ORIGINAL ARTICLE: EARLY PREGNANCY

Antimüllerian hormone as a risk factor for miscarriage in naturally conceived pregnancies

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Objective: To determine the association between antimüllerian hormone (AMH), a measure of ovarian reserve, and miscarriage among naturally conceived pregnancies.
Design: Prospective cohort study.
Setting: Not applicable.
Patient(s): Women (n = 533), between 30 and 44 years of age with no known history of infertility, polycystic ovarian syndrome, or endometriosis who conceived naturally.
Intervention(s): None.
Main Outcome Measure(s): Miscarriage, defined as an intrauterine pregnancy loss before 20 weeks' gestation.
Result(s): After adjusting for maternal age, race, history of recurrent miscarriage, and obesity, risk of miscarriage decreased as AMH increased (risk ratio per unit increase in natural log of AMH = 0.83; 95% confidence interval [CI], 0.73, 0.94). Women with severely diminished ovarian reserve (AMH $\leq$ 0.4 ng/mL) miscarried at over twice the rate of women with an AMH $\geq$ 1 ng/mL (hazard ratio, 2.3; 95% CI, 1.3, 4.3).
Conclusion(s): AMH levels are inversely associated with the risk of miscarriage. Women with severely diminished ovarian reserve are at an increased risk of miscarriage. (Fertil Steril® 2018;■:■–■. ©2018 by American Society for Reproductive Medicine.)
Key Words: Antimüllerian hormone, miscarriage, pregnancy loss, diminished ovarian reserve

Fecundity is a term that describes the reproductive capacity to both conceive and carry a pregnancy to term (1). As women age, fecundity declines due to an increase in both time to pregnancy and early pregnancy loss (2). Concomitant with the decline in fecundity is the decline in oocyte quality and quantity (commonly referred to as ovarian reserve).

One measure of oocyte quality is the number of chromosomes it contains, otherwise known as "ploidy." In oocytes from older women, homologous chromosomes paired during meiosis I have been shown to fail to segregate normally, a process called meiotic nondisjunction (3). Nondisjunction results in aneuploidy of the mature oocyte and the subsequent embryo and is thought to be the leading cause of the increased miscarriage rate in women over the age of 35 (4).

Markers of ovarian reserve, including both serum and ultrasound modalities, have been well studied in infertile populations and have proven to be efficacious in the quantitative assessment of ovarian reserve (5, 6). However, an adequate marker of oocyte quality has yet to be determined. Antimüllerian hormone (AMH) is a hormone produced by the granulosa cells in the preantral and early antral follicles. AMH is a marker of oocyte quantity (5) and declines accordingly with age (7). AMH has been shown to predict oocyte yield after controlled ovarian hyperstimulation (8) and age at menopause (9). However, whether AMH can be used as a putative marker of oocyte quality remains uncertain. The ability of AMH to predict conception either with or without assisted reproductive technologies remains controversial, and no prior studies have prospectively examined the association between AMH and miscarriage among women with no history of infertility (10, 11).

With more women electing to defer childbearing, increased inquiry into personal reproductive capacity (fecundity) is becoming common (1). Having

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a more robust marker of both oocyte quantity and quality would allow for better counseling in mid to late reproductive age women. Therefore, we sought to assess the association between AMH and miscarriage in spontaneously conceived pregnancies using a prospective cohort of women. We hypothesized that women with lower AMH would have increased risk of miscarriage, independent of age.

MATERIALS AND METHODS

Study Design

Time to Conceive was a prospective, time-to-pregnancy cohort study conducted between 2008 and 2016 that enrolled women between the ages of 30 and 44 years who were trying to conceive naturally. The cohort was constructed and previously used to examine the association between biomarkers of ovarian reserve and fecundability (12). Women were recruited from the Chapel Hill–Raleigh–Durham area of North Carolina. Eligible women had been attempting to conceive for 3 months or less (self-reported). Women were excluded if they reported a history of infertility, polycystic ovarian syndrome (PCOS), or endometriosis, had a partner with infertility, were currently breastfeeding, or did not speak English. Institutional Review Board approval was obtained for this research.

AMH Measurement

In the first menstrual cycle after enrollment, participants provided a blood sample on the second, third, or fourth menstrual day. Serum samples were stored at –30°C until analysis. They were assayed using sensitive and specific assays for AMH (Ultrasensitive AMH ELISA, Ansh). Interassay coefficients of variation ranged from 9% to 11% (lower limit of detection 0.078 ng/mL).

Pregnancy Detection and Miscarriage Ascertainment

Women completed a baseline questionnaire including information such as demographics, medical history, reproductive history, and lifestyle behaviors such as tobacco, alcohol, and caffeine use. They were given home pregnancy tests (sensitivity of 20 mIU hCG/mL). Women enrolled before April 2011 were instructed to perform the pregnancy test with missed menses. From April 2011 forward, women were instructed to test starting on menstrual cycle day 28 and every 3 days thereafter until a positive pregnancy test or menses occurred. Participants were asked to notify study staff when they observed a positive pregnancy test. They were scheduled for an endovaginal ultrasound between 6 0/7 and 8 0/7 weeks of gestation to confirm estimated date of delivery and fetal viability.

Participants who became pregnant completed a pregnancy outcome report at the end of their pregnancy. For women reporting a miscarriage, the survey queried the date the miscarriage was identified and whether or not dilation and curettage was performed. Women who did not report a pregnancy loss were contacted between 20 and 24 weeks of gestation to confirm continued viability of the pregnancy and to update contact information. Participants who were enrolled before April 2011 were not initially required to fill out a pregnancy outcome report but were contacted later, in 2016, to determine the outcome of their pregnancy.

Statistical Analysis

All women with a reported positive pregnancy test were included in the analysis (n = 533). For the purposes of our study, miscarriage was defined as a pregnancy loss before 20 weeks' gestation. Estimated date of delivery was defined by last menstrual period unless there was a greater than 2-week discrepancy when compared with first trimester ultrasound. If a greater than 2-week discrepancy existed, estimated date of delivery was defined by first trimester ultrasound. Miscarriage was categorized into the following groups: biochemical pregnancy (positive pregnancy test followed by a negative pregnancy test or menses $\leq$ 4 days after first positive pregnancy); early pregnancy loss (negative pregnancy test or menses $>$ 4 days after first test and before the pregnancy ultrasound); clinical pregnancy loss (pregnancies lost after documented viable pregnancy at the pregnancy ultrasound and before 20 weeks' gestational age).

AMH values below the limit of detection (0.078 ng/mL) were assigned a value of the limit of detection divided by the square root of two. AMH was log transformed, and age adjusted, bivariate analyses were conducted to examine relationships between AMH and covariates and using the Student's *t*-test and analysis of variance (ANOVA) for continuous variables and χ^2 for discrete variables.

Age-adjusted AMH values were compared among the types of miscarriage (biochemical pregnancy, early pregnancy loss, clinical pregnancy loss). Biochemical losses did not follow trends in the rest of our data. Participants who reported a biochemical loss (n = 9) were more likely to be overweight or obese and have an elevated AMH (median, 4 ng/mL; range, 1.8, 26.6 ng/mL). In addition, these women tended to have a long menstrual cycle length (median, 40 days; range, 27, 101 days).

Bivariate analyses were conducted to assess the relationship between [1] AMH and pregnancy outcome (miscarriage or live birth) and [2] covariates and pregnancy outcome using the Student's *t*-test and ANOVA for continuous variables and χ^2 tests for discrete variables. Multivariable binomial regression was used to calculate risk ratios (RRs) and 95% confidence intervals (CIs) for the association between AMH and clinical miscarriage (compared with live birth). This regression model excludes biochemical pregnancies (n = 9), pregnancies that end in stillbirth (n = 2), and pregnancies that end in induced abortion (n = 6). AMH was analyzed both as a linear (lnAMH) and categorical variable: AMH $\leq$ 0.4 (severe diminished ovarian reserve), AMH $>$ 0.4 and $<$ 1 (compromised ovarian reserve); AMH $\geq$ 1 (normal ovarian reserve). To adjust for potential confounders, the model incorporated covariates predictive of miscarriage including age, race, obesity, and history of recurrent pregnancy loss (history of three or more miscarriages) based on bivariate analyses ($P \leq .1$). Covariates were categorized as follows: maternal age at study enrollment (<35 years, 35–37 years, 3–40 years, and >40 years); white race (yes/no); obese (body mass index