Antimüllerian hormone and pregnancy loss from the Effects of Aspirin in Gestation and Reproduction trial

Shvetha M. Zarek, M.D.,[a,b] Emily M. Mitchell, Ph.D.,[a] Lindsey A. Sjaarda, Ph.D.,[a] Sunni L. Mumford, Ph.D.,[a] Robert M. Silver, M.D.,[c] Joseph B. Stanford, M.D., M.S.P.H.,[c] Noya Galai, Ph.D.,[d] Karen C. Schliep, Ph.D.,[d] Rose G. Radin, Ph.D.,[a] Torie C. Plowden, M.D.,[a,b] Alan H. DeCherney, M.D.,[b] and Enrique F. Schisterman, Ph.D.[a]

[a] Epidemiology Branch, Division of Intramural Population Health Research, and [b] Program in Reproductive and Adult Endocrinology, *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, Bethesda, Maryland; [c] Department of Obstetrics and Gynecology, University of Utah and Intermountain Healthcare, Salt Lake City, Utah; and [d] Department of Statistics, University of Haifa, Haifa, Israel

Objective: To evaluate if antimüllerian hormone (AMH) is associated with pregnancy loss.
Design: Prospective cohort study within a block-randomized, double-blind, placebo-controlled trial of low-dose aspirin.
Setting: Not applicable.
Patient(s): Women (n = 1,228) were of ages 18–40 years with a history of one to two pregnancy losses and were actively attempting pregnancy without fertility treatment.
Intervention(s): Not applicable.
Main Outcome Measure(s): Pregnancy loss.
Result(s): Relative risks (and 95% confidence interval [CIs]) of human chorionic gonadotropin (hCG)-detected and clinical pregnancy loss were assessed with the use of log binomial models with robust variance and inverse probability weights adjusted for age, race, body mass index, income, trial treatment assignment, parity, number of previous losses, and time since most recent loss. AMH levels were defined as: low (<1.00 ng/mL; n = 124), normal (referent; 1.00–3.5 ng/mL; n = 595), and high (>3.5 ng/mL; n = 483). Of the 1,202 women with baseline AMH data, 19 (17.3%) with low AMH experienced a clinical loss, compared with 61 (11.4%) with normal AMH and 50 (11.8%) with high AMH levels. Low or high AMH levels, compared with normal AMH, were not associated with clinical loss. Results for hCG-detected pregnancy loss mirrored those of clinical loss.
Conclusion(s): AMH values were not associated with hCG-detected or clinical pregnancy loss in unassisted conceptions in women with a history of one to two previous losses. Our data do not support routine AMH testing for prediction of pregnancy loss.
Clinical Trial Registration Number: NCT00467363. (Fertil Steril® 2016;105:946–52. ©2016 by American Society for Reproductive Medicine.)
Key Words: Antimüllerian hormone, pregnancy loss, miscarriage, spontaneous abortion, aneuploidy

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Pregnancy loss is common, affecting up to 15% of all clinically confirmed pregnancies (1, 2), and is associated with increasing maternal age (3). After experiencing a loss, couples desire information about future risk of subsequent pregnancy loss. Whereas current practices of ultrasonography are extensively used

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Reprint requests: Enrique F. Schisterman, Ph.D., Epidemiology Branch, Division of Intramural Population Health Research, *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, 6100 Executive Blvd., 7B03, Rockville, Maryland 20854 (E-mail: schistee@mail.nih.gov).

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to predict real-time pregnancy viability (4), less is known about preconception biomarkers to predict the risk of loss in a future pregnancy.

Antimüllerian hormone (AMH) is a dimeric glycoprotein from the transforming growth factor β family (5, 6) and is well established as a predictor of ovarian reserve (7–10). It has been evaluated as a marker of ovarian aging (11, 12), with a decline in AMH linked to increasing maternal age (12). Although AMH has been postulated to be useful for predicting pregnancy loss (13) and aneuploidy risk (14–18), its association with pregnancy loss is unclear. Some investigators report a significant link between AMH and pregnancy loss (13), whereas others report no association (19, 20). Furthermore, the relationship between AMH and pregnancy loss has never been evaluated in unassisted conceptions in a cohort with well characterized reproductive history. Therefore, the objective of the present analysis was to investigate the association between preconception AMH and pregnancy loss and aneuploidy in fecund women with a history of pregnancy loss. Given that pregnancy loss is a common pregnancy outcome and AMH is a frequently used prognosticator of ovarian reserve, a potential association may have an important impact on reproductive-age women.

METHODS

Study Design and Population

This is a secondary analysis of a prospective cohort of the Effects of Aspirin in Gestation and Reproduction (EAGER) trial, a multicenter, double-blind, block-randomized, placebo-controlled trial evaluating the effect of low-dose aspirin (LDA) on live birth in 1,228 women with a history of one to two previous losses. The EAGER study was conducted from 2006 to 2012 at four clinical centers in the United States, and the design and methods have been previously described (21). Women in this cohort were attempting pregnancy, were ages 18–40 years, had regular menstrual cycles of 21–42 days in length, had a history of one to two previous pregnancy losses, and had no history of infertility, pelvic inflammatory disease, tubal occlusion, endometriosis, anovulation, uterine abnormality, or polycystic ovarian syndrome. Fertility monitors were used to assist with timing intercourse and scheduling clinic visits (Clearblue Easy Fertility Monitor; Inverness Medical). Eligible participants reported their reproductive history, which was classified by: 1) number of previous live births (none or any); 2) number of previous pregnancy losses (one or two); and 3) time since most recent loss (≤ 1 or >1 year). Written informed consent was provided by every participant. Institutional Review Board authorization was obtained for the Data Coordinating Center and at all clinical centers. Patient safety was optimized by the Data Safety and Monitoring Board, and the trial was registered with ClinicalTrials.gov, number NCT00467363.

AMH Assessment

Preconception AMH concentrations were measured in serum samples collected before conception at the randomization visit scheduled to coincide with day 2–4 of the menstrual cycle. AMH was analyzed in 2014 on samples that were collected from 2007 to 2012 and promptly stored at −80°C, with the use of the GEN II ELISA protocol with correction for complement interference (Beckman Coulter) (22). AMH levels were available for 1,202 (97.9%) of the 1,228 women in the cohort. All machine-observed concentrations were used without substitution of concentration below the limits of detection (0.006 ng/mL) to avoid bias (23). The interassay laboratory coefficients of variation were 6.2% and 6.6% at mean concentrations of 8.9 ng/mL and 3.1 ng/mL, respectively, for lyophilized manufacturer's control samples and 6.3% for an in-house pooled serum control. To maximize measurement precision of AMH, we evaluated out-of-range values for manufacturer-provided control samples with the use of a pooled standard curve and confirmed that sample recalibration was not required (24).

Outcome Measures

Human chorionic gonadotropin (hCG)-detected pregnancy and loss. Detection of an hCG pregnancy was defined as a positive result on a urine pregnancy test sensitive to an hCG level of 25 mIU/mL (Quickvue; Quidel). These urine hCG pregnancy tests were conducted at home or at the clinic if a participant reported a missed menses. In addition to the urine hCG tests, free β-hCG was measured in daily first-morning-urine collected at home from the last 10 days of each woman's first and second menstrual cycles of study participation, as well as on spot urine samples collected at study visits timed to coincide with day 2–4 of the expected next menstrual cycle to enable a more sensitive detection of very early pregnancy. Two laboratory assays for free β-hCG (initial test: catalog no. RIS0011R, Biovendor; confirmatory test: catalog no. 4221-16, Diagnostic Automation) were sequentially used to identify 21 additional pregnancies that were verified as very early positive tests for hCG-detected pregnancy. An hCG-detected loss was defined as the detection of an hCG pregnancy followed by the absence of signs of clinical pregnancy and ensuing menses.

Clinical pregnancy and detection of clinical loss. Clinically confirmed pregnancy was defined as a pregnancy identified with the use of early ultrasound at ~6–7 weeks of gestation. Clinically confirmed pregnancy losses included: 1) preembryonic loss; 2) embryonic loss; 3) fetal loss; 4) stillbirth; 5) ectopic pregnancy; and 6) pregnancy of unknown location (25) (definitions available in Supplemental Table 1, available online at www.fertstert.org).

Chromosomal analysis for aneuploidy of pregnancy losses. Genetic testing was conducted at two of the four centers participating in the study and was initiated on 82 of the 133 clinical pregnancy losses (26). Participants were given labeled sterile specimen containers and clean gloves when they had a positive pregnancy test or at the time of their ultrasound and instructed in the event of a pregnancy loss to place any passed tissue in a specimen container, keep it chilled, and contact the research nurse as soon as possible. Tissue was also obtained from several participants by means of dilatation and curettage. Determinate results were obtained in 55 (41%) of