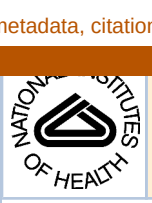

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The impact of smoking on antimüllerian hormone levels in women aged 38 to 50 years

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Abstract

Objective—Smoking is associated with increased follicle-stimulating hormone levels and early menopause. Smoking may directly accelerate ovarian follicular depletion or may act indirectly by increasing the pituitary production of follicle-stimulating hormone. Antimüllerian hormone (AMH), produced by ovarian follicles, is a more direct measure of ovarian reserve. The objective of our study was to determine the extent to which smoking influences ovarian reserve, as measured by AMH levels.

Methods—A community sample of 284 women aged 38 to 50 years completed a self-administered questionnaire including a detailed smoking history. Serum AMH levels were measured on day 2, 3, or 4 of the menstrual cycle. The association between AMH and smoking was analyzed using linear regression, adjusting for age and body mass index.

Results—Participants aged 38 to 42, 43 to 45, and 46 to 50 years had geometric mean AMH values of 6.7 pM (95% CI, 5.2–8.7 pM), 2.7 pM (95% CI, 1.9–3.8 pM), and 1.3 pM (95% CI, 1.0–1.7 pM), respectively. Current smokers, but not past smokers, had 44% lower AMH values than did the reference group (participants with neither active nor former or passive smoke exposure; $P = 0.04$). Passive smoking had no effect on AMH values when compared with the reference group ($P = 0.55$). The impact of smoking on AMH values was not dose dependent based on cigarettes per day ($P = 0.08$) or pack-years ($P = 0.22$). Finally, prenatal exposure to smoking (either maternal or paternal) had no impact on AMH levels ($P = 0.47$ and $P = 0.89$, respectively).

Conclusions—Active smoking, but not former smoking, is associated with decreased AMH values in late-reproductive-age and perimenopausal women, suggesting a possible direct effect of smoking

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In this study, the extent to which smoking influences ovarian reserve, as measured by antimüllerian hormone levels, was determined. Active smoking, but not former smoking, is associated with decreased antimüllerian hormone values in late-reproductive-age and perimenopausal women, suggesting a possible direct effect of smoking on the depletion of the antral but not primordial follicles.

on the depletion of the antral but not primordial follicles. The direct impact of active smoking on AMH levels in younger women requires further investigation.

Keywords

Smoking; Antimüllerian hormone; Müllerian inhibiting substance; Ovarian reserve; Menopause

A large body of literature has demonstrated that cigarette smoking adversely affects reproductive outcomes, including fecundability, miscarriage, and age of menopause. On average, smokers experience menopause more than 1 year earlier than nonsmokers do.[1,2] The mechanism, however, by which smoking influences ovarian function remains unclear.

In animal models, cigarette smoke and cigarette smoke toxicants cause oocyte destruction and ovarian failure.[3,4] In humans, previous work suggests that smoking affects follicular-phase follicle-stimulating hormone (FSH), a presumed measure of ovarian reserve (the size of a woman's remaining oocyte pool).[5] Smoking may cause oocyte depletion, resulting in a decline in serum inhibin B levels and a subsequent rise in pituitary FSH production. FSH, however, is produced by the pituitary gland and not the ovary, making it a surrogate marker of ovarian reserve. At least one study suggests that current smoking is associated with increased pituitary FSH levels but has no effect on direct markers of ovarian reserve, such as inhibin B, antral follicle counts, or estradiol.[6] It is plausible, therefore, that smoking stimulates the pituitary gonadotroph production of FSH but does not stimulate oocyte depletion. Thus, the association between smoking and increased FSH levels may or may not represent an effect of smoking on ovarian reserve.

In recent years, antimüllerian hormone (AMH), has exhibited significant promise as a potential direct marker of ovarian reserve.[7–10] AMH is expressed primarily by human granulosa cells of growing secondary, preantral, and early antral follicles,[11] and serum AMH levels correlate with the number of growing and primordial follicles in mice.[12] AMH does not seem to vary significantly across the menstrual cycle[13] or with cyclic changes in pituitary gonadotropins.[14]

Because smoking may have toxic effects on the ovary that accelerate the loss of reproductive function, we hypothesized that smokers will have less ovarian reserve, as measured by AMH levels, than nonsmokers will. Biologic evidence supports this concept, as animal literature suggests that polycyclic aromatic hydrocarbons (known carcinogens in cigarette smoke) cause oocyte destruction in mice.[15–17] In addition, increased levels of nicotine metabolites and cigarette carcinogens have been noted in the follicular fluid of active and passive smokers.[18,19] The objective of our study was to determine whether smoking influences a direct marker of ovarian reserve, by investigating the relationship between smoking status and AMH levels in a population of late-reproductive-age and perimenopausal women.

METHODS

To test our hypothesis, we conducted a secondary analysis of a previous cross-sectional study designed to study the effect of smoking, diet, and reproductive history on ovarian aging. The data collection and study design have been described previously.[5,20] For the original study, 290 women between ages 38 and 50 years, with an intact uterus and ovaries, were recruited from the community through advertisements and posters. The study was advertised in Durham and Orange counties in North Carolina from July 1992 to February 1993. Potential participants were excluded if they had undergone a hysterectomy or oophorectomy.

Participants completed a self-administered questionnaire including demographic information, smoking history, dietary habits, medical history, and reproductive history. These questionnaires were mailed to the participants at the time of enrollment and collected on the

day that their serum was obtained. Serum was obtained within 1 to 2 months of enrollment, depending on the woman's menstrual cycle. For those women with regular menstrual cycles, ovarian reserve was assessed with a serum sample drawn on the second, third, or fourth day of their menstrual cycle. A randomly timed sample was obtained from those participants who had not had a period for more than 2 months before the study. The serum was divided into 2-mL aliquots and stored at −80°C. In the initial analysis, FSH levels were measured using the Amerlite system of enhanced luminescence enzyme immunoassays (Amersham Corporation, Arlington Heights, IL).[5] Institutional review board approval was obtained for this research at the University of North Carolina at Chapel Hill.

For this analysis, six of the original participants were excluded because of insufficient stored serum. Of the 284 remaining participants, 2 had missing information on body mass index (BMI; kg/m^2) and were excluded from analyses adjusting for BMI. Serum AMH levels were quantified in duplicate in the 284 participants using a standard dual monoclonal antibody sandwich enzyme immunoassay (Immunotech, Beckman Coulter). This assay is specific for AMH and does not exhibit any significant cross-reactivity with related molecules. AMH's lower limit of sensitivity was 0.7 pM. Intra-assay and interassay coefficients of variation for AMH were 7% and 6%, respectively.

To assess the integrity of the stored samples for this study, FSH levels were determined in the residual serum in a subset of 40 participants using a standard dual monoclonal antibody sandwich enzyme immunoassay (Calbiotech, Spring Valley, CA). FSH's lower limit of sensitivity was 2.1 mIU/mL. The intra-assay coefficient of variation was 4%. After log transformation, correlation between the original FSH values and the new FSH values was tested using Pearson's correlation. There was a very high degree of correlation between the old and new FSH values ($r = 0.86$, $P < 0.0001$), consistent with previous work that has demonstrated AMH and FSH stability during long-term freezer storage.[21,22]

Participants were classified as current smokers if they were actively smoking within the previous 2 years. Past smokers included all participants who reported a history of smoking, with cessation occurring at least 2 years before the study. Participants were classified as passive smokers if they were currently living with someone who smoked in their home. Bivariate analyses revealed that the continuous variables (number of cigarettes smoked and pack-years) did not show a linear relationship with AMH, and polynomials (quadratic and cubic) did not reveal nonlinear relations. These variables were subsequently collapsed into three categories: none, above the median, and below the median. In our analysis, we controlled for age and BMI (kg/m^2), modeled as linear variables, two confounders known to be associated with AMH.[23,24] A two-sided *P* value of less than 0.05 was considered significant.

A commercial statistical software (STATA 10.0; StataCorp, College Station, TX) was used for the statistical analysis. A normal distribution of AMH values was achieved by natural log (ln) transformation. Geometric mean AMH values with 95% CIs were calculated for the entire population and according to age group (38–42, 43–45, and 46–50 y).[24] The adjusted relationship between tobacco use and (ln)AMH was determined by a Tobit regression model (Academic Technology Services, Statistical Consulting Group, University of California, Los Angeles, 2009), a linear regression model that accommodates left-censored data—in this case, AMH levels below assay sensitivity.[24] Exponentiation of the β coefficient produced a ratio of AMH levels for the exposed to the unexposed. An estimate of the percentage difference in AMH levels was obtained by subtracting 1 from the ratio and multiplying by 100. Adjusted geometric means were calculated using the models.[25]

RESULTS

The mean (SD) age of our 284 participants was 44.3 (3.0) years, and the mean BMI was 25.5 kg/m^2. A total of 101 (35.6%) participants were between ages 38 and 42 years; 93 (32.7%) participants were between ages 43 and 45 years; and 90 (31.7%) participants were between ages 46 and 50 years. The racial/ethnic breakdown of our sample was as follows: 226 (80%) white, 32 (11%) Black, 7 (2.5%) Asian, 3 (1%) Hispanic, and 6 (2%) Native American; 10 (3.5%) participants described themselves as other ethnicities not listed. Of the population, 23% graduated from college and an additional 38% had pursued postgraduate education. Regarding the participants' hormonal status, 10 women had experienced 12 or more months of amenorrhea, 18 were taking hormone therapy (HT), and 6 were taking oral contraceptives.

In terms of smoking, 129 (45.4%) participants had smoked regularly at some point in their lives. The mean (SD) age that they started smoking was 20.0 (4.9) years. A total of 31 (10.9%) participants described themselves as current smokers who had smoked within the previous 2 years. A total of 25 (8.8%) participants stated that they did not smoke but reported living with a smoker. These participants were classified as passive smokers.

AMH values for the entire population ranged from undetectable to 55.4 pM (geometric mean, 3.0 pM; 95% CI, 2.5–3.6 pM). Mean AMH values declined with age. Participants aged 38 to 42, 43 to 45, and 46 to 50 years had geometric mean AMH values of 6.7 pM (95% CI, 5.2–8.7 pM), 2.7 pM (95% CI, 1.9–3.8 pM), and 1.3 pM (95% CI, 1.0–1.7 pM), respectively. Of the 284 participants, 47 (16.5%) had undetectable AMH levels. AMH and FSH levels were significantly negatively correlated ($r = -0.47$, $P < 0.0001$).

To address our primary question of the impact of smoking on AMH values, we analyzed geometric mean AMH values according to smoking status, adjusting for age and BMI (summarized in Table 1). When women were categorized as either current smokers, passive smokers, or neither (the reference group), we found that current smokers had 44% lower AMH values than the reference group did ($P = 0.04$). When the women were further classified as current, passive, past, or never smokers, only current smoking was associated with lower AMH values ($P = 0.04$). Passive smoking had no effect on AMH values when compared with the reference group ($P = 0.55$). The geometric mean AMH for past smokers was almost identical to that of never smokers (3.24 vs 3.27 pM; $P = 0.97$).

A more detailed analysis related to number of cigarettes per day and smoking history showed that the impact of smoking on AMH values was not dose dependent. Smokers who currently smoked 15 cigarettes or more per day actually had AMH values 60% lower than those of smokers who smoked fewer than 15 cigarettes per day, but this difference was not statistically significant ($P = 0.08$). Similarly, pack-years of smoking did not influence AMH values. There was no difference between current smokers who had smoked for fewer than or more than 6 pack-years ($P = 0.22$).

Table 2 shows the detailed analysis of past smoking and AMH. Among past smokers, there was no difference between those who smoked 6 or fewer pack-years in the past versus those who previously smoked more than 6 pack-years ($P = 0.623$), nor was there a difference between those who quit before age 35 years and those who quit after age 35 years ($P = 0.398$). In addition, prenatal exposure to smoking—either maternal (n = 61) or paternal (n = 138)—had no impact on AMH levels ($P = 0.47$ and $P = 0.89$, respectively). Finally, a subgroup analysis including only premenopausal women who were not using oral contraceptives or HT (n = 250) did not alter the associations or significance of our findings between smoking and AMH.

DISCUSSION

To our knowledge, this study is the first to primarily determine the impact of smoking exposures on serum AMH levels. Current smoking was associated with reduced AMH levels, suggesting a direct effect of smoking on ovarian follicular depletion. Alternatively, smoking could simply influence follicular AMH gene expression, but this mechanism would not explain the finding that smokers experience menopause more than 1 year earlier than nonsmokers do.[1,2] Our results are consistent with previous work that has shown an association between smoking and measures of ovarian aging, such as FSH and age at menopause.[1,2,5,26] In addition, several studies have explored the relationship between smoking and inhibin B levels (another direct measure of ovarian reserve), but the findings have been inconsistent.[6,27,28]

Interestingly, past smoking did not influence AMH results at all, as there was no difference between past smokers and never smokers, even when analyzed according to the age at which they quit or the amount that they smoked previously. Similarly, when FSH values were examined in this population, there was also no effect of past smoking on FSH concentrations in women who quit smoking more than 2 years before the study.[5] Other studies have also shown that former smokers are similar to never smokers in terms of age of menopause.[29] In addition, past smokers seem to exhibit fecundity rates comparable with those of never smokers.[30]

A possible hypothesis to explain this discrepancy is that smoking leads to atresia of early growing, preantral, and antral follicles but not primordial follicles. The depletion of growing follicles could lead to a decline in AMH and inhibin B with an increase in FSH. If the primordial pool remained unaffected by smoking, smoking cessation could permit repopulation of the growing follicular pool and normalization of AMH, inhibin B, and FSH levels. The bulk of the available animal literature does not support this hypothesis, however. Rodent studies suggest that cigarette smoke toxicants, such as polyaromatic hydrocarbons and 7,12-dimethylbenz(a)anthracene, destroy primordial oocytes, but the relative effects of different exposure windows have not been firmly established.[3,4,15–17] Vahakangas et al[4] exposed pregnant mice to cigarette smoke and examined follicles in both the mothers and the offspring. Cigarette smoke exposure during gestation did not impact the number of primordial follicles in the ovaries of mothers but did decrease primordial oocyte counts in the offspring. These investigators concluded that fetal primordial oocytes may be more sensitive to cigarette smoke components than are those of adult mice. When taken together, these studies suggest that more than one mechanism may be involved, and the timing of exposures is an important consideration when interpreting the literature on this subject.

Other studies have shown a dose-dependent effect of smoking on FSH levels and timing of menopause.[1,5,26] Smoking also seems to adversely influence fertility in a dose-dependent fashion.[31–34] We had expected the association between smoking and AMH levels to be dose dependent. However, our findings did not support a link between AMH values and the quantity of cigarettes smoked or an individual's pack-year history. The discrepancy between our findings and previous literature suggests that these dose-dependent effects may be mediated in part through increased pituitary production of FSH, resulting in accelerated follicular depletion. Alternatively, the relatively small number of current smokers in our sample may have limited our ability to detect a concentration-dependent effect of smoking on AMH levels.

In addition to active smoking, we also explored the relationship between passive smoking and AMH values. We did not find an effect of passive smoke exposure on AMH. In the original study, the FSH values of passive smokers were significantly higher than those of nonsmokers.[5] Results of other studies on passive smoking are mixed. Whereas some authors have reported an association between passive smoking and earlier age at menopause,[35] others have found no

such relationship.[36,37] Larger studies are needed to further explore the reproductive effects of passive smoke exposure.

Neither maternal nor paternal prenatal smoking exposure seemed to influence AMH values. Self-reporting a prenatal exposure, however, may be subject to misclassification because of recall bias or response bias. The length of time from exposure to data collection and the reliance on family members for information may have also influenced the accuracy of responses, as many participants did not know if their mother or father smoked during pregnancy. In addition, with this analysis, we could not adjust for behaviors after delivery, such as passive smoking exposure during youth, which may have masked any possible relationship between prenatal smoking exposure and ovarian aging.

This study did not include a large enough sample of African American women to evaluate variation by race or ethnicity. One previous study found that both primary smoking and secondhand smoking were more strongly associated with risk of earlier menopause in black compared with white women.[35]

The limitations of this study include the small number of smokers and reliance on a self-administered questionnaire for smoking data. It is possible that some women were reluctant to acknowledge their true smoking status or may have underreported their smoking history. As a result, some participants may have been misclassified. If, for instance, smokers were misclassified as nonsmokers, then an association between smoking and AMH would have been weakened. In addition, approximately 8% of participants were using HT or oral contraceptives, which could have theoretically altered their AMH results. There are limited data regarding the interpretation of AMH values in the setting of postmenopausal HT. The available literature on AMH and oral contraceptives, however, suggests that AMH is not influenced by oral contraceptives and can be reliably measured in their presence.[38,39]

Longitudinal studies suggest that AMH levels begin to decline before FSH levels increase.[21,40] Because our population included late-reproductive-age and perimenopausal women, approximately 16% of participants had undetectable AMH levels. This substantial proportion of undetectable values may have limited our ability to detect more subtle differences in ovarian reserve between different subgroups. Unlike AMH, FSH has an unlimited range of abnormal values that can be detected, suggesting that FSH may have some advantages over AMH in older reproductive-age women.

Interpretation of our results is also limited by the cross-sectional study design. Theoretically, active smoking could cause a rise in FSH level, leading to oocyte depletion, and a subsequent decline in AMH. Animal models, however, have clearly shown that cigarette smoke toxicants directly cause oocyte destruction.[3,4,15–17] To truly demonstrate a direct toxic effect of smoking in humans, a longitudinal study would be needed. If smoking causes oocyte depletion directly, AMH would decline before the rise in FSH level occurs. In addition, it is unclear why former smokers do not seem as susceptible to the negative reproductive effects of smoking as current smokers are. If smoking does serve as a direct ovarian toxicant, it is plausible that younger women are less vulnerable to its effects because they have a larger follicular pool. Longitudinal studies of younger women using multiple measures of ovarian reserve and, if possible, ovarian tissue correlates are needed to further characterize smoking's effect on ovarian reserve.

CONCLUSIONS

Active smoking is associated with decreased AMH values in late-reproductive-age and perimenopausal women, suggesting a possible direct effect of smoking on ovarian follicular depletion. The direct impact of active smoking on AMH levels in younger women requires further investigation.

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TABLE 1

The impact of smoking on serum AMH

	N (%)	Geometric mean AMH, pM[a]	Difference in AMH, %[b]	95% CI of difference[b]
Never smokers	140 (49.3)	3.27	Reference group	Reference group
Past smokers[c]	88 (31.0)	3.24	−1	−32% to 44%
Passive smokers	25 (8.8)	2.73	−17	−54% to 52%
Current smokers	31 (10.9)	1.83	−44	−68% to −2%
Neither	228 (80.3)	3.26	Reference group	Reference group
Passive smokers	25 (8.8)	2.73	−16	−53% to 49%
Current smokers	31 (10.9)	1.83	−44	−67% to −4%
Current nonsmoker	253 (89.1)	3.20	Reference group	Reference group
Currently ≤15 cigarettes/d	13 (4.6)	1.09	−66	−85% to −24%
Currently >15 cigarettes/d	18 (6.3)	2.69	−16	−58% to 66%
Current nonsmoker	253 (89.1)	3.20	Reference group	Reference group
Currently ≤6 pack-years	5 (1.7)	1.07	−67	−90% to 13%
Currently >6 pack-years	26 (9.2)	2.06	−36	−64% to 15%

AMH, antimüllerian hormone; BMI, body mass index.

[a] Geometric means of AMH values were derived using linear regression analysis with left censoring at the lower limit of AMH assay sensitivity (0.7 pM), adjusting for age and BMI.

[b] Ratios were obtained from the model to compare the geometric means for each exposed group to the unexposed reference group. Estimates of the percentage difference in AMH levels and 95% CIs were obtained by subtracting 1 from each ratio and multiplying by 100.

[c] Past smokers without current passive exposure.

TABLE 2

The impact of past smoking on serum AMH

	N (%)	Geometric mean AMH, pM[a]	Difference in AMH, %[b]	95% CI of difference[b]
Never smokers[c]	140 (61.4)	3.27	Reference group	Reference group
Past smokers ≤6 pack-years[d]	50 (22.0)	3.46	6%	−33% to 66%
Past smokers >6 pack-years[d]	38 (16.6)	2.98	−9%	−45% to 51%
Never smokers[c]	140 (61.4)	3.27	Reference group	Reference group
Past smokers, quit before age 35 y[d]	62 (27.2)	2.99	−9%	−40% to 39%
Past smokers, quit after age 35 y[d]	26 (11.4)	3.93	20%	−33% to 116%

AMH, antimüllerian hormone; BMI, body mass index.

[a] Geometric means of AMH values were derived using linear regression analysis with left censoring at the lower limit of AMH assay sensitivity (0.7 pM), adjusting for age and BMI.

[b] Ratios were obtained from the model to compare the geometric means for each exposed group to the unexposed reference group. Estimates of the percentage difference in AMH levels and 95% CIs were obtained by subtracting 1 from each ratio and multiplying by 100.

[c] Never smokers with no current passive exposure.

[d] Past smokers, including those with current passive exposure.