Published in final edited form as:

Fertil Steril. 2015 August ; 104(2): 347–55.e3. doi:10.1016/j.fertnstert.2015.04.044.

Changes in Anti-Mullerian Hormone Levels in Early Pregnancy are Associated with Preterm Birth

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Abstract

Objective—To determine the association of preterm birth with anti-Mullerian hormone (AMH) levels, both in isolation and in combination with other markers of feto-placental health commonly measured during Integrated Prenatal Screening (IPS) for aneuploidy.

Design—Retrospective case-control study

Setting—State of Iowa

Patients—All pregnant women in Iowa who elected to undergo IPS and who subsequently delivered in Iowa were eligible for inclusion. Cases included women giving birth at <37 weeks gestation, controls included those who delivered at ≥37 weeks gestation.

Interventions—none

Main outcome measure(s)—Probability of a preterm birth

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Disclosures: BJS is a current employee of Merck & Co., Whitehouse Station, New Jersey. However, Merck & Co. provided no financial assistance for this project.

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Results—2nd trimester AMH levels were not associated with preterm birth, either independently or after controlling for other markers of feto-placental health. AMH difference was not associated with preterm birth when modeled alone, but a statistically significant association was found after adjusting for maternal serum alpha-fetoprotein (MSAFP) and maternal weight change between the 1st and 2nd trimester. After stratifying the model by MSAFP level, most of the risk for preterm birth was identified in women with an MSAFP >1 Multiple of the Median (MoM) and who had a stable or rising AMH level in early pregnancy.

Conclusions—A lack of decline in the AMH level in early pregnancy can be used to identify women with a high probability for preterm birth, especially when MSAFP levels are >1 MoM. Monitoring changes in the AMH level between the 1st and 2nd trimesters of pregnancy may help identify women who would benefit from interventional therapies such as supplemental progesterone.

Keywords

Preterm birth; Anti-Mullerian Hormone (AMH); Maternal Serum Alpha-fetoprotein (MSAFP); ovarian biomarkers; placental biomarkers

Introduction

In 2010, approximately 14.9 million (11.1%) (1) of all births worldwide delivered prior to 37 complete weeks of gestation (259 days, meeting the World Health Organization (WHO) definition of PTB (2). PTB accounts for 75% of perinatal mortality and over half of long-term perinatal morbidity (3), and has an estimated annual cost in the United States of over 26 billion dollars (4). Yet despite efforts to identify and treat women at risk for PTB, rates continue to increase in most of the world (1, 3, 5). Attempts to identify women at risk for PTB using individual risk models based on obstetric and demographic risk factors such as race, smoking during pregnancy, and previous history of a PTB (3, 4) have been largely unsuccessful (6-8). This lack of success is due, in part, to the multiple pathways that can lead to an early delivery (9) as well as a general lack of understanding about the underlying pathophysiology of the disease.

One proposed pathway to PTB includes disruption of placentation early in gestation as the result of inflammation and/or infection (3, 10, 11). A critical period for placentation is the late 1st and early 2nd trimesters when trophoblasts invade and remodel the myometrial junctional zone (12). Disruption of this process leads to utero-placental ischemia (12) and up-regulation of the pro-inflammatory pathways (10), which alters the integrity of the maternal-placental barrier. This allows fetal proteins such as alpha-fetoprotein to cross into the maternal circulation (13), and may induce or suppress production of placental hormones such as human Chorionic Gonadotropin (hCG) and inhibin A (14). Several adverse obstetric outcomes have been associated with abnormal maternal levels of these proteins (9, 13, 15, 16), but the usefulness of feto-placental hormonal biomarkers in identifying women at risk for PTB is limited because statistically significant associations are only seen at very high or very low levels (9, 15, 16).

In early pregnancy, the corpus luteum (CL) of the ovary produces the majority of the progesterone required for pregnancy maintenance, and without this CL-derived progesterone, pregnancies fail (17, 18). Yet, serum progesterone is not a useful biomarker because serum levels do not accurately reflect placental (19). Therefore, other methods of determining the need for CL-derived progesterone during the transition of progesterone production from the ovary to the placenta are needed. Anti-Müllerian hormone (AMH) is a candidate ovarian hormone that might provide information on overall ovarian activity during pregnancy.

AMH is a dimeric glycoprotein produced in the granulosa cells of the ovary (20-22). Originally thought to be stable throughout pregnancy (23), it is now known that AMH levels decline rapidly between the 13th and 15th weeks of gestation (24, 25). This timing corresponds to two key pregnancy events: the 2nd phase of deep placentation and the involution of the CL (18, 26). The speed of the AMH decline, in addition to the rapid increase to near pre-pregnancy levels within days of delivery (27, 28) suggests that AMH is being suppressed (28) as the result of physiologic cross-talk between the placenta and the ovary. Because of the timing of this decline, it is possible that changes in AMH levels during pregnancy are related to the need for increased progesterone production when placentation is disrupted, making it possible that AMH levels are useful as a biomarker of feto-placental health. The purpose of this study is to determine the association of PTB with AMH levels, both as a single biomarker and in combination with other feto-placental biomarkers commonly measured in early pregnancy. Specifically we will determine if AMH levels in the 2nd trimester, or alternatively the decline in AMH levels between the 1st and 2nd trimester, are associated with PTB.

Materials and Methods

We used a case-control study design to predict the odds of PTB in pregnant women undergoing Integrated Prenatal Screening (IPS) for aneuploidy in Iowa between 2009 and 2010. Pregnancy information and maternal serum samples were obtained from an existing maternal-fetal serum tissue bank at the University of Iowa. The Human Subjects Office at the University of Iowa approved the study (201106791), as did the Iowa Department of Public Health Research and Ethics Review Committee (RA2682), who granted permission to use birth certificate data. The Congenital and Inherited Disorders Advisory Committee for the state of Iowa granted permission for use of the maternal serum samples.

Development of the Serum Tissue Bank

All women in Iowa can elect to undergo IPS in the 1st and 2nd trimesters of pregnancy. IPS provides risk calculations for the presence of Down Syndrome, Trisomy 18, and for open neural tube defects in the fetus based on levels of maternal serum markers, including MSAFP, hCG, inhibin A, PAPP-A and estriol. Testing requires two timed serum samples, the first drawn between 10-13 6/7 weeks gestation and the second drawn between 15-20.9 weeks gestation. A fetal ultrasound is required for all pregnancies to confirm gestational age.

The State Hygienic Laboratory (SHL) at the University of Iowa performs all IPS testing in Iowa. After testing was completed, the remaining serum was stored in a serum tissue bank at

the University of Iowa at -80°C. After the child delivered, samples were linked to birth certificate data. This bank contained over 12,000 maternal samples linked to birth certificate data, and yielded over 3,000 paired 1st and 2nd trimester samples. Maternal biomarker information obtained during IPS was also available for these women.

Development of the Dataset

All women with samples in the serum tissue bank were eligible for inclusion. Inclusion criteria included women of any age who delivered a singleton after 20 weeks gestation and who had paired serum samples. Exclusion criteria were designed to limit the dataset to women with a spontaneous PTB, which are shown in Figure 1. Cases and controls were defined using the WHO definition of PTB. At completion of this initial screening, there were a total of 117 cases and 924 controls available.

Sample Size

Case-control selections were 1:1 frequency-matched based on the following age groups: <21, 21-22.9, 23-24.9, 25-26.9, 27-28.9, 29-30.0, 31-32.9, 33-34.9, 35-36.9, and 37. Sample size analysis indicated a sample size of 100 cases and 100 controls would provide over 80% power to find a 1.0 ng/mL difference in AMH levels with a two-sided alpha of 0.05. Because of concerns over misclassification related to the cause of PTB, a known limitation when working with birth certificate data (29, 30), the sample size in each arm was increased to 112 women in order to increase the study power.

After completion of AMH testing, several reports were published noting differences in the AMH levels based on race (3, 31). In reviewing the racial distribution in this dataset, all but 24 women had self-identified as white. As this number was too small to adequately address possible confounding associated with maternal race, the dataset was further restricted to white women. This did not change the bivariate associations observed in the unrestricted dataset. The case-control ratio in the restricted dataset was 105 to 95, which resulted in a slight skew in the number of cases to controls by age group. Specifically, the following age groups had one or two more cases than controls: < 21, 25-<27, 27 to <29, 29 to <31, 31 to <33, 33 to <35, and 35 to < 37. In addition, there was one less case than control in the 21 to <23 age group. The imbalance in age was addressed by controlling for maternal age during model development.

Anti-Mullerian Hormone Measurement

Stored samples had a single freeze-thaw cycle and then remained frozen at -80°C (minimum storage time of two years) until the time of analysis. Testing was not done on a freshly drawn or freshly frozen sample as all samples were shipped at room temperature to the SHL for testing as per the instructions for the IPS. A single reference laboratory (ReproSource, Inc., Woburn, Massachusetts) specializing in AMH testing performed a blinded analysis of all samples using the laboratory-developed ReproSource AMH assay. This assay is based on research-use-only materials and reagents from Beckman Coulter-DSL (Chaska, MN). Intra- and interassay coefficients of variation with serum controls were 5%-9% and 7%-12%, respectively. Serum AMH values below 0.1 ng/mL, the reported clinical level of detection, were considered to be zero but were reported as 0.05 in the analysis.

Beckman Coulter assays were used for MSAFP, hCG, inhibin A, PAPP-A and estriol measurements. All testing was done on the Beckman UniCel Dxl 600 Access Immunoassay System. Inter- and intra-assay variable for each test can be found in supplemental Table 1.

Statistical analysis

Univariate and bivariate analyses (t-test and χ^2 test statistics) were used to determine normality of the data as appropriate. AMH levels in the 1st and 2nd trimesters were not normally distributed. These variables were transformed and the models rerun, but no difference in the results were seen with the transformed data; therefore, the original variables were used in the final models as these more easily translated to the clinical setting.

Models were constructed using two different AMH exposure variables: 2nd trimester AMH level and as the mathematical difference between the 1st and 2nd trimester AMH levels, used to identify the magnitude of decline in AMH. Covariates included as potential confounders were: prior history of PTB (3), fetal gender (31), maternal age (32), maternal weight gain between the 1st and 2nd trimesters (33, 34), and smoking during the pregnancy (3). To control for differences in the timing of the blood draws, gestational age (days) at the time of the blood draw was included in models using 2nd trimester AMH level, and the length of time between blood the 1st and 2nd blood draw (days) was used in models using AMH difference between trimesters. The contribution of each covariate was assessed using backward, stepwise logistic regression with a threshold to retain the covariate of a 15% change in the β coefficient of the AMH variable used in the model. Receiver operating characteristic (ROC) curve and Hosmer-Lemeshow testing were used to determine goodness of fit. Conditional (fixed-effects) logistic regression, matched on age group, produced nearly identical results in all models. Therefore, only results from the multivariate logistic regression models are shown.

After completing the models with AMH levels only, MSAFP, inhibin A, hCG, estriol and PAPP-A levels were added to the existing model and the analyses were repeated to determine if model performance improved. Placental biomarkers (hCG, estriol, and inhibin A) with missing values had the levels imputed. All statistical analyses were performed using Stata version 11.2 (College Station, TX).

Results

Table 1 includes demographic and bivariate findings for both the unrestricted and race restricted datasets. In the restricted dataset, the average gestational age at delivery was 34.0 ± 2.9 weeks for cases vs. 38.7 ± 1.0 weeks for controls. On average, case fetuses were two days older at the time of 1st trimester screening compared to controls, and were one day older at 2nd trimester screening. The average MSAFP was significantly higher in cases than controls. The difference between the 1st and 2nd trimester AMH level was significantly higher in controls, indicating they experienced a greater drop in AMH levels. However, the average 1st trimester and 2nd trimester AMH levels were not significantly different between groups.

Because of previous reports of correlations between body mass index (BMI) and AMH levels (35), a correlation analysis was performed between 2nd trimester AMH levels and BMI and between AMH difference between trimesters. No correlations were found between BMI and either AMH variable ($r = -0.06$, $p = 0.39$ for 2nd trimester AMH; $r = 0.04$, $p = -0.62$, AMH difference between trimesters). Therefore BMI was not considered as a covariate in any of the models.

Models using only AMH

The association between AMH alone and probability of PTB was explored using the exposure variable of 2nd trimester AMH level (Table 2). The full model was adjusted for maternal age, fetal gender, maternal weight change between trimesters, previous preterm delivery, smoking status in pregnancy and gestational age at the time of the blood draw. All covariates except fetal gender were dropped in the final model, and no interactions were found. Even after controlling for fetal gender, the OR did not reach statistical significance (Table 2). The Hosmer-Lemeshow goodness of fit test for this model was not significant ($p = 0.68$), and the ROC curve was 0.57.

Next, the difference between AMH levels in the 1st and 2nd trimesters was considered as the exposure variable. The same covariates were included in this model except that time difference between blood draws replaced the time of the 2nd trimester blood draw. Again, the only covariate that remained in the model was fetal gender. The association between AMH difference and PTB did reach statistical significance after adjusting for fetal gender (Figure 2a and Table 2), indicating that larger declines in AMH levels decreased the odds for PTB. However, the model was better at predicting women with a low risk for PTB rather than a high risk (OR: 0.74, 95% CI: 0.52, 0.98). The Hosmer-Lemeshow test confirm a good fit ($p = 0.24$), and the ROC curve for this model was 0.61.

To evaluate a possible interaction between AMH difference and fetal sex, the model was stratified by fetal gender. No significant interactions were seen, although there was a difference in the patterns of AMH decline (Figure 2b).

Models combining AMH with other Markers

The same modeling strategy was repeated with MSAFP, hCG, PAPP-A, estriol, and inhibin A included as covariates. Twelve women were missing levels for inhibin A, estriol, and hCG in the dataset, so imputed values were used. In the final model using the 2nd trimester AMH level as the exposure variable, all covariates were dropped except MSAFP level and maternal weight change between trimesters. However, the association between 2nd trimester AMH and PTB was not significant (Table 2). The Hosmer-Lemeshow statistic indicated a good fit ($p = 0.25$) and the ROC curve was 0.63.

In the second model, the difference between the 1st and 2nd trimesters AMH levels was used as the exposure variable, adjusting for MSAFP, hCG, inhibin A, estriol, and PAPP-A. Again, only MSAFP and weight change between trimesters remained in the final model. The overall model was highly significant (Figure 2c and Table 2), but the majority of the risk was found in the association between MSAFP and PTB (OR: 2.27, 95% CI: 1.11, 4.64). The Hosmer-

Lemeshow statistic was not significant ($p=0.11$) and the ROC curve indicated a moderate fit at 0.66. This model was also better at predicting women at low risk of PTB rather than high risk; thus limiting its clinical usefulness.

Finally, a correlation analysis was performed between MSAFP and AMH difference between trimesters to determine if these variables were related. No significant correlation was found between these variables ($r=0.1206$, $p=0.09$). This is shown graphically in supplemental Figure 1.

In each of the above models, MSAFP was strongly associated with PTB, with an OR over 2. To further evaluate the contribution of MSAFP to the PTB model using AMH difference, MSAFP was dichotomized at ≤ 1 MoM or >1 MoM and evaluated as a class variable (Figure 2d). Fifty-three (50.5%) cases vs. 24 (25.3%) controls had an MSAFP of >1 MoM. According to this model, the risk for PTB was greatest with an MSAFP level >1 MoM (Figure 2d). The addition of AMH difference provided further discrimination of the risk in women with an elevated MSAFP so that when the AMH level increased instead of decreasing between the 1st and 2nd trimester, the probability of PTB was as high as 82%.

This probability of PTB applies to a case-control population with a 1:1 match and not to the general population. However, the results can be adapted to estimate the probability of PTB in a prospectively collected population if the incidence rate in the general population is known. The following formula is used to convert the case-control probability: $P_p = kP_R / (1 + kP_R - P_R)$, where P_p is the incidence probability in the prospective population (1), P_R is the probability based on a fitted regression model from a case-control study, and k is the ratio that depends on the case-control sampling rates (in the case of a 11% incidence, it would be $(5/89)/(5/11)$). Based on this formula, women with an MSAFP of >1 MoM and an increase in AMH levels between trimesters would have a probability of PTB of 36%, compared to a probability of 6% in women with a decline in AMH of 2.0 ng/mL and an MSAFP of ≤ 1 MoM.

Women with a Previous Preterm Birth

Although a history of previous PTB did not have significant influence in these models, we further analyzed the contribution of this variable in our models by stratifying the results by history of previous PTB. In the bivariate analysis, the only variable that was statistically different between these two groups of women was gestational age at delivery, with women with a history of a previous PTB delivering significantly earlier than those without this history. Bivariate comparisons for all variables can be found in supplemental Table 2. The ORs for each model were recalculated with the previous history of PTB variable in the model, but there were no changes in any of the observed associations. These results can be found in Table 2.

Discussion

A minimal decline or an increase in AMH between the 1st and 2nd trimesters of pregnancy can help identify women with a high probability of PTB, but only when the MSAFP is also elevated. Significant associations were noted with minor changes in either hormone, a

finding that is unique to these models. In addition, significant associations were found in women without a previous history of PTB, making it easier to apply the results to the general population. Because the decline in AMH between trimesters occurs in the same time periods used for IPS, these findings could be easily adapted into current clinical practice. It should be noted that these results apply only to white women, and should be validated in women of other races.

These models improve on previous attempts of PTB prediction using feto-placental biomarkers. Metcalfe et al. (7) reported that biomarkers currently used to screen for aneuploidy have detection rates too low to identify women at risk for adverse obstetrical outcomes. Menon et al. (36) reviewed 116 different biomarkers assayed 578 times in 217 studies, and found that no single biomarker could reliably predict PTB. Jelliffe-Pawlowski et al. (37) developed a model that predicted PTB with an OR of 2.3-3.6, but only with extreme biomarker levels (PAPPA-A the 5th percentile and/or MSAFP the 95th percentile, and/or 2nd trimester inhibin A 95th percentile). Finally, a meta-analysis by Yuan et al. (9) on predictive ability of MSAFP on PTB did find a relationship with MSAFP when levels exceeded 2.0 MoM, but only when other biomarkers are also abnormal.

The improved sensitivity of PTB models using an ovarian biomarker, and the need to control for fetal gender in some models, indicates that cross-talk may be occurring between the ovary and the feto-placental unit during the critical periods of placentation. Specifically, this suggests the existence of a fetal/placental/ovarian axis for progesterone control. The contribution of the ovary, or more specifically the corpus luteum (CL), to the maintenance of a healthy pregnancy after the first eight weeks of gestation has been largely ignored. While the “rescued” CL is required for progesterone production prior to the shift to placenta production at 8 weeks gestation (18, 26, 38), the reason the CL persists until 13-15 weeks gestation remains unclear. Recently, Baird postulated that when the placenta progesterone production is insufficient, the CL might be recruited for additional progesterone support. She theorized that continued and prolonged CL stimulation is an attempt to lessen an existing progesterone deficit and salvage a marginal pregnancy (18). Therefore, an AMH levels that does not decline in the 2nd trimester of pregnancy may indicate an increased progesterone need and/or abnormal placentation. The lack of decline in AMH levels in women with a corresponding increase in MSAFP who were identified to be at high risk for PTB in our model supports this theory.

The use of banked samples was a major strength of this study by allowing us to test 1st and 2nd trimester serum in women with a known birth outcome. As all of the samples came from one laboratory (SHL) and were handled according to a single protocol, there was less potential for bias based on sample processing. In addition, all AMH testing was performed in a blinded manner by a single reference laboratory with extensive experience in AMH testing that had strict quality control measures in place (ReproSource), thus the results were less likely to be influenced by variations in the testing procedure.

However, this study has weaknesses. The use of birth certificate data to identify risk factors might have led to misclassification based on inaccurate reporting of other PTB etiologies. We recognized this possibility and oversampled in order to address this potential bias. In

addition, this misclassification would have likely led to a bias towards the mean and a negative result, which was not the outcome of our study. All participants elected to undergo IPS so these women may be fundamentally different from the rest of the women in Iowa. Further, we only studied white women so results cannot be generalized to the non-white population. But this restriction could also be a strength as it limited the heterogeneity in the data and may have eliminated confounding based on race. In addition, the sample size was relatively small, and the results should be validated in a larger sample of women. Finally, the ELISA kit used for AMH testing has reported abnormal findings due to complement interaction when testing was performed in freshly drawn or freshly frozen samples. However, none of these samples were freshly drawn at the time there were frozen and remained frozen for a minimum of two years. This would have minimized the impact of complement interference during AMH testing.

Conclusions

In conclusion, AMH levels in early pregnancy may improve the sensitivity of PTB models. The lack of decline in AMH levels, along with elevation in MSAFP, suggests an increased need for CL-derived progesterone when abnormal placentation has occurred. Therefore, consideration of AMH changes in early pregnancy in PTB risk models may help identify women who would benefit from interventional therapies such as supplemental progesterone (39, 40). However, additional studies are needed to validate these findings and to develop a predictive model for PTB that could be used in the clinical setting.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We would like to thank Charles Lynch, MD, James Torner, PhD, Lars Fuortes, MD and Joseph Cavanaugh, PhD for their comments and support of this protocol, Dr. Kristi Borowski for the use of the information and samples in the serum tissue bank, and Dr. Stephen Hunter for his advice and assistance. This work was supported by the Iowa Women's Reproductive Health Research Career Development Award (K12 HD063117), the Reproductive Scientist Development Program NIH (K12 HD00849) and the Institute for Clinical and Translational Science at the University of Iowa (5 KL2 RR024980 and 1U54TR0001013). The Institute for Clinical and Translational Science at the University of Iowa is supported by the National Institutes of Health (NIH) Clinical and Translational Science Award (CTSA) program, grant KL2 TR000444. The CTSA program is led by the NIH's National Center for Advancing Translational Sciences (NCATS). This publication's contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIH.

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Figure 1. Selection of cases and controls

*premature rupture of membranes is defined as rupture of membranes prior to the onset of labor

Figure 2. Probabilities of preterm birth based on difference in AMH levels between the 1st and 2nd trimesters of pregnancy.

- a. Probability of preterm birth based on AMH difference, adjusted for fetal gender,
- b. Probability of preterm birth based on AMH difference, stratified by fetal gender.

Probability of preterm birth based on AMH levels and MSAFP

- c. Probability of preterm birth based on AMH difference, adjusted for MSAFP and weight change between trimesters,
- d. Probability of preterm birth based on AMH differences between the 1st and 2nd trimesters, stratified by MSAFP (class variable) and adjusted for weight change between trimesters.

Table 1
Demographic and univariate/bivariate data, unrestricted dataset and dataset restricted to white women only

Continuous variables	Unrestricted Dataset				Restricted to white women only			
	Cases Mean (SD) N=112	Controls Mean (SD) N=112	Odds Ratio (95% CI)	Cases Mean (SD) N=105	Controls Mean (SD) N=95	Odds ratio (95% CI)		
Maternal age ^a	28.6 (5.4)	28.7 (5.3)	1.0 (0.9, 1.0)	28.8 (5.2)	28.8 (5.5)	1.0 (0.9, 1.1)		
Gestational age at delivery ^b	33.7 (3.4)	38.7 (1.0)	NA ^k	34.0 (2.9)	38.7 (1.0)	NA ^k		
Gestational age at 1 st trimester screen ^c	84.7 (7.0)	82.6 (5.8)	1.1 (1.0, 1.1)	84.6 (6.9)	82.6 (5.8)	1.1 (1.0, 1.1)		
Gestational age at 2 nd trimester screen ^c	119.0 (9.0)	117.7 (9.2)	1.0 (0.9, 1.0)	119.2 (9.2)	118.0 (9.1)	1.0 (0.9, 1.0)		
Maternal weight change between the 1 st and 2 nd trimester ^d	3.3 (5.9)	1.9 (5.8)	1.1 (1.0, 1.1)	3.5 (5.9)	2.5 (4.1)	1.0 (1.0, 1.1)		
MSAFP ^e at 2 nd trimester ^f	1.2 (0.5)	1.0 (0.4)	2.5 (1.3, 4.9)	1.2 (0.5)	1.0 (0.4)	2.3 (1.2, 4.6)		
Maternal hCG ^g at 2 nd trimester ^{f, h}	1.1 (0.8)	1.1 (0.5)	1.1 (0.7, 1.6)	1.2 (0.8)	1.1 (0.5)	1.1 (0.7, 1.7)		
Maternal PAPP-A ⁱ at 1 st trimester ^{f, h}	1.0 (0.5)	1.1 (0.6)	0.6 (0.4, 1.0)	1.0 (0.5)	1.1 (0.6)	0.7 (0.4, 1.2)		
Maternal Estriol at 2 nd trimester ^{f, h}	1.0 (0.3)	1.0 (0.3)	1.1 (0.5, 2.6)	1.0 (0.3)	1.0 (0.3)	0.9 (0.4, 2.1)		
Maternal Inhibin A at 2 nd trimester ^h	1.3 (1.3)	1.1 (0.6)	1.4 (0.9, 2.1)	1.3 (1.3)	1.0 (0.6)	1.5 (0.9, 2.3)		
AMH at 1 st trimester screening ^j	3.2 (2.4)	3.7 (2.8)	0.9 (0.8, 1.0)	3.1 (2.4)	3.6 (2.6)	0.9 (0.8, 1.0)		
AMH at 2 nd trimester screening ^h	2.9 (2.1)	3.1 (2.5)	1.0 (0.9, 1.1)	2.8 (2.1)	3.0 (2.3)	1.0 (0.8, 1.1)		
Difference between 1 st and 2 nd trimester AMH levels ^j	0.3 (1.4)	0.6 (1.8)	0.7 (0.5, 0.9)	0.4 (1.4)	0.6 (1.6)	0.7 (0.5, 0.9)		
Categorical Variables	Cases n (%)	Controls n (%)	Odds ratio (95% CI)	Cases n (%)	Controls n (%)	Odds ratio (95% CI)		
Infant Gender								
Male	64 (57.1)	50 (44.6)		60 (57.1)	44 (46.3)			
Female	48 (42.9)	62 (55.4)	0.6 (0.4, 1.0)	45 (42.9)	51 (53.4)	0.7 (0.4, 1.1)		
Previous Preterm Birth								
Yes	11 (9.8)	7 (6.3)		10 (9.5)	6 (6.3)			
No	101 (90.2)	105 (93.8)	1.6 (0.6, 4.4)	95 (90.5)	89 (93.7)	1.6 (0.5, 4.5)		
Smoked during pregnancy								
Yes	20 (17.9)	15 (13.4)		19 (18.1)	13 (13.7)			

Continuous variables	Unrestricted Dataset			Restricted to white women only		
	Cases Mean (SD) N=112	Controls Mean (SD) N=112	Odds Ratio (95% CI)	Cases Mean (SD) N=105	Controls Mean (SD) N=95	Odds ratio (95% CI)
Maternal race	No	92 (82.1)	97 (86.6)	86 (81.9)	82 (86.3)	1.4 0.6, 3.0)
	White	105 (93.8)	95 (84.8)			
	Black	3 (2.7)	8 (7.1)			
	Asian	1 (1.0)	3 (2.7)			
	Other	3 (2.7)	6 (5.4)	0.7 (0.4, 1.1)		
^a years,						
^b weeks of gestation						
^c days of gestation						
^d pounds						
^e Maternal Serum Alfa-fetoprotein						
^f Measures of the Median (MoM)						
^g Maternal human Chorionic Gonadotropin						
^h 12 missing values (6 cases and 6 controls)						
ⁱ Maternal plasma protein of pregnancy-A						
^j ng/mL,						
^k Odds ratio could not be calculated as this variable used for case-control selection						

Table 2
Odds ratios for preterm birth by 2nd trimester AMH levels and the difference in AMH levels between trimesters

Model	OR	95% CI	p-value
2 nd trimester AMH (crude)	1.0	0.9, 1.1	0.6
2 nd trimester AMH adjusted for fetal gender			0.3
2 nd trimester AMH	1.0	0.9, 1.1	0.7
Fetal gender	0.7	0.4, 1.2	0.1
2 nd trimester AMH adjusted for fetal gender			0.4
2 nd trimester AMH	1.0	0.9, 1.1	0.7
Fetal gender	0.7	0.4, 1.2	0.2
History of previous PTB	1.5	0.5, 4.3	0.5
2 nd trimester AMH adjusted for MSAFP			0.03
2 nd trimester AMH	1.0	0.9, 1.1	0.6
MSAFP	2.5	1.3, 5.1	0.01
Weight change between trimesters	1.1	1.0, 1.1	0.1
2 nd trimester AMH adjusted for MSAFP			0.046
2 nd trimester AMH	1.0	0.9, 1.1	0.6
MSAFP	2.5	1.2, 5.0	0.01
Weight change between trimesters	1.1	1.0, 1.1	0.1
History of previous PTB	1.4	0.5, 4.1	0.6
AMH difference (crude)	0.7	0.5, 1.0	0.04
AMH difference adjusted for fetal gender			0.03
AMH difference	0.7	0.5, 1.0	0.03
Fetal gender	0.6	0.4, 1.1	0.1
AMH difference adjusted for fetal gender			0.06
AMH difference	0.7	0.5, 1.0	0.04
Fetal gender	0.6	0.4, 1.1	0.1
History of previous PTB	1.4	0.5, 4.1	0.5
AMH difference adjusted for MSAFP and weight change			0.006
AMH difference	0.7	0.5, 1.0	0.06
MSAFP	2.4	1.2, 4.9	0.02
Weight change between trimesters	1.1	1.0, 1.1	0.09
AMH difference adjusted for MSAFP and weight change			0.01
AMH difference	0.7	0.5, 1.0	0.07
MSAFP	2.4	1.2, 4.8	0.02
Weight change between trimesters	1.1	1.0, 1.1	0.09
History of previous PTB	1.4	0.5, 4.1	0.6