception in the exposed group compared with the reference group in a given attempt cycle.

[d] Per 1-pg/mL increase in inhibin B.

cumulative probability of conceiving after 6 cycles of attempt (63%; 95% CI, 50%-73%) compared with women with normal values (62%; 95% CI, 57%-66%) or after 12 cycles of attempt (82% [95% CI, 70%-89%] vs 75% [95% CI, 70%-78%]). Kaplan-Meier curves comparing adjusted cumulative probabilities of conception by categories of ovarian reserve biomarkers are presented in **Figure 2**. Although the curves suggest longer times to pregnancy in women with higher AMH values and for those with lower FSH values, confidence intervals overlap for both biomarkers. High and normal urinary FSH value curves are almost indistinguishable from one another.

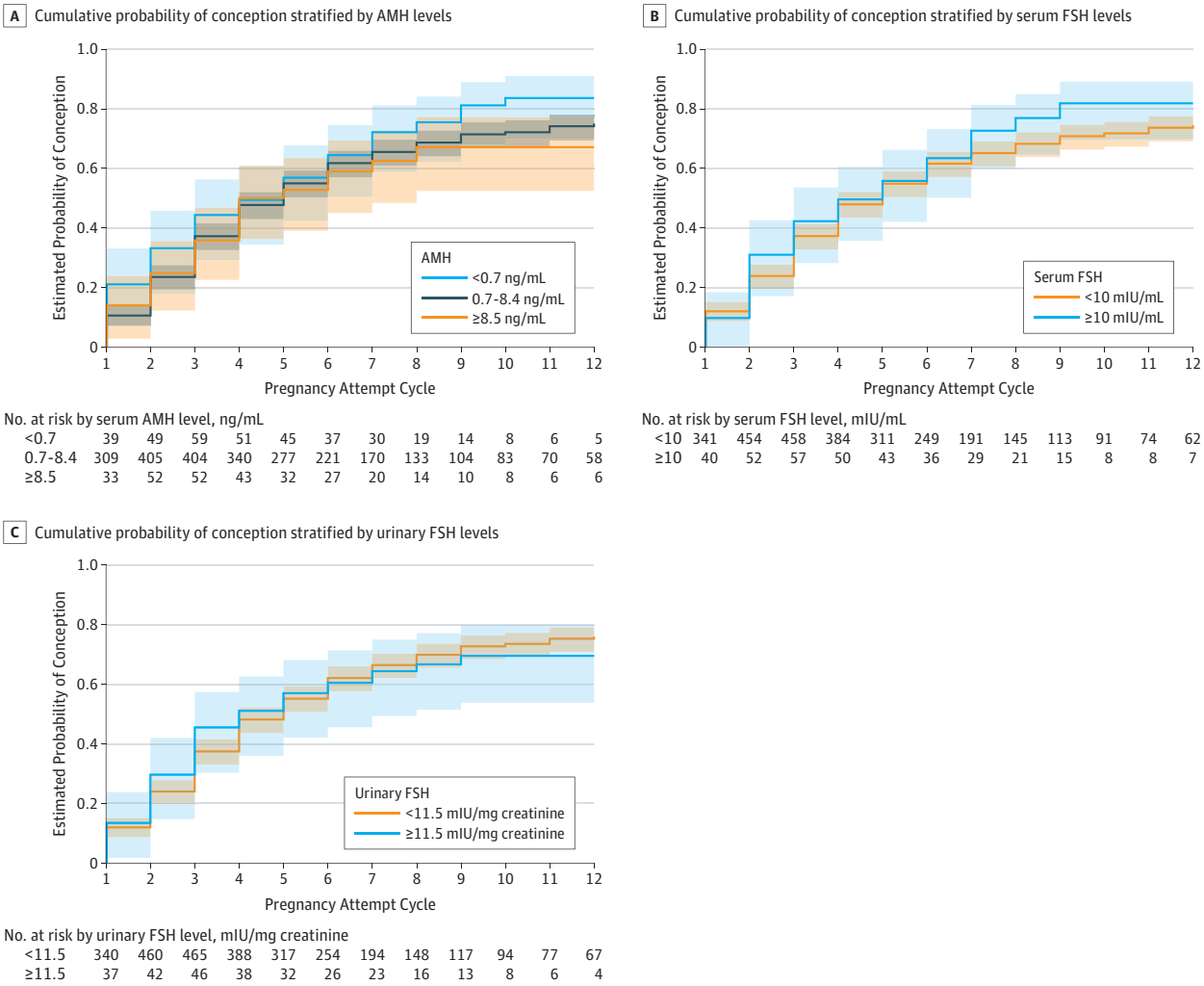
Relative fecundability according to biomarker values as calculated from the discrete-time Cox models is presented as HRs in Table 3. Women with low AMH values or high serum FSH values, which suggest diminished ovarian reserve, did not have reduced fecundability as had been hypothesized. Inhibin B levels (n = 737) were also not associated with the probability of conceiving in a given cycle (HR per 1-pg/mL increase, 0.999; 95% CI, 0.997-1.001).

Secondary Analyses

Planned subgroup analyses by age and parity were conducted (**Table 4**). In every age group, low AMH was not associated with diminished fecundability. Point estimates suggested higher fecundability among women with low AMH at any age. The relationship between high AMH and fecundability appeared to differ by a woman's age. In younger women, high AMH suggested reduced fecundability. However, among older women, high AMH suggested higher fecundability. Although these point estimates differed, the confidence intervals overlap and the age interaction was not found to be statistically significant (*P* = .35). Subsequent subgroup analysis by pregnancy history also did not reveal significant effect modification by pregnancy history (Table 4).

In sensitivity analyses, different cutoff values for AMH were examined. Women with AMH values of 0.4 ng/mL or lower had an HR of 1.40 (95% CI, 0.95-2.07) compared with women with AMH values between 0.4 ng/mL and 5.0 ng/mL. Women with AMH values of 1.0 ng/ml or lower had an HR of

Figure 2. Adjusted Kaplan-Meier Curves for Time to Pregnancy by AMH, Early-Follicular-Phase Serum FSH, and Early-Follicular-Phase, Creatinine-Corrected, Urinary FSH Levels



AMH indicates antimüllerian hormone; FSH, follicle-stimulating hormone. 95% CIs are shown as shading. Model adjusted for age, body mass index, race, current smoking status, history of pregnancy, and hormonal contraceptive use in the preceding year. The median number of cycles each woman contributed was 4 (interquartile range, 2-6).

1.16 (95% CI, 0.89-1.50) compared with women with AMH values between 1.0 ng/mL and 5.0 ng/mL. Also after adjusting for hormonal contraception use in the preceding 3 months (information available for 552 women), the HRs were further from the null but remained statistically nonsignificant (for low AMH: HR, 1.30 [95% CI, 0.92-1.86]; for high AMH: HR, 0.75 [95% CI, 0.51-1.10]). Restricting the analysis to women who entered into the study at cycles 1, 2, or 3 of pregnancy attempt did not change the findings.

Discussion

In this cohort of women of older reproductive age attempting to conceive naturally, biomarkers of diminished ovarian reserve (low AMH or high FSH) were not associated with reduced fecundability or a lower cumulative probability of conceiving by 6 or 12 cycles of pregnancy attempt. Early-follicular-phase inhibin B levels were also not associated with fertility outcomes.

In an earlier, small pilot study (n=100 women), we found that low AMH (≤0.7 ng/mL) as measured using the Diagnostic Systems Laboratory assay was associated with a 60% reduction in the day-specific probability of conception.[13] Those findings are different from current findings in this larger cohort, most likely because of sample size. Also, the pilot study used a day-specific probability analysis. This method uses information on intercourse patterns around the time of ovulation that relied on a calendar method that could have led to misclassification. Additionally, a different AMH assay was used. There is some evidence that the Ansh assay results in higher values compared with other assays.[18] However,

Table 4. Associations Between Antimüllerian Hormone and Probability of Conceiving by Age and Pregnancy History in the Time to Conceive Cohort Study

Antimüllerian Hormone Level, ng/mL	No. (%) of Participants	Conceived During Study, No. (%)[a]	Cumulative Probability of Conception, % (95% CI)[b]		Adjusted Hazard Ratio (95% CI)[b,c]
			By 6 Cycles	By 12 Cycles	
Age, y					
30-34					
<0.7	32 (6)	22 (69)	71 (48-84)	87 (60-96)	1.16 (0.75-1.81)
0.7-8.4	414 (81)	296 (71)	68 (63-73)	83 (78-87)	1 [Reference]
≥8.5	64 (13)	39 (61)	63 (48-74)	73 (57-83)	0.87 (0.62-1.22)
35-37					
<0.7	24 (18)	16 (67)	53 (26-71)	75 (45-89)	1.33 (0.76-2.33)
0.7-8.4	104 (77)	60 (58)	58 (45-67)	64 (52-74)	1 [Reference]
≥8.5	7 (5)	4 (57)	68 (0-91)	N/a	1.14 (0.40-3.25)
38-44					
<0.7	28 (31)	15 (54)	41 (15-59)	61 (18-79)	1.24 (0.61-2.51)
0.7-8.4	61 (66)	25 (41)	32 (16-49)	38 (20-51)	1 [Reference]
≥8.5	3 (3)	1 (33)	NA	NA	6.85 (0.71-65.9)
Prior pregnancy					
No					
<0.7	40 (11)	22 (55)	61 (37-77)	90 (59-98)	1.43 (0.89-2.29)
0.7-8.4	277 (77)	169 (61)	55 (48-62)	69 (61-75)	1 [Reference]
≥8.5	44 (12)	22 (55)	51 (32-64)	NA	0.85 (0.55-1.31)
Yes					
<0.7	44 (12)	31 (70)	66 (47-79)	81 (62-90)	1.09 (0.73-1.63)
0.7-8.4	302 (80)	212 (70)	66 (60-72)	80 (73-85)	1 [Reference]
≥8.5	30 (8)	20 (67)	69 (47-82)	NA	1.04 (0.65-1.66)

Abbreviation: NA, not applicable because small sample size did not allow for predicted values.

[a] Women were followed up for up to 12 months in the study (independent of the number of menstrual cycles of attempt at or during enrollment). This includes any pregnancies conceived while enrolled in the study.

[b] Predicted from Cox models, which adjusted for age, body mass index, race, nulligravidity, current smoking status, and recent hormonal contraceptive use by setting them to the mean of the cohort.

[c] A hazard ratio less than 1 suggests a lower probability of conception in the exposed group compared with the reference group in a given attempt cycle. A hazard ratio greater than 1 suggests a higher probability of conception in the exposed group compared with the reference group in a given attempt cycle.

a sensitivity analyses with the higher (AMH ≤1.0 ng/mL) and lower (AMH ≤0.4 ng/mL) cutoff values was conducted, and there was still no evidence of reduced fecundability in either of the low AMH groups.

Three other publications examined AMH and fecundability in women attempting to conceive naturally and none reported significant associations.[19-21] In a prospective study of 186 Danish women, Hagen et al[19] found that fecundability was not significantly reduced in women with low AMH (≤10 pmol/L; approximately ≤1.4 ng/mL) compared with women with normal AMH levels (HR, 0.81; 95% CI, 0.44-1.40). The cohort included women aged in their mid-20s and had a smaller sample size (n = 186). A secondary analysis of the EAGER study, which was a randomized clinical trial of aspirin for women (average age, 28.7 [SD, 4.8] years) with a prior pregnancy loss, found no statistically significant association between low AMH and fecundability using the Gen II assay with a cutoff value of 1.0 ng/mL (HR, 1.13; 95% CI, 0.85-1.49).[20]

Given that prior studies were small or based on secondary analyses, the finding in the current study that women with diminished ovarian reserve did not have reduced fertility was surprising and contrary to the hypothesis. Although both ovarian reserve and fertility decline with chronological age when looking at cross-sectional data, there may be little association between a given woman's ovarian reserve and factors that affect her fertility, such as egg quality. Antimüllerian hormone and FSH levels may, however, affect follicular recruitment in those with diminished ovarian reserve. It is possible that low AMH allows for a greater proportion of the remaining primordial follicle pool to activate and become growing follicles. Additionally, high FSH seen in women with low reserve could lead to "superovulation" with multifollicular ovulation, increasing the odds of pregnancy. It has previously been shown that women of advanced maternal age are at higher risk of dizygotic twins.[22]

It was hypothesized that younger women with diminished ovarian reserve might not have decreased fertility but that older women would. However, the exploratory subgroup analysis did not support this. Neither the younger (30-35 years of age) nor the older women with diminished ovarian reserve (as measured by AMH) showed reduced fecundability. However, high AMH was nonsignificantly associated with reduced fecundability in the younger women and increased fecundability in the older women. Hagen et al similarly found that young women with high AMH levels had reduced fecundability (HR, 0.62; 95% CI, 0.39-0.99).[19] Antimüllerian hormone

is not only a marker of ovarian reserve but also a potential marker for polycystic ovarian syndrome. While the appropriate AMH cutoff value for polycystic ovarian syndrome is debated, multiple studies have shown that AMH is elevated in women with polycystic ovarian syndrome.[18,23] In younger women, high AMH values may suggest undiagnosed polycystic ovarian syndrome. High AMH may inhibit follicle sensitivity to FSH and subsequent follicular recruitment.[24] In older women, high AMH may simply reflect higher-than-normal ovarian reserve. Further study of women with high AMH across various age groups and over time is warranted.

This study has several strengths. First, it was specifically designed to address an important public health question: Is diminished ovarian reserve a cause of infertility in women of late reproductive age? Second, the sample size is large enough to detect even relatively small effects. Third, its prospective design allows for biomarker testing at the appropriate time and inclusion of participants with the full range of natural fertility. Fourth, most women were enrolled during their first 3 menstrual cycles of attempting to conceive. Enrolling women later selects a less fertile cohort, as 50% of women are likely to conceive within the first 3 cycles.[25] Fifth, the age range studied (30-44 years) focuses on women at risk of diminished ovarian reserve. Sixth, the study protocol standardized the outcome measure (whether a woman conceived in any given menstrual cycle). This was done by providing women free pregnancy tests and instructing them on when to test for pregnancy. Thus, the sensitivity of the test was the same for all, and the set timing of testing minimized the potential for differential identification of pregnancies. Seventh, the ovarian reserve markers evaluated include urinary FSH, which is used in the commercially available test kits marketed for women to assess their natural fertility. Thus, the findings relate directly to the usefulness of such tests. Eighth, biomarkers were measured in all study participants during the early follicular phase, minimizing potential variation in biomarkers due to the phase of the menstrual cycle.

This study has several limitations. First, conception, not live birth, was the primary outcome. Fecundity, the capacity to reproduce, is composed of the ability to both conceive and carry a fetus to viability. Diminished ovarian reserve could affect fecundity by increasing the risk of miscarriage, perhaps through an effect on egg quality. Prior studies to date have failed to show such an association.[26,27] Second, not all women remained in the study for 12 cycles of attempt. This was anticipated given the older-reproductive-age cohort. Current recommendations advise women older than 35 years to obtain an infertility evaluation after 6 months of attempt. The median attempt cycle at which women started infertility treatment in the study was 8 cycles. For this reason, conception by 6 cycles of attempt was calculated, and Cox models, which allow participants who initiate fertility medications to contribute time to the analysis until they are censored for their fertility medication use, were constructed. Third, ovulation was not assessed. This information would have allowed us to evaluate the strictest definition of fecundability (the probability of conceiving in a given *ovulatory* menstrual cycle). Fourth, male partners did not provide a semen sample for analysis. However, there is no reason to believe that women with diminished ovarian reserve would be more or less likely to be partnered with a man with abnormal semen parameters. Fifth, not all women were enrolled in their first, second, or third cycle of attempt; however, when the less than 10% of women who entered after their third cycle of attempt were excluded, the findings did not differ. Sixth, although various AMH cutoff values were explored, the study was not powered to look at very low (≤0.1 ng/mL) AMH values, which reflect diminished ovarian reserve more consistent with the late perimenopausal transition. It is possible that in such advanced stages, fecundability may be affected, especially if it results in frequent anovulation.

Conclusions

Among women aged 30 to 44 years without a history of infertility who had been trying to conceive for 3 months or less, biomarkers indicating diminished ovarian reserve compared with normal ovarian reserve were not associated with reduced fertility. These findings do not support the use of urinary or blood FSH tests or AMH levels to assess natural fertility for women with these characteristics.

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Acquisition, analysis, or interpretation of data: All authors.
Drafting of the manuscript: Steiner.
Critical revision of the manuscript for important intellectual content: All authors.
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