

# **Title Page: Demographic, lifestyle, and other factors in relation to anti-müllerian hormone levels in premenopausal women**

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**Running title:** Correlates of anti-müllerian hormone

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**Abbreviations:** AMH, Anti-Müllerian Hormone; BMI, body mass index; DHEAS, dehydroepiandrosterone sulfate; PCOS, polycystic ovary syndrome; SHBG, sex hormone-binding globulin

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Capsule summary: In this study examining correlates of Anti-Müllerian Hormone (AMH), lower AMH concentrations were associated with older age, a younger age at menarche and current use of oral contraceptives.

## ABSTRACT

**Objective:** To identify reproductive, lifestyle, hormonal and other correlates of circulating Anti-Müllerian Hormone (AMH) concentrations

**Design:** Cross-sectional study

**Setting:** Nine cohorts that participated in the Prospective Study of AMH and Gynecologic Cancer Risk

**Patient(s):** 671 premenopausal women not known to have cancer.

**Intervention(s):** None

**Main Outcome Measure(s):** AMH concentrations were measured in a single laboratory using the picoAMH enzyme-linked immunosorbent assay. Multivariable-adjusted median (and interquartile range) AMH concentrations were calculated using quantile regression for several potential correlates.

**Results:** Older women had significantly lower AMH concentrations ( $\geq 40$ ,  $n=444$  vs.  $<35$  years,  $n=64$ , multivariable-adjusted median: 0.73 ng/mL vs. 2.52 ng/mL). AMH concentrations were also significantly lower among women with earlier age at menarche ( $<12$ ,  $n=96$  vs.  $\geq 14$  years,  $n=200$  : 0.90 ng/mL vs. 1.12 ng/mL) and among current users of oral contraceptives ( $n=27$ ), compared to never or former users ( $n=468$ ) (0.36 ng/mL vs. 1.15 ng/mL). Race, body mass index, education, height, smoking status, parity and menstrual cycle phase were not significantly associated with AMH concentrations. There were no significant associations between AMH concentrations and androgen or sex hormone-binding globulin concentrations or with factors related to blood collection (e.g., sample type, time, season, and year of blood collection).

**Conclusions:** Among premenopausal women, lower AMH concentrations are associated with older age, a younger age at menarche and currently using oral contraceptives, suggesting these factors are related to a lower number or decreased secretory activity of ovarian follicles.

**Key Words:** anti-müllerian hormone, demographic, lifestyle, reproductive factors, ovarian reserve

## Introduction

Anti-Müllerian Hormone (AMH) is a member of the transforming growth factor- $\beta$  superfamily, produced by the granulosa cells of preantral and small antral ovarian follicles (1-4). Studies show a strong positive correlation between circulating AMH concentrations and the number of follicles (5) and age at menopause (6-8). The correlation of the number of ovarian oocytes retrieved during in-vitro fertilization with AMH is reported to be higher than with follicle-stimulating hormone, inhibin B, or estradiol (1). AMH is relatively stable throughout the menstrual cycle (1, 9-14), compared to other ovarian hormones (1). Thus, AMH appears to be sensitive and stable markers of ovarian reserve in premenopausal women.

Numerous studies have demonstrated that AMH is associated with ovulatory disorders such as primary ovarian insufficiency, polycystic ovary syndrome (PCOS), and ovarian hyperstimulation syndrome (15, 16). Women with a low AMH concentration respond poorly to fertility treatment (17). AMH also decreases progressively with increasing age, becoming undetectable a few years before menopause (6-8). Therefore, AMH is a valuable reference in both clinical and research settings for prediction of ovulatory disorders, fertility, and reproductive lifespan. Animal and experimental studies reported that AMH may inhibit the development of cancer, particularly in organs that are of Müllerian origin and/or express AMH receptors (18), while recent epidemiologic studies found significant positive associations between AMH concentrations and breast cancer risk (19-21), but not with ovarian or prostate cancer risks (22, 23).

Evidence on individual characteristics associated with AMH has been inconsistent. Some studies have reported significantly lower AMH concentrations associated with oral

contraceptive use (9, 24-27), higher body mass index (BMI) (28-33), earlier age at menarche (26, 27, 34, 35), parity (35), alcohol consumption (36), and smoking (27, 37, 38), but these associations have not been consistent in other studies (8-10, 26, 27, 35, 36, 39-44). Many earlier studies included women who were infertile or who had PCOS, which may have influenced associations and reduces generalizability more broadly to normal premenopausal women. The present study examined potential correlates of AMH in controls of premenopausal women within the nested case-control studies of the Prospective Study of AMH and Gynecologic Cancer Risk.

## **Material and methods**

### ***Study population***

The Prospective Study of AMH and Gynecologic Cancer Risk is an ongoing project funded by the National Cancer Institute (USA). The aim of that study is to examine associations between AMH concentrations and ovarian and endometrial cancer risks using blood samples and covariate data from nine cohorts including the Columbia, Missouri Serum Bank (USA) (19), the Campaign Against Cancer and Heart Disease (CLUE I/II; USA) (45), the European Prospective Investigation into Cancer and Nutrition (EPIC; Europe) (46), the Guernsey Cohort Study (UK) (47), the New York University Women's Health Study (NYU-WHS; USA) (48), the Nurses' Health Study and Nurses' Health Study II (NHS/NHSII; USA) (49), the Hormones and Diet in the Etiology of Breast Cancer (ORDET; Italy) (50), the Northern Sweden Health and Disease Study (NSHDS; Sweden) (51), and the Shanghai Women's Health Study (SWHS; China) (52).

Within cohorts participating in the Prospective Study of AMH and Gynecologic Cancer Risk, one or two controls were matched per case by age and date of blood collection, as well as other cohort specific matching factors. This analysis included 671 premenopausal women who were cancer free at the time of blood collection and who remained cancer free at least until the age of the matched case's cancer diagnosis. Consent was provided by all participants at baseline in each of the cohorts. The institutional review boards of all collaborating institutions approved the present study.

### ***Blood sampling***

Blood samples were collected during visits to the study centers (19, 45-48, 50-52) or by mailing phlebotomy kits to the laboratory via overnight couriers (49) in each of the original cohorts. These blood samples were processed, separated into serum (19, 45-48) or plasma (45, 46, 49-52) using EDTA (51, 52), heparin (45, 49, 50) or multiple anticoagulants (e.g., EDTA, heparin, citrate) (46) at each cohort and archived in freezers at -70°C or colder except in Guernsey where samples were stored at -20°C.

### ***Laboratory assays***

#### ***AMH***

Plasma (45, 46, 49-52) or serum samples (19, 45-48) stored in each cohort were sent to a single laboratory at the Massachusetts General Hospital (Boston, MA, USA) for AMH assays. Case-control pair samples blinded to case and control status were randomly ordered and assayed together within batches for AMH using a picoAMH Enzyme-Linked Immunosorbent Assay (ELISA) kit (Ansh Catalog no. AL-124, Webster, TX). The coefficient of variation (CV) for AMH concentration measured in pooled masked quality control samples was 15.5%.



The limit of detection (LOD) of the AMH assay is 20 pg/mL; samples with less than LOD values were assigned to 10 pg/mL.

### ***Androgens and SHBG***

Subsets of samples from endometrial cancer and control participants from six cohorts (Columbia, EPIC, Guernsey, NYU-WHS, NSHDS, and ORDET) were sent to the German Cancer Research Center (DKFZ; Heidelberg, Germany) and assayed for androstenedione, dehydroepiandrosterone sulfate (DHEAS), testosterone and sex hormone-binding globulin (SHBG); for samples not assayed at DKFZ as part of the current study, we alternatively used the available androgen and SHBG concentrations measured previously for a subset of case-control pairs in CLUEI/II, NYU-WHS and EPIC (45, 46, 53). Testosterone, androstenedione and DHEAS were assayed with direct radioimmunoassays (Beckman-Coulter) and SHBG with an immunoradiometric assay (Cisbio) except for some of the earlier measurements of DHEAS (RIA, Wein Laboratories) or androstenedione (RIA, Diagnostic System Laboratories) in CLUEI/II (45, 53); and those of testosterone and DHEAS (RIA, Immunotech) and androstenedione (RIA, Diagnostic System Laboratories) from EPIC. The CVs for samples assayed at DKFZ for the current study were 11.7 % for androstenedione, 21.8 % for DHEAS, 15.0 % for testosterone and 20.5 % for SHBG; CVs of previously measured androgen data are reported elsewhere (45, 53).

### ***Data collection***

Each cohort provided data on potential correlates of AMH that were collected closest to the time of blood collection. Information on demographics, lifestyles, reproductive and menstrual history, and medical history was obtained via self-report (19, 45, 46, 48-51) or both self-report and interview (47, 52). We calculated BMI using height and weight information that

were either measured in Guernsey, ORDET, and SWHS or self-reported via questionnaires in all other cohorts. All cohorts provided information on age at blood draw, smoking status, season of blood draw, and use of oral contraceptives. Information on race (19, 45, 47-52) and education (45, 46, 48-52) was available from most cohorts.

### ***Statistical analyses***

After excluding one woman with an AMH value greater than 10 standard deviations above the median, 671 premenopausal women were available for this analysis. Primary data were harmonized for each variable to be expressed in uniform units or categories across cohorts. AMH values measured in citrate plasma (N = 4 samples) were converted to the corresponding AMH values from serum using an equation provided by Ansh Labs. To account for potential study related variability (e.g., blood collection procedures, transport, processing and storage) in biochemical markers, AMH, androgens and SHBG data were adjusted for cohort using the method by Rosner et al (54, 55); in brief, log-transformed hormones were regressed on age at blood collection (yrs, continuous) and study (Columbia, CLUEI/II, EPIC, Guernsey, NYU-WHS, NHS/NHSII, ORDET, NSHDS, SWHS). Then, we calculated study-specific correction factors by subtracting the average of study beta coefficients from study specific-beta coefficients. These study-specific correction factors were then subtracted from the log-transformed hormones to generate study-corrected log-transformed hormone data, which were back-transformed and used for all subsequent analyses.

In this cross-sectional analyses to evaluate associations of demographic, lifestyle and other factors with AMH concentrations, adjusted estimates of the median of cohort-adjusted AMH concentration and its interquartile range (IQR) were calculated for women within each category of the factors using age-adjusted and multivariable-adjusted quantile regression to

account for the skewness of AMH data (56). The multivariable model includes age at blood draw, a known correlate of AMH, as well as current oral contraceptive use and age at menarche, which were suggestively correlates of AMH in our age-adjusted model and have supporting biologic plausibility. For androgens and SHBG, we restricted analyses to women who were not current oral contraceptive users because of their influence on circulating concentrations of sex hormones and SHBG (57, 58); cohort-adjusted androgens and SHBG were categorized into common quartiles based on the distribution in these women. All women with non-missing data on the factor being evaluated were included in analysis. The only covariates retained in multivariable models were age, current use of oral contraceptives, and age at menarche; missing indicators were used when these variables were included as covariates for adjustment in multivariable models. P-values were calculated by an F-test with bootstrap variances, using a continuous term for continuous variables or a nominal term for categorical variables; p-value calculated using a continuous term (e.g., height, BMI, and sex hormones) can also be considered as p-trend. The between-study heterogeneity in the association of AMH with each factor was tested using the Q statistic from a meta-analysis assuming random effects (59, 60).

In sensitivity analyses, we restricted analysis to women who were not current users of oral contraceptives. Analyses stratified by age (<40 vs.  $\geq$ 40 years) were also conducted for associations between AMH and demographic and lifestyle factors; their interaction with age was tested by adding the cross-product term between each factor and age.

STATA version 13.0 (College Station, TX, USA) was used for analyses. All tests were two-sided and considered significant if  $P < 0.05$ .

## Results

This cross-sectional analysis included 671 women in their late premenopausal years (**Table 1**). The median age at blood draw was 40.9 years with a range of 19.3-46.7 years, though most were in their late thirties to early forties (IQR = 39.0-43.8 years). Mean height and BMI were 162.2 cm and 24.5 kg/m<sup>2</sup>, respectively. The majority of women were Caucasian (61%) and never smokers (56%). Some women (30%) had attended college. Most women (64%) were parous. Few were current users of hormonal contraceptives (4%). The median AMH concentration (IQR) was 1.01 ng/mL (0.32-2.28 ng/mL). The study-specific participant characteristics are presented in **Supplementary Table 1**. In brief, the median age ranged from 38.7 years in the CLUE I/II to 43.6 years in the SWHS and the NHS/NHS II. The current use of oral contraceptives in each cohort was low because aliquots of blood were mostly collected from premenopausal women who were included in earlier studies that evaluated associations between sex hormones and gynecologic cancer risk.

**Table 2** shows associations between AMH and demographic, lifestyle, and reproductive factors. As expected, we observed significantly lower AMH concentrations among older women. The multivariable-adjusted median AMH concentrations (IQR) were 2.52 ng/mL (0.61-4.63 ng/mL) in women aged <35 years, 1.55 ng/mL (0.66-2.97 ng/mL) in women aged 35-39 years, and 0.73 ng/mL (0.25-1.45 ng/mL) in women aged ≥40 years ( $P<0.001$ ). Race, smoking status, education, height, and BMI were not significantly associated with AMH concentration.

Of the reproductive and menstrual factors examined (**Table 2**), younger age at menarche was significantly associated with a lower AMH concentration (<12 vs. ≥14 years: 0.90 ng/mL vs.

1.12 ng/mL;  $P = 0.04$ ). AMH concentrations were significantly lower in women who were current users of oral contraceptives compared to never or former users (0.36 ng/mL vs. 1.15 ng/mL;  $P=0.04$ ). Adjusted median AMH concentrations were similar in never and former oral contraceptive users (1.17 ng/mL vs. 1.13 ng/mL) (data not shown). AMH concentrations did not significantly vary by parity or phase of the menstrual cycle.

We further examined the associations between AMH concentration and concentrations of androgens and SHBG (**Table 3**) and factors related to blood collection (**Table 4**). SHBG concentrations were positively associated with AMH with a borderline significance (lowest vs. highest SHBG quartile: 0.97 vs. 1.43 ng/mL;  $P = 0.05$ ). Androgens, including androstenedione, DHEAS and testosterone, and the testosterone/SHBG ratio were not significantly associated with AMH concentrations. Sample type and the time of day, season, and calendar year at blood collection, which, because AMH was measured in all samples at the same time, reflects the length of storage for blood specimens, were also not significantly associated with AMH concentrations.

Between-study heterogeneity for the association between AMH concentrations and each of the factors examined was not significant ( $P_{\text{heterogeneity}} \geq 0.21$ ) except for age ( $P_{\text{heterogeneity}} < 0.001$ ); the significant heterogeneity for the association between age and AMH concentrations disappeared when we excluded NSHDS (51). Restricting analyses to women who were not current oral contraceptive users did not alter results materially (data not shown). Additional adjustment for BMI, smoking status, and storage temperature in multivariable-adjusted models yielded similar results, though the significant associations between age at menarche and oral contraceptive use with AMH concentration were slightly attenuated; nonetheless, the direction of associations were largely consistent (age at menarche  $< 12$  vs.

$\geq 14$  years: 0.98 ng/mL vs. 1.09 ng/mL;  $P=0.07$ ; oral contraceptive current vs. never/former users: 0.41 ng/mL vs. 1.18 ng/mL;  $P=0.06$ ) (data not shown). The associations between AMH and demographic and lifestyle factors were not significantly modified by age, except for oral contraceptive use. Significantly lower AMH concentrations among current oral contraceptive users compared to never/former users were observed in women aged less than 40 years (0.46 ng/mL vs. 2.08 ng/mL;  $P=0.02$ ), but not in women aged greater than 40 years (0.61 vs. 0.72 ng/mL;  $P=0.76$ ;  $P_{\text{interaction}} = 0.03$ ). (data not shown).

## Discussion

In this cross-sectional analysis of 671 premenopausal women not known to have cancer from nine cohorts, older age was significantly associated with lower AMH concentrations.

Younger age at menarche and current oral contraceptive use were also associated with lower AMH concentrations. Race, BMI, smoking status, height, parity, and phase of menstrual cycle were not significantly associated with AMH concentrations. AMH concentrations also were not significantly associated with androgens or with factors related to blood collection and had a borderline significant association with SHBG.

Although few correlates of AMH are known, the decrease of AMH concentrations with increasing age in adult premenopausal women and the stability of AMH throughout the menstrual cycle are well established. The pool of follicles determined at birth progressively decreases with age, which results in a decline in the total number of granulosa cells that produce AMH. Consistent with this, age was reported to account for 84% variation of the number of follicles in a previous study of women aged less than 51 years (61). Our findings aligns with the majority of longitudinal and cross-sectional studies (8, 9, 27, 28, 36, 62-65)

that reported an inverse association between age and AMH concentrations. With regard to the menstrual cycle, AMH concentrations are suggested not to exhibit the large fluctuations typical of other ovarian hormones before menopause. In our study and most, though not all (66-69), other studies, AMH concentrations have been reported to be relatively stable across the menstrual cycle (1, 9-14), possibly reflecting the continuous recruitment of antral follicles, independent of gonadotropins, during the menstrual cycle (70, 71).

The association between age at menarche and AMH concentration in adulthood suggests early life influences on later ovarian function. Our finding of higher AMH concentrations in women with older compared to younger ages at menarche is consistent with two large studies (27, 34), while other smaller studies reported inverse (26, 35) or no associations (9). In a study of 502 women (34), those less than 12 years old at menarche were 1.6 times more likely to have a lower AMH concentration (below the 25<sup>th</sup> age-specific percentile) in adulthood than those older than 13.4 years of age at menarche (34). The Doetinchem Cohort Study, which included 2,030 healthy women, also suggested a positive association between AMH concentrations and age at menarche, although the results were only marginally significant (27). The fact that follicular recruitment peaks during puberty and declines thereafter (72) may imply that earlier menarche might lead to earlier follicular depletion at middle age, thereby explaining our observation. Future large studies are warranted to replicate our finding.

Associations of AMH with oral contraceptive use have been hypothesized because of their influence on follicle-stimulating hormone. In particular, oral contraceptives suppress follicle-stimulating hormone and reduce ovary size, which might decrease follicle recruitment, impair follicle functionality and result in lower antral follicle number and size (73-75). In the present

study, we observed substantially lower AMH concentrations in current oral contraceptive users compared to never and former users. Although our results were based on a small number of current oral contraceptive users, our observation is consistent with the majority of clinical trials (24, 25) and cross-sectional studies (9, 25-27), although not all (10, 39).

It has been speculated that long anovulatory periods associated with higher parity might slow the exhaustion of available follicles and alter AMH concentration (27). But evidence for the association between parity and AMH is lacking. Our finding of no association between parity and AMH level is consistent with four (8, 9, 27, 36) earlier studies, but not with one study that noted a significant inverse association (35).

The association of obesity with adverse reproductive outcomes has been hypothesized to be mediated, at least in part, through its effect on ovarian function (76, 77). Of 11 studies that reported results from healthy women on the association of BMI with AMH concentrations (9, 27, 28, 31-33, 36, 41-44), seven reported no significant association (9, 27, 36, 41-44), whereas four observed a significant inverse association (28, 31-33). Our non-significant association between BMI and AMH is consistent with the results from most studies of healthy women. While obese premenopausal women are more likely to experience anovulation than normal weight women (78), which may increase the number of small antral follicles that secrete AMH (79), obesity also increases adipokines and/or inflammatory markers in the ovaries, thereby potentially impairing follicle function which could lead to decreased AMH (76, 77). These opposite effects could explain the lack of clear association between AMH and BMI. Further investigation is warranted given that PCOS status, not available in our study, may interact with BMI (80).



Animal and experimental studies (81, 82) have suggested a possible adverse effect of smoking on the ovarian follicular pool. However, the association between smoking and ovarian reserve as evidenced by AMH concentrations has been mixed (8, 9, 27, 36-38, 40, 83). Our finding of no association between smoking and AMH is consistent with some studies (8, 9, 36, 83), though not others that reported significantly lower (27, 37, 38) or higher AMH concentrations (40) with smoking. Inconsistent results might have arisen from crude assessment of smoking status using three (9, 38) or two categories (8, 36, 40, 83) (e.g., never/past/current or never/ever) in most studies including ours, which does not take into account the duration and quantity of smoking that might differ across studies. In one study that collected a detailed smoking history, women who smoked  $\geq 10$  pack-years had significantly lower age-specific AMH concentrations compared to those who smoked  $< 5$  pack-years (27). Further, previous results are difficult to reconcile because of different adjustment factors across studies. Additional studies are warranted given the limitations of ours and other studies.

Racial differences associated with AMH concentrations are largely unknown. Our result for AMH concentrations in Asian vs. White women is inconsistent with that of a previous study (84). Whereas we found no significant variations in AMH concentrations between Asian women, mostly living in China, and White women living in the US and western Europe, AMH concentrations were 22% lower in Chinese women than in White women living in the US in one other study (84). More research is needed to fully understand potential racial differences in AMH concentrations. Finally, lack of an association of AMH concentrations with height (9) and education (36) observed in previous studies are consistent with our results.

Previously, eight studies (9, 28, 42-44, 65, 85, 86) examined associations of AMH with testosterone, three with androstenedione (42, 44, 86), and two with SHBG (9, 86). Our statistically non-significant testosterone results are consistent with three studies (9, 43, 44) but not with five others that reported significant positive associations between AMH and testosterone (28, 42, 65, 85, 86). Similarly, our statistically non-significant androstenedione results are consistent with one study (44) but not with two others that reported positive associations (42, 86). Use of a direct assays with no prior extraction step (87) or inclusion of data from multiple laboratories might have contributed to lack of associations of AMH with testosterone and androstenedione in our study. No study that we are aware of previously examined associations of AMH with DHEAS, and overall evidence for the possible role of DHEA supplements on ovarian reserve in trials has been inconclusive (88). Evidence for the association between SHBG and AMH has been inconsistent. While we observed a suggestively positive association between AMH and SHBG, others reported no association (9) or significant inverse associations (86).

The different blood collection and processing methods used by participating cohorts might introduce systematic differences in biomarker concentrations. Although our results were based on AMH concentrations adjusted for possible cohort variability using Rosner's method (54, 55), our finding of no significant association between AMH concentrations and season, time, and calendar year of blood collection is consistent with a previous study (9). AMH concentrations measured in serum and Li-heparin plasma specimens have been reported to be highly correlated (89), which is consistent with our observation of no difference in concentrations by matrix – serum, EDTA-plasma and heparin-plasma. All of these results suggest that AMH is not sensitive to differences in blood collection and processing when done under strict, albeit different protocols used by participating cohorts.

Our study has several strengths. It comprehensively examined the association of premenopausal AMH concentrations with numerous factors, including demographics, lifestyle, circulating androgen concentrations, and blood collection methods, while adjusting for important confounding factors that were not adjusted for in prior studies. Quantile regression, used for analysis, though less powerful than linear regression provides valid estimates of central tendency and effectively reduces the influence of outliers and, thus, gives a more accurate picture of correlates of badly skewed biomarkers such as AMH (56). Finally, all samples were analyzed for AMH in a single laboratory using a new ultrasensitive assay with demonstrated good validity and reproducibility (90, 91).

Our study also has some limitations. A single measurement of AMH might be subject to random measurement error, due to biological and assay variability, attenuating associations. Nonetheless, AMH concentrations have been shown to be relatively stable between a woman's menstrual cycles (92) and track over time (93). Furthermore, the analytical performance of the Ansh picoAMH assay that we used is excellent as mentioned above (90, 91). We also accounted for age-related changes in AMH adjusting for age in all of our analyses. Despite data collection at multiple sites using different protocols, we obtained primary data and uniformly harmonized those and biochemical markers were adjusted for cohort (54, 94). Our study included few women younger than 35 years of age (a median age of 40.4 years; interquartile range: 39.0-43.8 years). Given higher AMH concentrations in early adulthood, many of non-significant results might be attributed to the age range in our study population. We are not aware of the prevalence of PCOS or infertility in our study population because these data were not collected by most of the contributing cohorts. Nonetheless, the prevalence of PCOS in the 1990's and earlier (95), when most of blood

samples in our study were collected, was <10% and the PCOS prevalence is reported to decrease in late premenopausal women(96), as our study population. Even so, generalizing our results to all US women requires caution.

In conclusion, our result confirms a decline of AMH with increasing aging. We also observed significant lower AMH concentrations with earlier age at menarche and current oral contraceptive use, but not with any of the other lifestyle, reproductive or hormonal factors investigated. Although further large studies are warranted our results suggest that early life factors like age at menarche as well as current use of oral contraceptives may influence ovarian function by lowering the number or decreasing the secretory activity of follicles.

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**Table 1. Characteristics of control participants in the Prospective Study of AMH and Gynecologic Cancer Risk (N=671)**

Characteristics	N	Mean (SD) or Median (IQR)
Anti-Müllerian Hormone, ng/mL	671	1.01 (0.32-2.28)
Androstenedione (ng/dL) <sup>a,b</sup>	187	142 (95-207)
DHEAS (µg/dL) <sup>a,b</sup>	189	103.8 (70-161.0)
Testosterone (ng/dL) <sup>a,b</sup>	181	38 (27-48)
SHBG (nmol/L) <sup>a,b</sup>	171	56.5 (42.2-80.1)
Age at blood draw, yrs	671	40.9 (39.0-43.8)
Height <sup>a</sup> , cm	578	162.2 (6.1)
Body mass index <sup>a</sup> , kg/m <sup>2</sup>	576	24.5 (4.4)
Age at menarche <sup>a</sup> , yrs	530	13.1 (1.7)
<b>Percentage</b>		
Race		
White	411	61 %
Asian	82	12 %
Black or other	7	1 %
Unknown	171	25 %
Education		
High school or less	340	51 %
Vocational school	58	9 %
Attended college	201	30 %
Unknown	72	11 %
Smoking status		
Never	376	56 %
Past	113	17 %
Current	139	21 %
Unknown	43	6 %
Oral contraceptive use		
Never	204	30 %
Past	285	42 %
Current	27	4 %
Unknown	155	23 %
Total number of pregnancy		
0	86	13 %
1	77	11 %
2	181	27 %
≥3	174	26 %
Unknown	153	23 %
Menstrual phase		
Follicular	227	34 %
Luteal	229	45 %
Unknown	145	22 %

<sup>a</sup> The N for this variable is less than the study N because of missing information.<sup>b</sup> Androgens or SHBG values were from non-current users of oral contraceptives.

**Table 2. Adjusted median and the interquartile range of anti-müllerian hormone (ng/mL) across demographics and lifestyle factors in the Prospective Study of AMH and Gynecologic Cancer Risk**

Variable	N	Age-adjusted model <sup>a</sup>	P-value <sup>b</sup>	Multivariable model <sup>c</sup>	P-value <sup>b</sup>
Age (yrs)					
<35	64	2.62 (0.61, 4.37)	<0.001	2.52 (0.61, 4.63)	<0.001
≥35-<40	163	1.54 (0.64, 3.13)		1.55 (0.66, 2.97)	
≥40	444	0.72 (0.24, 1.50)		7.29 (0.26, 1.45)	
Race <sup>d</sup>					
White	411	1.14 (0.46, 2.01)	0.77	1.16 (4.93, 2.16)	0.62
Asian	82	1.18 (0.49, 2.41)		1.09 (4.64, 2.35)	
Education <sup>e</sup>					
High school or less	340	1.16 (0.45, 2.18)	0.56	1.13 (0.46, 2.18)	0.56
Vocational school	58	1.36 (0.46, 2.88)		1.48 (0.46, 2.71)	
Attended college	201	1.09 (0.43, 1.98)		1.11 (0.47, 2.16)	
Height (cm)					
≤157.7	145	0.82 (0.31, 1.95)	0.22	0.86 (0.31, 1.88)	0.15
>157.7-≤162	146	1.09 (0.39, 2.32)		1.10 (0.41, 2.22)	
>162-≤167	152	0.89 (0.39, 1.57)		0.88 (0.40, 1.67)	
167	134	1.12 (0.53, 2.31)		1.12 (0.51, 2.20)	
BMI (kg/m <sup>2</sup> )					
<20	53	1.059 (0.46, 1.79)	0.80	1.03 (0.46, 1.84)	0.48
≥20-<25	319	0.947 (0.32, 2.03)		0.95 (0.34, 1.93)	
≥25-<30	139	1.060 (0.35, 2.40)		1.05 (0.38, 2.30)	
≥30	64	1.145 (0.63, 2.08)		1.16 (0.57, 1.96)	
Smoking status					
Never	376	1.16 (0.490, 2.38)	0.29	1.15 (0.49, 2.31)	0.73
Past	113	0.99 (0.368, 1.92)		1.04 (0.36, 2.14)	
Current	139	1.14 (0.460, 1.99)		1.13 (0.47, 1.92)	
Current uses of oral contraceptives					
No	582	1.17 (0.56, 2.22)	0.08	1.15 (0.55, 2.16)	0.04
Yes	27	0.47 (<LOD <sup>f</sup> , 1.48)		0.36 (<LOD <sup>f</sup> , 1.55)	
Parity					
by Never/Ever					
Nulliparous	86	1.05 (0.33, 1.73)	0.52	1.07 (0.39, 1.86)	0.43
Parous	432	0.95 (0.38, 2.05)		0.95 (0.42, 2.00)	
by Total number of childbirths					
Nulliparous	86	1.04 (0.34, 1.77)	0.33	1.04 (0.37, 1.88)	0.41

**Table 2. Continued.**

Variable	N	Age-adjusted model <sup>a</sup>	P-value <sup>b</sup>	Multivariable model <sup>c</sup>	P-value <sup>b</sup>
1 child	77	0.83 (0.30, 1.45)		0.82 (0.32, 1.58)	
2 children	181	0.88 (0.38, 2.11)		0.91 (0.42, 2.01)	
≥3 children	174	1.15 (0.43, 2.13)		1.11 (0.46, 2.06)	
Age at menarche, yrs					
<12	96	0.88 (0.31, 1.73)	0.06	0.90 (0.36, 1.76)	0.04
12-<13	106	0.87 (0.30, 2.06)		0.86 (0.33, 2.04)	
13-<14	127	1.01 (0.42, 1.90)		1.01 (0.46, 1.84)	
≥14	200	1.11 (0.38, 2.17)		1.12 (0.42, 2.17)	
Menstrual cycle					
Follicular	227	1.11 (0.48, 2.07)	0.42	1.13 (0.50, 2.18)	0.78
Luteal	299	1.20 (0.53, 2.33)		1.16 (0.56, 2.23)	

<sup>a</sup> Model adjusted for age (continuous, yrs).

<sup>b</sup> P-values were calculated using an F-test, using a continuous term for continuous variables (age, height, body mass index, number of childbirths and age at menarche) or a nominal term for categorical variables (race, education, smoking status, current uses of oral contraceptives, ever parity, menstrual cycle).

<sup>c</sup> Model adjusted for age (continuous, yrs), current use of oral contraceptives (yes, no, missing) and age at menarche (<12, 12-<13, 13-<14, ≥14 yrs, missing).

<sup>d</sup> The European Prospective Investigation into Cancer and Nutrition Cohort study was excluded from this analysis, because race information was not collected in this study.

<sup>e</sup> The Columbia, Missouri study and the Guernsey Cohort Study were excluded from this analysis, because education was not collected in these studies.

<sup>f</sup> LOD represents the assay limit of detection.

**Table 3. Adjusted median and the interquartile range of anti-müllerian hormone (ng/mL) across endogenous level of androgens and SHBG in the Prospective Study of AMH and Gynecologic Cancer Risk<sup>a</sup>**

Hormones	N	Age-adjusted model <sup>b</sup>	P-value <sup>c</sup>	Multivariable model <sup>d</sup>	P-value <sup>c</sup>
Androstenedione (ng/dL)					
<125.0	47	1.03 (0.45, 1.75)	0.11	1.08 (0.45, 1.82)	0.15
≥125.0-<168.7	47	1.03 (0.35, 1.77)		0.99 (0.37, 1.76)	
≥169.9-<240.0	47	1.35 (0.57, 2.80)		1.37 (0.43, 2.82)	
≥ 240.0	46	1.33 (0.45, 2.63)		1.46 (0.44, 2.92)	
DHEAS (μg/dL)					
<76.4	48	1.06 (0.49, 2.37)	0.51	1.07 (0.44, 2.25)	0.78
≥76.6- <112.1	47	1.02 (0.34, 2.00)		1.06 (0.38, 1.93)	
≥112.5-<163.0	47	1.42 (0.57, 2.28)		1.44 (0.67, 2.21)	
≥ 164.5	47	1.19 (0.47, 2.36)		1.14 (0.41, 2.22)	
Testosterone (ng/dL)					
<31.2	46	0.90 (0.30, 1.74)	0.11	0.95 (0.28, 1.76)	0.36
≥31.2-39.9	45	0.99 (0.46, 1.82)		1.04 (0.47, 1.85)	
≥40.0-<54.0	45	1.36 (0.43, 2.44)		1.33 (0.44, 2.49)	
≥53.9	45	1.21 (0.57, 2.62)		1.27 (0.65, 2.76)	
Testosterone/SHBG ratio					
<0.45	41	1.03 (0.53, 1.56)	0.76	1.05 (0.50, 1.59)	0.73
≥0.45-<0.71	41	1.40 (0.39, 2.15)		1.41 (0.33, 2.41)	
≥0.71-<1.16	41	1.37 (0.55, 2.61)		1.32 (0.54, 2.65)	
≥1.16	40	1.01 (0.52, 2.78)		1.07 (0.51, 2.4 <sup>e</sup> )	
SHBG (nmol/L)					
<41.5	43	1.03 (0.46, 2.45)	0.54	0.97 (0.40, 2.47)	0.05
≥41.6-<54.3	43	1.21 (0.62, 2.35)		1.25 (0.57, 2.09)	
≥54.4-<74.2	43	1.13 (0.45, 2.68)		1.31 (0.35, 2.67)	
≥74.2	42	1.11 (0.53, 1.96)		1.43 (0.57, 2.00)	

Abbreviation: DHEAS, Dehydroepiandrosterone-sulfate; SHBG, sex hormone-binding globulin

<sup>a</sup> This analysis was conducted among women who do not currently use oral contraceptives, because of the influence of contraceptives on endogenous levels of sex hormones.

<sup>b</sup> Model adjusted for age (continuous, yrs).

<sup>c</sup> P-values were calculated using an F-test, using a continuous term for androgens and SHBG.

<sup>d</sup> Model adjusted for age (continuous, yrs), and age at menarche (<12, 12-<13, 13-<14, ≥14 yrs, missing).

**Table 4. Adjusted median and the interquartile range of anti-müllerian hormone (ng/mL) across blood collection and processing methods in the Prospective Study of AMH and Gynecologic Cancer Risk**

Variable	<i>N</i>	Age-adjusted model <sup>a</sup>	P-value <sup>b</sup>	Multivariable model <sup>c</sup>	P-value <sup>b</sup>
Season of blood collection					
Winter	128	1.09 (349, 1.91)	0.96	1.13 (383, 2013)	0.94
Spring	145	1.15 (479, 2.24)		1.18 (510, 2217)	
Summer	180	1.11 (448, 1.97)		1.12 (481, 2166)	
Fall	218	1.15 (460, 2.30)		1.09 (475, 2254)	
Calendar year of blood collection					
≤1985	168	1.12 (565, 1.94)	0.60	1.10 (538, 1946)	0.60
>1985-≤1990	177	1.07 (374, 1.93)		1.08 (396, 2001)	
>1990-≤1997	204	1.08 (438, 2.40)		1.09 (441, 2300)	
>1997	122	1.25 (477, 2.53)		1.25 (526, 2427)	
Type of samples <sup>d</sup>					
Serum	354	1.11 (451, 2.04)	0.43	1.13 (0.48, 2.22)	0.99
EDTA plasma	194	1.08 (445, 2.39)		1.12 (0.47, 2.21)	
Heparin plasma	119	1.26 (437, 2.14)		1.13 (0.47, 2.22)	
Time of blood collection					
≤9 a.m.	158	1.05 (0.51, 2.02)	0.50	1.02 (0.52, 2.05)	0.44
>10 a.m.-<12 p.m.	148	1.07 (0.50, 2.24)		1.06 (0.50, 2.16)	
≥12 p.m.-<4 p.m.	141	1.22 (0.55, 2.24)		1.22 (0.54, 2.23)	
≥4 p.m.	108	1.16 (0.58, 2.41)		1.19 (0.60, 2.18)	

<sup>a</sup> Model adjusted for age (continuous, yrs).

<sup>b</sup> P-values were calculated using a F-test, using a nominal term for categorical variables for fasting status, season of blood collection, calendar year of blood collection, type of samples, time of blood collection.

<sup>c</sup> Model adjusted for age (continuous, yrs), current use of oral contraceptives (yes, no, missing) and age at menarche (<12, 12-<13, 13-<14, ≥14 yrs, missing).

<sup>d</sup> For this analyses, we did not convert AMH value measured from citrate plasma to the corresponding AMH value from serum using the equation provided by Ansh Lab.

**Supplementary Table 1. Cohort-specific characteristics of study population in the Prospective Study of AMH and Gynecologic Cancer Risk**

	Columbia	CLUE I/II	EPIC	Guernsey	NHS/NHSII	NYU-WHS	NSHDS	ORDET	SWHS
	(N = 17)	(N = 138)	(N = 149)	(N = 27)	(N = 35)	(N = 96)	(N = 94)	(N = 34)	(N = 81)
<i>Mean (SD) or median (IQR)</i>									
Anti-Mullerian Hormone <sup>a</sup> , ng/mL	1.18 (0.68-1.71)	1.22 (0.43-3.04)	1.03 (0.32-3.68)	0.60 (0.18-1.16)	0.74 (0.12-1.73)	1.13 (0.48-2.36)	0.97 (0.36-2.24)	1.04 (0.37-1.96)	0.44 (0.18-1.29)
Anti-Mullerian Hormone <sup>b</sup> , ng/mL	1.43 (0.72-2.82)	1.17 (0.50-2.10)	1.15 (0.47-2.39)	1.11 (0.50-1.89)	1.26 (0.43-2.40)	0.99 (0.44-1.77)	1.12 (0.49-2.35)	1.09 (0.44-1.71)	1.12 (0.46-2.35)
Andronestendione, ng/dL	141 (114-175.5)	83.9 (58.8-128.0)	134.7 (85.5-178.9)	463 (360-567)	-	145 (107-212.5)	138 (118-214)	237.5 (207-400)	-
DHEAS, µg/dL	95.1 (69.0-144.2)	109 (83-174)	73.6 (50.8-110.7)	183.4 (158.1-223.3)	-	124.9 (82.5-180.5)	102.6 (64.6-141.7)	129.7 (89.4-239.5)	-
Testosterone, ng/dL	41 (31-56.5)	40 (22.0-47.2)	39 (27.5-48.2)	53 (50-84)	-	32 (26-44)	35 (28-38)	47.5 (39-60)	-
SHBG, nmol/L	69.7 (43.5-91.0)	60.6 (47.6-82.8)	61.8 (44.1-79.7)	53.9 (39.1-68.4)	-	55.7 (44.0-74.8)	53.8 (37.8-62.7)	42.2 (32.9-56.9)	-
Age at blood draw, yrs	40.8 (39.5-42.7)	38.7 (33.6-42.7)	41.9 (39.6-44.6)	40.5 (36.5-43.7)	43.6 (41.3-45.4)	40.8 (38.6-43.2)	40.1 (39.9-40.3)	42.3 (38.9-43.8)	43.6 (42.1-44.7)
Height, cm	164.5 (4.4)	163.2 (6.1)	160.7 (6.7)	162.8 (4.2)	164.6 (6.4)	162.3 (5.4)	165.3 (5.0)	158.1 (5.7)	160.4 (5.1)
Body mass index, kg/m <sup>2</sup>	25.2 (6.9)	26.8 (6.0)	24.7 (4.3)	24.8 (5.0)	24.9 (5.6)	23.5 (3.5)	24.7 (3.8)	25.1 (4.2)	23.1 (3.1)
Age at menarche, yrs	12.6 (1.2)	12.3 (1.9)	12.6 (1.5)	13.0 (1.2)	12.9 (1.7)	12.4 (1.6)	13.3 (1.5)	12.9 (1.6)	14.9 (1.7)
<i>Percentage</i>									
Race									
White	100 %	99 %	-	100 %	97 %	73 %	98 %	100 %	0 %
Asian	0 %	0 %	-	0 %	3 %	0 %	0 %	0 %	100 %
Black or other	0 %	1 %	-	0 %	0 %	6 %	0 %	0 %	0 %
Missing	0 %	0 %	-	0 %	0 %	21 %	2 %	0 %	0 %
Education									
High school or less	-	73 %	52 %	-	0 %	16 %	52 %	62 %	95 %

Vocational school	-	0 %	26 %	-	0 %	4 %	11 %	6 %	4 %
Attended college	-	26 %	22 %	-	100 %	64 %	26 %	32 %	1 %
Missing	-	1 %	0 %	-	0 %	17 %	12 %	0 %	0 %

**Supplementary Table 1. Continued.**

	Columbia	CLUE I/II	EPIC	Guernsey	NHS/NHSII	NYU-WHS	NSHDS	ORDET	SWHS
Smoking status									
Never	53 %	55 %	54 %	48 %	57 %	42 %	36 %	68 %	100
Past	18 %	15 %	19 %	4 %	31 %	32 %	15 %	12 %	0
Current	29 %	30 %	27 %	26 %	11 %	17 %	20 %	21 %	0
Missing	0 %	0 %	1 %	22 %	0 %	9 %	29 %	0 %	0
Oral contraceptive use									
Never	29 %	14 %	23 %	37 %	20 %	23 %	20 %	44 %	89 %
Past	59 %	19 %	58 %	52 %	80 %	67 %	30 %	56 %	11 %
Current	12 %	7 %	6 %	11 %	0 %	0 %	3 %	0 %	0 %
Missing	0 %	60 %	12 %	0 %	0 %	10 %	47 %	0 %	0 %
Total number of pregnancy									
0	0 %	3 %	11 %	19 %	17 %	45 %	10 %	9 %	0 %
1	24 %	2 %	12 %	7 %	9 %	8 %	9 %	15 %	32 %
2	12 %	10 %	30 %	41 %	31 %	34 %	22 %	47 %	36 %
≥3	65 %	9 %	33 %	33 %	43 %	7 %	37 %	29 %	32 %
Missing	0 %	76 %	15 %	0 %	0 %	5 %	22 %	0 %	0 %
Menstrual phase									
Follicular	24 %	47 %	40 %	48 %	3 %	53 %	-	0 %	42 %
Luteal	65 %	53 %	38 %	48 %	54 %	47 %	-	100 %	58 %
Missing	12 %	0 %	22 %	4 %	43 %	0 %	-	0 %	0 %

Abbreviations: Columbia, the Columbia, Missouri (USA); the CLUE I/II, Campaign Against Cancer and Heart Disease (USA); EPIC, the European Prospective Investigation into Cancer and Nutrition Cohort study (Europe); Guernsey, the Guernsey Cohort Study (UK); NYU-WHS, the New York University Women's Health Study (USA); NHS/NHSII, the Nurses' Health Studies (USA); ORDET, the Hormones and Diet in the Etiology of Breast Cancer study (Italy); NSHDS, Northern Sweden Health and Disease Study (Sweden); SWHS, the Shanghai Women's Health Study (China)

<sup>a</sup>AMH values are unadjusted raw values.

<sup>b</sup>AMH values are adjusted for age at blood draw and current use of oral contraceptives.