



# Advancing research:

*One cell at a time*

*One scientist at a time*

*One discovery at a time*

**Proven solutions  
that further science**

BD Accuri™ C6 Plus

BD FACSCelesta™

BD LSRFortessa™

**Discover more>**



[www.bdbiosciences.com/us/go/research-solutions](http://www.bdbiosciences.com/us/go/research-solutions)



## Title Page: Anti-Müllerian Hormone and risk of ovarian cancer in nine cohorts

Seungyoun Jung<sup>1</sup>, Naomi Allen<sup>2</sup>, Alan A. Arslan<sup>3,4</sup>, Laura Baglietto<sup>5,6</sup>, Aurelio Barricarte<sup>7,8,9</sup>, Louise A. Brinton<sup>10</sup>, Brian L. Eggleston<sup>11</sup>, Roni T. Falk<sup>10</sup>, Renée T. Fortner<sup>12</sup>, Kathy J. Helzlsouer<sup>13,14</sup>, Yutang Gao<sup>15</sup>, Annika Idahl<sup>16</sup>, Rudolph Kaaks<sup>12</sup>, Vittorio Krogh<sup>17</sup>, Melissa A. Merritt<sup>18</sup>, Eva Lundin<sup>19</sup>, N. Charlotte Onland-Moret<sup>20</sup>, Sabina Rinaldi<sup>21</sup>, Helena Schock<sup>12</sup>, Xiao-Ou Shu<sup>22</sup>, Patrick M. Sluss<sup>23</sup>, Paul N. Staats<sup>24</sup>, Carlotta Sacerdote<sup>25</sup>, Ruth C. Travis<sup>26</sup>, Anne Tjønneland<sup>27</sup>, Antonia Trichopoulou<sup>28,29</sup>, Shelley S. Tworoger<sup>30,31</sup>, Kala Visvanathan<sup>14</sup>, Elisabete Weiderpass<sup>32,33,34,35</sup>, Anne Zeleniuch-Jacquotte<sup>4</sup>, Joanne F. Dorgan<sup>1</sup>

<sup>1</sup> Department of Epidemiology and Public Health, University of Maryland School of Medicine, Baltimore, MD, USA

<sup>2</sup> Clinical Trial Service Unit and Epidemiological Studies Unit, Nuffield Department of Population Health, University of Oxford, UK

<sup>3</sup> Department of Obstetrics and Gynecology, New York University School of Medicine, NY, USA

<sup>4</sup> Departments of Population Health and Environmental Medicine and Perlmutter Cancer Center, New York University School of Medicine, New York, NY, USA

<sup>5</sup> Cancer Epidemiology Centre, Cancer Council of Victoria, Melbourne, Australia

<sup>6</sup> Centre for Epidemiology and Biostatistics, School of Population and Global Health, University of Melbourne, Australia

<sup>7</sup> Navarra Public Health Institute, Pamplona, Spain

<sup>8</sup> Navarra Institute for Health Research (IdiSNA) Pamplona, Spain

<sup>9</sup> CIBER Epidemiology and Public Health CIBERESP, Spain

<sup>10</sup> Division of Cancer Epidemiology and Genetics, National Cancer Institute, MD, USA

<sup>11</sup> Fox Chase Cancer Center, PA, USA

<sup>12</sup> Division of Cancer Epidemiology, German Cancer Research Cancer, Heidelberg, Germany

<sup>13</sup> Division of Cancer Control and Population Sciences, National Cancer Institute, MD, USA

<sup>14</sup> Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA

<sup>15</sup> Department of Epidemiology, Shanghai Cancer Institute, Shanghai, China.

<sup>16</sup> Department of Clinical Sciences, Obstetrics and Gynecology, Umeå University, Umeå, Sweden

<sup>17</sup> Fondazione IRCCS Istituto Nazionale dei Tumori, Milano, Italy

<sup>18</sup> Department of Epidemiology and Biostatistics, School of Public Health, Imperial College London, London, United Kingdom

<sup>19</sup> Department of Medical Biosciences, Pathology, and Public Health and Clinical Medicine: Nutritional Research, Umeå University, Umeå, Sweden

<sup>20</sup> Department of Epidemiology, UMC Utrecht Julius Center, Utrecht, Netherlands

<sup>21</sup> International Agency for Research on Cancer, Lyon, France

<sup>22</sup> Department of Epidemiology, Vanderbilt University School of Medicine, Nashville, Tennessee, USA

<sup>23</sup> Department of Pathology, Harvard Medical School, Boston, MA, USA

<sup>24</sup> Department of Pathology, University of Maryland School of Medicine, Baltimore, MD, USA

<sup>25</sup> Unit of Cancer Epidemiology, Città della Salute e della Scienza University-Hospital and Center for Cancer Prevention (CPO), Turin, Italy

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1002/ijc.31058

<sup>26</sup> Cancer Epidemiology Unit, University of Oxford, Oxford United Kingdom

<sup>27</sup> Danish Cancer Society Research Center, Copenhagen, Denmark

<sup>28</sup> Hellenic Health Foundation, Athens, Greece;

<sup>29</sup> WHO Collaborating Center for Nutrition and Health, Unit of Nutritional Epidemiology and Nutrition in Public Health, Dept. of Hygiene, Epidemiology and Medical Statistics, University of Athens Medical School, Greece

<sup>30</sup> Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, MA, USA

<sup>31</sup> Channing Division of Network Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA

<sup>32</sup> Department of Community Medicine, Faculty of Health Sciences, University of Tromsø, The Arctic University of Norway, Tromsø, Norway.

<sup>33</sup> Department of Research, Cancer Registry of Norway, Institute of Population-Based Cancer Research, Oslo, Norway.

<sup>34</sup> Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden

<sup>35</sup> Genetic Epidemiology Group, Folkhälsan Research Center, Helsinki, Finland

**Key Words:** Anti-Müllerian hormone, ovarian cancer, epidemiology, ovarian function

### Corresponding author

**Dr. Joanne F. Dorgan**

University of Maryland School of Medicine

Howard Hall 102E, Baltimore, MD 21201

Tel: 410-706-1602

Fax: 410-706-8013

Email: [jdorgan@som.umaryland.edu](mailto:jdorgan@som.umaryland.edu)

**Word count:** Abstract = 198; Text = 2773

**Tables/Figure:** 5 main tables/ 3 supplementary tables; Supplementary Figure 1

**Abbreviations:** AMH, Anti-Müllerian Hormone; BMI, body mass index; CI, confidence interval; OR, odds ratio

**Grant support:** This work was supported by U.S. National Institutes of Health (NIH) grant R01 CA163018 to J.F. Dorgan. The Nurses' Health Study is supported by grant UM1 CA186107, R01 CA49449, and P01 CA87969, while the Nurses' Health Study II is supported by grant UM1 CA17672. The intramural program of the National Cancer Institute, National Institutes of Health also supported this project. The NYU Women's Health Study is supported by grants R01 CA178949, UM1 CA182934 and center grants P30 CA016087 and P30 ES000260. The coordination of EPIC is financially supported by the European Commission (DG-SANCO) and the International Agency for Research on Cancer. The national cohorts are supported by Danish Cancer Society (Denmark); Ligue Contre le Cancer, Institut Gustave Roussy, Mutuelle Générale de l'Éducation Nationale, Institut National de la Santé et de la Recherche Médicale (INSERM) (France); German Cancer Aid, German Cancer Research Center (DKFZ), Federal Ministry of Education and Research (BMBF), Deutsche Krebshilfe, Deutsches Krebsforschungszentrum and

Federal Ministry of Education and Research (Germany); the Hellenic Health Foundation (Greece); Associazione Italiana per la Ricerca sul Cancro-AIRC-Italy and National Research Council (Italy); Dutch Ministry of Public Health, Welfare and Sports (VWS), Netherlands Cancer Registry (NKR), LK Research Funds, Dutch Prevention Funds, Dutch ZON (Zorg Onderzoek Nederland), World Cancer Research Fund (WCRF), Statistics Netherlands (The Netherlands); ERC-2009-AdG 232997 and Nordforsk, Nordic Centre of Excellence programme on Food, Nutrition and Health (Norway); Health Research Fund (FIS), PI13/00061 to Granada; , PI13/01162 to EPIC-Murcia), Regional Governments of Andalucía, Asturias, Basque Country, Murcia and Navarra, ISCIII RETIC (RD06/0020) (Spain); Swedish Cancer Society, Swedish Research Council and County Councils of Skåne and Västerbotten (Sweden); Cancer Research UK (14136 to EPIC-Norfolk; C570/A16491 and C8221/A19170 to EPIC-Oxford), Medical Research Council (1000143 to EPIC-Norfolk, MR/M012190/1 to EPIC-Oxford) (United Kingdom). The Guernsey Study is supported by Cancer Research UK and the Lloyds TSB Charitable Foundation for the Channel Islands. The Shanghai Women's Health Study is supported by NIH grants R37 CA070867 and UM1 CA182910.

For information on how to submit an application for gaining access to EPIC data and/or biospecimens, please follow the instructions at <http://epic.iarc.fr/access/index.php>

### **Novelty and Impact statements**

Anti-Müllerian hormone (AMH) is a member of the transforming growth factor-beta superfamily and serves as a marker of ovarian reserve. This pooled analysis of nine cohorts of mostly late premenopausal women provides the largest epidemiologic results testing the hypothesized protective role of AMH against ovarian carcinogenesis. Contrary to animal and experimental studies, we found no associations between AMH and ovarian cancer risk. Whether AMH reduces ovarian cancer risk in humans remains to be established.

## ABSTRACT

Animal and experimental data suggest that anti-Müllerian hormone (AMH) serves as a marker of ovarian reserve and inhibits the growth of ovarian tumors. However, few epidemiologic studies have examined the association between AMH and ovarian cancer risk. We conducted a nested case-control study of 302 ovarian cancer cases and 336 matched controls from nine cohorts.

Prediagnostic blood samples of premenopausal women were assayed for AMH using a picoAMH enzyme-linked immunosorbent assay. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using multivariable-adjusted conditional logistic regression. AMH concentration was not associated with overall ovarian cancer risk. The multivariable-adjusted OR (95% CI), comparing the highest to the lowest quartile of AMH, was 0.99 (0.59-1.67) ( $P_{\text{trend}}: 0.91$ ). The association did not differ by age at blood draw or oral contraceptive use (all  $P_{\text{heterogeneity}} \geq 0.26$ ).

There also was no evidence for heterogeneity of risk for tumors defined by histologic developmental pathway, stage, and grade, and by age at diagnosis and time between blood draw and diagnosis (all  $P_{\text{heterogeneity}} \geq 0.39$ ). In conclusion, this analysis of mostly late premenopausal women from nine cohorts does not support the hypothesized inverse association between prediagnostic circulating levels of AMH and risk of ovarian cancer.

## Introduction

Anti-Müllerian hormone (AMH) is a member of the transforming growth factor- $\beta$  superfamily secreted exclusively by the gonads. In males, AMH promotes regression of Müllerian ducts in-utero, while in females it regulates recruitment of primordial ovarian follicles and is a sensitive marker of ovarian reserve<sup>1</sup>. Minimal fluctuations in AMH concentration within the menstrual cycle makes AMH a more reliable biomarker than most other ovarian hormones<sup>2</sup>.

The protective effect of AMH against development of tumors of the female reproductive tract was first hypothesized in the early 1980s<sup>3</sup>. The Müllerian ducts of female fetuses evolve into the fallopian tubes, endometrium, and endocervix. Ovarian “surface epithelial” neoplasms are hypothesized in most cases to arise from Müllerian epithelium (e.g. fallopian tube, endometrium) or ovarian surface mesothelium that has undergone Müllerian transformation<sup>4</sup>. Based on the inhibitory role of AMH on the Müllerian ducts during sexual differentiation, AMH was speculated to inhibit ovarian cancer development. *In vitro* and animal studies subsequently demonstrated that AMH decreases ovarian cell proliferation rates, tumor growth, and steroid synthesis, while increasing apoptosis in ovarian cancer cell lines, though at supra-physiologic levels<sup>5-9</sup>. Mechanistic studies have also shown that AMH binds to AMH receptors that are expressed in the ovaries<sup>10-12</sup> and, in turn, initiates a series of intracellular cascades that alter cell cycle regulating proteins and levels of transcription factors involved in cell proliferation and differentiation<sup>13-16</sup>.

However, there is sparse evidence in human populations for an association between circulating AMH concentration and subsequent risk of ovarian cancer. To date, the association has been evaluated in only one small nested case-control study of pregnant women and that study reported no overall association between AMH concentration and ovarian cancer risk<sup>17</sup>. Using a sensitive AMH assay<sup>18, 19</sup>, we examined the association between prediagnostic circulating levels of AMH in premenopausal women and the subsequent risk of ovarian cancer in nine cohorts.

## Methods

### *Study population*

Participants derive from the nine cohorts within the Prospective Study of AMH and Gynecologic Cancer Risk; the participating cohorts were the Columbia, Missouri Serum Bank (USA)<sup>20</sup>, the Campaign Against Cancer and Heart Disease (CLUE I/II; USA)<sup>21</sup>, the European Prospective Investigation into Cancer and Nutrition (EPIC; Europe)<sup>22</sup>, the Guernsey Cohort Study (UK)<sup>23</sup>, the New York University Women's Health Study (NYUWHS; USA)<sup>24</sup>, the Nurses' Health Study and Nurses' Health Study II (NHS/NHSII; USA)<sup>25</sup>, the Hormones and Diet in the Etiology of Breast Cancer (ORDET; Italy)<sup>26</sup>, the Northern Sweden Health and Disease Study (NSHDS; Sweden)<sup>27</sup>, and the Shanghai Women's Health Study (SWHS; China)<sup>28</sup>. All participants provided informed consent. The current study was approved by the institutional review boards of each of the collaborating institutions and the University of Maryland, Baltimore.

### *Case-control selection*

This analysis included premenopausal women aged less than 47 years with no history of cancer (other than non-melanoma skin cancer) at blood draw. This age cut-off was implemented because of the high frequency of AMH values below the assay limit of detection after 47 years of age in our earlier study of breast cancer<sup>20</sup>. Incident cases of ovarian cancer were ascertained through self-report and medical record review<sup>25</sup>, linkage to cancer registries<sup>21-23, 26-28</sup>, death registries<sup>20, 23, 24</sup> or all three methods<sup>20, 24</sup>. Information on tumor characteristics, including histology, stage and grade, were obtained through cancer registries<sup>20, 21, 24, 27</sup>, pathology reports<sup>22-24</sup> and medical records<sup>25, 26, 28</sup>. Histology and grade were used to classify tumors as type I or type II<sup>29, 30</sup>. Type II tumors were defined as “high grade serous” or “serous, not otherwise specified (NOS)” (ICD-O-2 codes: 8461, 8441, 8450, 8460) with grade $\geq$ 2; for Shanghai Women’s Health Study (for which grade data were not available), type II tumors were defined as “high grade serous” or “serous, NOS” with documentation of death from ovarian cancer. All other tumors (8310, 8323, 8380, 8442, 8451, 8462, 9014, 8470, 8741, 8480, 8481, 8472, 8473, 8640, 8800, 8950, 8951, 8980), except “carcinomas, NOS” and “sarcomas”, which were set to missing for type I/II tumor categorization, were defined as type I tumors.

Eligible controls were premenopausal women less than 47 years old at blood draw who had no history of cancer other than non-melanoma skin cancer. One or two controls from NSHDS and one control from all other cohorts were matched per case by age at blood draw (88% were matched within  $\pm 1$  years; max 2.4 years) and date of blood draw ( $\pm 1$  year), and other study-specific matching factors<sup>20-28</sup> (Supplementary Table 1). Of the 304 cases and 339 matched controls initially identified, 2 cases of granulosa cell tumors and their matched controls (N = 3)



were excluded because of the well-known positive association of AMH with these tumors<sup>31</sup>. The final sample included 302 cases and 336 controls.

### ***Measurement of circulating AMH***

Each cohort selected plasma<sup>21, 22, 25-28</sup> or serum samples<sup>20-24</sup> from eligible women, which were labelled to be indistinguishable as to case-control status. Samples were sent to a single location where they were organized into study-specific batches before being shipped to the Massachusetts General Hospital Clinical Laboratory Research Core (Boston, MA, USA) for assay. Case-control sets were randomly ordered within cohort and assayed together using a picoAMH enzyme-linked immunosorbent assay kit (Ansh Catalog no. AL-124, Webster, TX). The coefficient of variation of the AMH assay, estimated from 61 blinded quality control replicates from a common pool of control samples, was 15.5%. The assay limit of detection was 20 pg/mL and <5% (N= 29) had values below the assay limit of detection.

### ***Demographic and lifestyle data***

Cohorts collected participants' data on demographics, lifestyle, reproductive and menstrual histories, and medical history before or at the time of blood collection by self-report<sup>20-22, 24-26, 32</sup> and/or interview<sup>23, 28</sup>. For the present analysis, data were available for age at blood draw, race, education, height, weight, body mass index (BMI), smoking status, age at menarche, total number of pregnancies, and oral contraceptive use.

### ***Statistical analyses***

Primary data obtained from the nine cohorts were harmonized to be uniformly defined and categorized. AMH values (N=2) measured in citrate plasma were converted to the corresponding AMH serum values using an equation provided by Ansh Labs to correct for dilution by citrate (personal communication with Ansh Labs). AMH concentration was categorized in study-specific quartiles based on the distribution in the controls; secondary analyses were also conducted using (i) study-wide common quartiles of AMH concentration, and (ii) common quartiles of cohort-adjusted AMH concentration, using the method of Rosner<sup>33</sup>. Study-specific deciles of AMH, based on the distribution in the controls in each cohort, were also used to compare women with extreme AMH levels in relation to ovarian cancer risk.

The odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using conditional logistic regression models. Tests for trend were conducted using the Wald statistic of the ordinal value of AMH quartiles. The following factors were initially considered as potential confounding factors in the multivariable model: BMI, ever use of oral contraceptives, total number of pregnancies, age at menarche, smoking status, education, and race. Missing values, for which there was a high proportion for some variables (Table 1), were handled in one of two ways. Missing values for BMI, ever use of oral contraceptives, total number of pregnancies, age at menarche and smoking status were imputed<sup>34</sup> using a prediction model to create 10 multiply imputed datasets, which included the imputed variables, age at blood collection, cohort, current oral contraceptive use, race, and education as predictors based on their distributions within each cohort. Otherwise, we used missing indicators for variables that were not collected in some cohorts (i.e. education in the Columbia and Guernsey cohorts; race in EPIC).

Of the potential confounding factors studied, age at menarche and oral contraceptive use were found to be associated with AMH and were retained in the final multivariable model. Age at blood draw (continuous) was also included in the final model, because of its strong inverse association with AMH. We obtained final pooled results by averaging results of analysis from each of the 10 multiply imputed datasets using Rubin's rule<sup>35</sup>. The between-study heterogeneity of the pooled risk estimates from 10 multiply imputed datasets was tested using the Q statistic assuming random effects<sup>36</sup>.

Separate conditional logistic models were fit to evaluate associations of AMH with risk for ovarian tumor subtypes defined by tumor development pathway (Type I vs. Type II), stage (early [=stage 1] vs. late [= stage  $\geq 2$ ]), and grade (low [=grade 1] vs. high [=grade  $\geq 2$ ]), and by age at ovarian cancer diagnosis (<50 yrs vs.  $\geq 50$  yrs) and time between blood collection and diagnosis ( $\leq 5$  yrs, >5-<10 yr,  $\geq 10$  yrs). The heterogeneity of the observed associations across subgroups was tested using a contrast test<sup>37</sup>, comparing the risk estimates of the ordinal trend terms from the conditional logistic regression models.

In subgroup analyses stratified by age at blood draw (<40 yrs vs.  $\geq 40$  yrs) and oral contraceptive use (never vs. ever), unconditional logistic regression models were used to preserve the number of subjects included in analyses. Models were fit using robust variances that adjusted for within-cohort correlation<sup>38</sup>, while additionally adjusting for the matching factors common to all cohorts (age and year of blood collection). Interaction with AMH concentration was tested using the cross-product term between stratifying factor and AMH concentration. Inferences from unconditional logistic models did not differ from conditional models.

We also conducted several sensitivity analyses. To evaluate the potential effect of subclinical ovarian cancer on AMH concentration, we excluded cases diagnosed within one year of the date of blood draw (N cases = 10). We also excluded women who were current users of oral contraceptives (N cases =7; N controls=12), and separately, those from one cohort (Guernsey) that stored blood at -20° C (N cases =16; N controls=16).

All analyses were conducted using STATA version 13.0 (College Station, TX, USA) or SAS version 9.4 (SAS Institute, Cary, North Carolina, USA). All tests were two-sided and considered significant if P-value <0.05.

## Results

**Table 1** shows the population characteristics of the 302 cases and 336 controls included in this analysis. The majority of cases and controls were White. The mean age at blood draw was 40.6 years (range: 25.4-46.0 years) for the cases and 40.5 years (range: 24.1-46.8 years) for the controls. Compared to the controls, cases had a higher BMI, slightly earlier age at menarche, were less likely to be current or ever users of oral contraceptives, and were more likely to be never smokers. The median (interquartile range) AMH concentration was 1035 pg/mL (300-2230 pg/mL) in the cases and 1025 pg/mL (330-2235 pg/mL) in the controls. The median age at diagnosis for the cases was 50.5 years, and the median time from blood draw to ovarian cancer diagnosis was 9.2 years (**Table 2**). Ninety percent of ovarian cancer cases had data on histology; 51% had data on grade, and 70% had data on stage; of characterized tumors, 49% were serous,



55% were poorly differentiated (grade  $\geq 3$ ), and 65% had spread beyond the ovaries (stage  $\geq 2$ ).

Cohort-specific participant characteristics are summarized in **Supplementary Table 2**. Age-adjusted median AMH concentration in controls ranged from 530 pg/mL in SWHS to 1605 pg/mL in CLUE I/II, while mean age at blood draw ranged from 38.3 years in CLUE I/II to 43.1 years in SWHS.

We observed no significant association between prediagnostic concentrations of AMH and the overall risk of ovarian cancer in either simple- or multivariable-adjusted models (OR<sub>Q4vsQ1</sub> [95% CIs]: 0.99 [0.59-1.67],  $P_{\text{trend}}$ : 0.91,  $P_{\text{heterogeneity across studies}}$ : 0.48; **Table 3**). Additional adjustments for BMI, smoking status, race, total number of pregnancies, and education did not change results. Exclusion of ovarian cancer cases diagnosed within one year of blood collection, current users of oral contraceptives or samples from one cohort that were stored at -20° C, in separate sensitivity analyses, did not substantially alter the results (data not shown). The associations were similar when using common study-wide AMH quartiles or common quartiles of cohort-adjusted AMH (**Supplementary Table 3**). Results also were similar when comparing more extreme AMH concentrations using deciles (**Supplementary Figure 1**). Repeating analyses using missing indicators for all covariates instead of imputed values for some yielded similar non-significant associations (data not shown).

The non-significant associations of AMH concentration with the risk of overall ovarian cancer did not vary significantly according to age at blood draw (OR<sub>Q4vsQ1</sub> [95% CIs] <40 years: 1.17 [0.52-2.62];  $\geq 40$  years, 1.06 [0.70-1.60]) or oral contraceptive use (never oral contraceptive

users: 1.20 [0.46-3.15]; ever oral contraceptive users, 1.01 [0.55-1.88]) (All  $P_{\text{trend}} \geq 0.39$ ; all  $P_{\text{heterogeneity}} \geq 0.26$ ; **Table 4**).

We also examined associations with ovarian cancer defined by tumor characteristics and by age at and time to diagnosis (**Table 5**). AMH concentrations were not significantly associated with risk of ovarian cancer subtypes defined by Type I/II classification (OR<sub>Q4vsQ1</sub> [95% CIs], Type I tumors, 0.98 [0.44-2.15]; Type II tumors, 0.86 [0.27-2.68]), stage (early stage, 0.97 [0.30-3.48]; late stage, 1.07 [0.48-2.39]), or grade (low grade, 1.63 [0.24-11.03]; high grade, 0.76 [0.32-1.81]) (all  $P_{\text{trend}} \geq 0.68$ ; all  $P_{\text{heterogeneity}} \geq 0.61$ ). The associations with AMH and ovarian cancer also did not differ by age at diagnosis (OR<sub>Q4vsQ1</sub> [95% CIs], <50 yrs: 1.37 [0.58-3.25];  $\geq 50$  yrs, 0.72 [0.41-1.28]) or time between blood draw and diagnosis ( $\leq 5$ yr, 2.75 [0.70-10.78], for >5-<10 yr, 0.69 [0.25-1.93];  $\geq 10$  yr, 1.00 [0.48-2.11]) (all  $P_{\text{trend}} \geq 0.43$ ; all  $P_{\text{heterogeneity}} \geq 0.39$ ).

## Discussion

In this prospective analysis including nine cohorts, prediagnostic circulating concentration of AMH was not associated with overall ovarian cancer risk and did not differ by oral contraceptive use or age at blood draw. There also was no association with risk by specific ovarian tumor subtypes defined by Type I/II classification, stage, grade, age at diagnosis or time from blood draw to diagnosis.

To date, only one prior nested case-control study (the Finnish Maternity Cohort, with 107 cases), not included in the current pooled analyses<sup>17</sup>, examined the association between AMH and risk

of ovarian cancer. That study reported that AMH concentration in blood collected during the first trimester of pregnancy from women in their early thirties was not associated with subsequent risk of overall ovarian cancer ( $OR_{T3vs\ T1}$ : 0.93, 95% CIs: 0.49-1.77,  $P_{trend}$ : 0.83). This result is consistent with our findings in older non-pregnant premenopausal women.

In contrast, these epidemiological findings are not consistent with results from animal and *in vitro* studies, which suggest that AMH suppresses the growth of ovarian tumors<sup>5-9, 14-16</sup>. While experimental studies have the advantage of being better able to control the exposure as well as potential confounding factors, their wider applicability to humans is not always clear, particularly when supra-physiologic concentrations of AMH are used<sup>5-9, 14-16</sup>. Additionally, even physiologic concentrations of AMH observed to regress the Müllerian ducts during the sexual differentiation<sup>39</sup>, are much higher than circulating concentrations in premenopausal women under normal circumstances<sup>6, 14</sup>.

To our knowledge, our study is the first to evaluate associations of prediagnostic AMH concentration measured in blood collected from non-pregnant women with risk of subsequent ovarian cancer. The prospective design minimized potential for reverse causality as well as potential for biased recall of confounding factors. All AMH assays were performed in a single laboratory blinded to case-control status and using a new ultrasensitive AMH assay that is valid and reproducible<sup>18, 19</sup>. The primary data obtained at multiple sites was uniformly harmonized, standardizing covariate categorization for the statistical analysis.

A limitation of our study is that AMH was measured in a single blood sample collected during late menopause and may not reflect long-term concentrations or the relevant etiologic period.. However, AMH concentrations track over time; the intra-class correlation of AMH over one year period in late premenopausal women was 0.87 in a previous study<sup>40</sup>, suggesting that misclassification of AMH concentration due to temporal variation in our study is likely to be small. Furthermore, circulating AMH concentrations could be less relevant for ovarian carcinogenesis, compared to intra-ovarian levels. Nonetheless, AMH is known to bind to AMH receptors expressed in the ovaries<sup>10-12</sup> to stimulate AMH signaling pathways.<sup>41, 42</sup> Despite the inclusion of nine cohorts from North America, Europe and China, we had limited power to examine associations of AMH with specific subtypes of ovarian cancer (Table 5) or to detect differences in stratified analysis. While we cannot rule out residual or unmeasured confounding, the similarity of the simple and multivariable models, suggest this is unlikely to be a major limitation.

In conclusion, in this analysis of nine cohorts of late premenopausal women, prediagnostic concentrations of AMH were not associated with risk of overall ovarian cancer or its specific subtypes. Large epidemiologic studies of younger premenopausal women, where AMH concentrations are higher, are warranted to confirm the etiologic relevance of AMH in relation to ovarian carcinogenesis.

### Acknowledgement

We would like to thank the participants and staff of the Nurses' Health Study and Nurses' Health Study II for their valuable contributions as well as the following state cancer registries for their



help: AL, AZ, AR, CA, CO, CT, DE, FL, GA, ID, IL, IN, IA, KY, LA, ME, MD, MA, MI, NE, NH, NJ, NY, NC, ND, OH, OK, OR, PA, RI, SC, TN, TX, VA, WA, WY.

Cancer incidence data for CLUE were provided by the Maryland Cancer Registry, Center for Cancer Surveillance and Control, Department of Health and Mental Hygiene, 201 W. Preston Street, Room 400, Baltimore, MD 21201, <http://phpa.dhmh.maryland.gov/cancer>, 410-767-4055.

We acknowledge the State of Maryland, the Maryland Cigarette Restitution Fund, and the National Program of Cancer Registries of the Centers for Disease Control and Prevention for the funds that support the collection and availability of the cancer registry data.

The authors assume full responsibility for analyses and interpretation of these data.

## References

1. Durlinger AL, Visser JA, Themmen AP. Regulation of ovarian function: the role of anti-Mullerian hormone. *Reproduction (Cambridge, England)* 2002;**124**: 601-9.
2. Visser JA, de Jong FH, Laven JS, Themmen AP. Anti-Mullerian hormone: a new marker for ovarian function. *Reproduction (Cambridge, England)* 2006;**131**: 1-9.
3. MacLaughlin DT, Donahoe PK. Mullerian inhibiting substance/anti-Mullerian hormone: a potential therapeutic agent for human ovarian and other cancers. *Future oncology (London, England)* 2010;**6**: 391-405.
4. Auersperg N, Wong AS, Choi KC, Kang SK, Leung PC. Ovarian surface epithelium: biology, endocrinology, and pathology. *Endocrine reviews* 2001;**22**: 255-88.
5. Chin TW, Parry RL, Donahoe PK. Human mullerian inhibiting substance inhibits tumor growth in vitro and in vivo. *Cancer research* 1991;**51**: 2101-6.
6. Stephen AE, Pearsall LA, Christian BP, Donahoe PK, Vacanti JP, MacLaughlin DT. Highly purified mullerian inhibiting substance inhibits human ovarian cancer in vivo. *Clinical cancer research : an official journal of the American Association for Cancer Research* 2002;**8**: 2640-6.
7. Chang HL, Pieretti-Vanmarcke R, Nicolaou F, Li X, Wei X, MacLaughlin DT, Donahoe PK. Mullerian inhibiting substance inhibits invasion and migration of epithelial cancer cell lines. *Gynecologic oncology* 2011;**120**: 128-34.
8. Donahoe PK, Fuller AF, Jr., Scully RE, Guy SR, Budzik GP. Mullerian inhibiting substance inhibits growth of a human ovarian cancer in nude mice. *Annals of surgery* 1981;**194**: 472-80.
9. Kim JH, Seibel MM, MacLaughlin DT, Donahoe PK, Ransil BJ, Hametz PA, Richards CJ. The inhibitory effects of mullerian-inhibiting substance on epidermal growth factor induced proliferation and progesterone production of human granulosa-luteal cells. *The Journal of clinical endocrinology and metabolism* 1992;**75**: 911-7.

10. Song JY, Chen KY, Kim SY, Kim MR, Ryu KS, Cha JH, Kang CS, MacLaughlin DT, Kim JH. The expression of Mullerian inhibiting substance/anti-Mullerian hormone type II receptor protein and mRNA in benign, borderline and malignant ovarian neoplasia. *International journal of oncology* 2009;**34**: 1583-91.
11. Bakkum-Gamez JN, Aletti G, Lewis KA, Keeney GL, Thomas BM, Navarro-Teulon I, Cliby WA. Mullerian inhibiting substance type II receptor (MISIIR): a novel, tissue-specific target expressed by gynecologic cancers. *Gynecologic oncology* 2008;**108**: 141-8.
12. Masiakos PT, MacLaughlin DT, Maheswaran S, Teixeira J, Fuller AF, Jr., Shah PC, Kehas DJ, Kenneally MK, Dombkowski DM, Ha TU, Preffer FI, Donahoe PK. Human ovarian cancer, cell lines, and primary ascites cells express the human Mullerian inhibiting substance (MIS) type II receptor, bind, and are responsive to MIS. *Clinical cancer research : an official journal of the American Association for Cancer Research* 1999;**5**: 3488-99.
13. Meirelles K, Benedict LA, Dombkowski D, Pepin D, Preffer FI, Teixeira J, Tanwar PS, Young RH, MacLaughlin DT, Donahoe PK, Wei X. Human ovarian cancer stem/progenitor cells are stimulated by doxorubicin but inhibited by Mullerian inhibiting substance. *Proceedings of the National Academy of Sciences of the United States of America* 2012;**109**: 2358-63.
14. Stephen AE, Masiakos PT, Segev DL, Vacanti JP, Donahoe PK, MacLaughlin DT. Tissue-engineered cells producing complex recombinant proteins inhibit ovarian cancer in vivo. *Proceedings of the National Academy of Sciences of the United States of America* 2001;**98**: 3214-9.
15. Ha TU, Segev DL, Barbie D, Masiakos PT, Tran TT, Dombkowski D, Glander M, Clarke TR, Lorenzo HK, Donahoe PK, Maheswaran S. Mullerian inhibiting substance inhibits ovarian cell growth through an Rb-independent mechanism. *The Journal of biological chemistry* 2000;**275**: 37101-9.
16. Pieretti-Vanmarcke R, Donahoe PK, Szotek P, Manganaro T, Lorenzen MK, Lorenzen J, Connolly DC, Halpern EF, MacLaughlin DT. Recombinant human Mullerian inhibiting substance inhibits long-term growth of MIS type II receptor-directed transgenic mouse ovarian cancers in vivo. *Clinical cancer research : an official journal of the American Association for Cancer Research* 2006;**12**: 1593-8.

17. Schock H, Lundin E, Vaarasmaki M, Grankvist K, Fry A, Dorgan JF, Pukkala E, Lehtinen M, Surcel HM, Lukanova A. Anti-Mullerian hormone and risk of invasive serous ovarian cancer. *Cancer causes & control : CCC* 2014;**25**: 583-9.
18. Burks HR, Ross L, Oppen N, Paulson E, Stanczyk FZ, Chung K. Can highly sensitive antimullerian hormone testing predict failed response to ovarian stimulation? *Fertility and sterility* 2015;**104**: 643-8.
19. Welsh P, Smith K, Nelson SM. A single-centre evaluation of two new anti-Mullerian hormone assays and comparison with the current clinical standard assay. *Human reproduction (Oxford, England)* 2014;**29**: 1035-41.
20. Dorgan JF, Stanczyk FZ, Eggleston BL, Kahle LL, Shaw CM, Spittle CS, Godwin AK, Brinton LA. Prospective case-control study of serum mullerian inhibiting substance and breast cancer risk. *Journal of the National Cancer Institute* 2009;**101**: 1501-9.
21. McSorley MA, Alberg AJ, Allen DS, Allen NE, Brinton LA, Dorgan JF, Kaaks R, Rinaldi S, Helzlsouer KJ. Prediagnostic circulating follicle stimulating hormone concentrations and ovarian cancer risk. *International journal of cancer Journal international du cancer* 2009;**125**: 674-9.
22. Ose J, Fortner RT, Rinaldi S, Schock H, Overvad K, Tjonneland A, Hansen L, Dossus L, Fournier A, Baglietto L, Romieu I, Kuhn E, et al. Endogenous androgens and risk of epithelial invasive ovarian cancer by tumor characteristics in the European Prospective Investigation into Cancer and Nutrition. *International journal of cancer Journal international du cancer* 2015;**136**: 399-410.
23. Fentiman IS, Hanby A, Allen DS, Key T, Meilahn EN. Hormone dependency of breast tumours developing in the Guernsey Cohort study. *Breast cancer research and treatment* 2006;**97**: 205-8.
24. Zeleniuch-Jacquotte A, Akhmedkhanov A, Kato I, Koenig KL, Shore RE, Kim MY, Levitz M, Mittal KR, Raju U, Banerjee S, Toniolo P. Postmenopausal endogenous oestrogens and risk of endometrial cancer: results of a prospective study. *British journal of cancer* 2001;**84**: 975-81.



25. Fortner RT, Eliassen AH, Spiegelman D, Willett WC, Barbieri RL, Hankinson SE. Premenopausal endogenous steroid hormones and breast cancer risk: results from the Nurses' Health Study II. *Breast cancer research : BCR* 2013;**15**: R19.
26. Lukanova A, Lundin E, Akhmedkhanov A, Micheli A, Rinaldi S, Zeleniuch-Jacquotte A, Lenner P, Muti P, Biessy C, Krogh V, Berrino F, Hallmans G, et al. Circulating levels of sex steroid hormones and risk of ovarian cancer. *International journal of cancer Journal international du cancer* 2003;**104**: 636-42.
27. Hallmans G, Agren A, Johansson G, Johansson A, Stegmayr B, Jansson JH, Lindahl B, Rolandsson O, Soderberg S, Nilsson M, Johansson I, Weinehall L. Cardiovascular disease and diabetes in the Northern Sweden Health and Disease Study Cohort - evaluation of risk factors and their interactions. *Scandinavian journal of public health Supplement* 2003;**61**: 18-24.
28. Ma X, Beeghly-Fadiel A, Shu XO, Li H, Yang G, Gao YT, Zheng W. Anthropometric measures and epithelial ovarian cancer risk among Chinese women: results from the Shanghai Women's Health Study. *British journal of cancer* 2013;**109**: 751-5.
29. Shih Ie M, Kurman RJ. Ovarian tumorigenesis: a proposed model based on morphological and molecular genetic analysis. *The American journal of pathology* 2004;**164**: 1511-8.
30. Koshiyama M, Matsumura N. Recent concepts of ovarian carcinogenesis: type I and type II 2014;**2014**: 934261.
31. La Marca A, Volpe A. The Anti-Mullerian hormone and ovarian cancer. *Human reproduction update* 2007;**13**: 265-73.
32. Julin B, Wolk A, Akesson A. Dietary cadmium exposure and risk of epithelial ovarian cancer in a prospective cohort of Swedish women. *British journal of cancer* 2011;**105**: 441-4.
33. Birmann BM, Neuhauser ML, Rosner B, Albanes D, Buring JE, Giles GG, Lan Q, Lee IM, Purdue MP, Rothman N, Severi G, Yuan JM, et al. Prediagnosis biomarkers of insulin-like growth factor-1, insulin, and interleukin-6 dysregulation and multiple myeloma risk in the Multiple Myeloma Cohort Consortium. *Blood* 2012;**120**: 4929-37.

34. Raghunathan TE, Lepkowski JM, Van Hoewyk J, Solenberger P. A multivariate technique for multiply imputing missing values using a sequence of regression models. *Survey methodology* 2001;**27**: 85-96.
35. Rubin DB. *Multiple imputation for nonresponse in surveys*. New York: Wiley, 1987.
36. DerSimonian R, Laird N. Meta-analysis in clinical trials. *Control Clin Trials* 1986;**7**: 177-88.
37. Zhang X, Spiegelman D, Baglietto L, Bernstein L, Boggs DA, van den Brandt PA, Buring JE, Gapstur SM, Giles GG, Giovannucci E, Goodman G, Hankinson SE, et al. Carotenoid intakes and risk of breast cancer defined by estrogen receptor and progesterone receptor status: a pooled analysis of 18 prospective cohort studies. *The American journal of clinical nutrition* 2012;**95**: 713-25.
38. Williams RL. A note on robust variance estimation for cluster-correlated data. *Biometrics* 2000;**56**: 645-6.
39. Kim JH, MacLaughlin DT, Donahoe PK. Mullerian inhibiting substance/anti-Mullerian hormone: A novel treatment for gynecologic tumors. *Obstetrics & gynecology science* 2014;**57**: 343-57.
40. Dorgan JF, Spittle CS, Eggleston BL, Shaw CM, Kahle LL, Brinton LA, Dorgan JF, Spittle CS, Eggleston BL, Shaw CM, Kahle LL, Brinton LA. Assay reproducibility and within-person variation of Mullerian inhibiting substance. *Fertil Steril* 2010;**94**: 301-4.
41. di Clemente N, Josso N, Gouedard L, Belville C. Components of the anti-Mullerian hormone signaling pathway in gonads. *Molecular and cellular endocrinology* 2003;**211**: 9-14.
42. Josso N, di Clemente N, Gouedard L. Anti-Mullerian hormone and its receptors. *Molecular and cellular endocrinology* 2001;**179**: 25-32.

Table 1. Population characteristics at blood collection

Characteristics	Controls (N=336 )		Cases (N= 302)	
	N	Mean (SD)	N	Mean (SD)
Age at blood draw, yrs	336	40.5 (4.0)	302	40.6 (4.1)
Body mass index <sup>a</sup> , kg/m <sup>2</sup>	310	24.5 (4.5)	277	25.0 (4.9)
Age at menarche, yrs	279	13.1 (1.8)	259	12.8 (1.6)
	N	Percentage	N	Percentage
Current use of oral contraceptives				
No	277	82%	265	88%
Yes	12	4%	7	2%
Missing	47	14%	30	10%
Ever use of oral contraceptives				
Never	91	27%	93	31%
Ever	190	57%	165	55%
Missing	55	16%	44	14%
Total number of pregnancy				
0	40	12%	44	15%
1	39	12%	42	14%
2	103	31%	94	31%
3	52	15%	43	14%
≥4	40	12%	30	10%
Missing	62	18%	49	16%
Smoking status				
Never	184	55%	181	60%
Past	59	17%	55	18%
Current	66	20%	53	18%
Missing	27	8%	13	4%
Education				
High school or less	160	48%	134	44%
Vocational school	27	8%	38	13%
Attended college	110	33%	96	32%
Missing	39	11%	34	11%
Race				
White	209	62%	178	59%
Other <sup>b</sup>	35	11%	34	11%
Missing	92	27%	90	30%
	N	Median (IQR)	N	Median (IQR)
AMH, pg/ml	336	1025 (330-2235)	302	1035 (300-2230)

Abbreviation: AMH, anti-Müllerian hormone

<sup>a</sup> Information on body mass index was missing for 26 controls and 25 cases (a total of 8% of all women).

<sup>b</sup> Among other races, 34 controls and 33 cases were Asian and 1 control and 1 case were Black or African American.

Accepted Article



Table 2. Characteristics of ovarian cancer at diagnosis (*N*=302)

Cancer characteristics	<i>N</i>	Percentage
Histology		
Carcinoma, NOS	49	16%
Clear cell	16	5%
Endometriod	37	12%
Serous, NOS	88	29%
Serous, low grade/LMP	23	8%
Serous, high grade	20	7%
Mucinous carcinoma/Mucinous, LMP	31	10%
Other <sup>a</sup>	7	3%
Missing	31	10%
Grade		
1	30	10%
2	40	13%
≥3	84	28%
Missing	148	49%
Stage (FIGO)		
1	74	25%
2	39	13%
3	67	22%
4	30	10%
Missing	92	30%
	<i>N</i>	Median (IQR)
Age at diagnosis, yrs	302	50.5 (46.5-55.0)
Time between blood draw and diagnosis	302	9.2 (5.2-13.9)

<sup>a</sup> Other histology includes mixed cell adenocarcinoma (*N*=1), sarcoma (*N*=4) and Brenner tumor (*N*=2).

Table 3. Odds ratio and 95% confidence interval for the association between AMH concentration (pg/mL) and risk of ovarian cancer

	Quartiles of AMH <sup>a</sup>				P-trend <sup>b</sup>
	Q1	Q2	Q3	Q4	
N cases/controls	76/88	75/82	78/84	73/82	
Simple model <sup>c</sup>	1.00 (ref)	1.01 (0.65-1.59)	1.07 (0.67-1.72)	1.01 (0.61-1.69)	0.90
MV model <sup>d</sup>	1.00 (ref)	1.03 (0.65-1.62)	1.14 (0.70-1.85)	0.99 (0.59-1.67) <sup>e</sup>	0.91

Abbreviations: AMH; anti-Müllerian hormone; MV, multivariable

<sup>a</sup> Study-specific quartiles of AMH concentration, defined among controls, were used.

<sup>b</sup> Test for trend was conducted using the Wald statistic of the ordinal value of study-specific quartiles of AMH concentration, modeled as a continuous term.

<sup>c</sup> Conditional logistic regression model was conditioned on case/control matching factors and adjusted for age at blood draw (continuous, yrs).

<sup>d</sup> Conditional logistic regression model was conditioned on case/control matching factors and adjusted for age at blood draw (continuous, yrs), oral contraceptive use (never, ever) and age at menarche (11-<12yrs, 12-<13yrs, 13-<14yrs, ≥ 14yrs).

<sup>e</sup> Heterogeneity across cohorts was not significant ( $P_{\text{heterogeneity across studies}} = 0.48$ )

Table 4. Odds ratio and 95% confidence intervals for the associations between AMH concentration (pg/mL) and risk of ovarian cancer according to participants' characteristics

	Quartiles of AMH <sup>a</sup>				P-trend <sup>b</sup>	P-interaction <sup>c</sup>
	Q1	Q2	Q3	Q4		
By age at blood draw <sup>d</sup>						
<40 yrs						
N cases/controls	14/19	15/26	34/30	37/42		
MV model <sup>d</sup>	1.00 (ref)	0.77 (0.41-1.44)	1.56 (0.62-3.91)	1.17 (0.52-2.62)	0.39	0.26
≥40 yrs						
N cases/controls	62/69	60/56	44/54	36/40		
MV model <sup>d</sup>	1.00 (ref)	1.21 (0.79-1.84)	1.01 (0.60-1.70)	1.06 (0.70-1.60)	0.96	
By oral contraceptive use						
Never						
N cases/controls	23/22	26/25	21/25	23/19		
MV model <sup>d</sup>	1.00 (ref)	1.00 (0.46-2.21)	0.83 (0.33-2.08)	1.20 (0.46-3.15)	0.82	0.38
Ever						
N cases/controls	40/48	40/46	45/46	40/50		
MV model <sup>d</sup>	1.00 (ref)	1.01 (0.51-1.98)	1.31 (0.80-2.16)	1.01 (0.55-1.88)	0.74	

Abbreviations: AMH; anti-Müllerian hormone; MV, multivariable

<sup>a</sup> Study-specific quartiles of AMH concentration, defined among controls, were used.

<sup>b</sup> Test for trend was conducted using the Wald statistic of the ordinal value of study-specific quartiles of AMH concentration, modeled as a continuous term.

<sup>c</sup> Test for interaction was conducted using the Wald statistic of the cross-product term between each of the stratification factors and AMH concentration

<sup>d</sup> Unconditional logistic regression model adjusted for age at blood draw (continuous, yrs), year of blood draw (quintiles, yrs), oral contraceptive use (never, ever) and age at menarche (11-<12yrs, 12-<13yrs, 13-<14yrs, ≥ 14yrs).

Table 5. Odds ratio and 95% confidence intervals for the associations between AMH concentration (pg/mL) and risk of ovarian cancer by the tumor characteristics and by age at and time to diagnosis

		Quartiles of AMH <sup>a</sup>				P-trend <sup>b</sup>	P-heterogeneity <sup>c</sup>
		Q1	Q2	Q3	Q4		
By tumor development pathway <sup>d</sup>							
Type I							
N cases/controls		31/41	33/40	41/44	32/37		
MV model <sup>e</sup>		1.00 (ref)	1.00 (0.48-2.07)	1.18 (0.59-2.38)	0.98 (0.44-2.15)	0.89	0.72
Type II							
N cases/controls		22/19	20/21	17/24	22/23		
MV model <sup>e</sup>		1.00 (ref)	0.80 (0.28-2.31)	0.66 (0.21-2.01)	0.86 (0.27-2.68)	0.74	
By tumor stage <sup>f</sup>							
Early stage							
N cases/controls		15/17	16/15	23/31	20/21		
MV model <sup>e</sup>		1.00 (ref)	1.23 (0.34-4.44)	0.87 (0.26-2.90)	0.97 (0.30-3.48)	0.74	0.61
Late stage							
N cases/controls		39/43	32/37	37/35	28/32		
MV model <sup>e</sup>		1.00 (ref)	1.04 (0.52-2.08)	1.35 (0.66-2.74)	1.07 (0.48-2.39)	0.68	
By tumor grade <sup>g</sup>							
Low grade							
N cases/controls		5/8	9/6	8/12	8/8		
MV model <sup>e</sup>		1.00 (ref)	5.24 (0.57-48.52)	0.95 (0.15-5.90)	1.63 (0.24-11.03)	0.75	0.87
High grade							
N cases/controls		36/33	25/33	34/35	29/32		
MV model <sup>e</sup>		1.00 (ref)	0.62 (0.28-1.35)	0.87 (0.38-1.97)	0.76 (0.32-1.81)	0.79	

By age at diagnosis

<50 yrs

N cases/controls	21/38	37/37	45/44	43/47		
MV model <sup>e</sup>	1.00 (ref)	1.57 (0.71-3.48)	1.67 (0.77-3.60)	1.37 (0.58-3.25)	0.54	0.39

≥50 yrs

N cases/controls	55/50	38/45	33/40	30/35		
MV model <sup>e</sup>	1.00 (ref)	0.94 (0.75-1.17)	1.03 (0.64-1.66)	0.72 (0.41-1.28)	0.56	

By time between blood draw and age at diagnosis

≤5 yrs

N cases/controls	12/23	24/19	19/22	14/16		
MV model <sup>e</sup>	1.00 (ref)	3.27 (1.00-10.69)	2.09 (0.69-6.32)	2.75 (0.70-10.78)	0.43	0.73

>5 yrs- <10 yrs

N cases/controls	22/23	25/31	27/23	21/25		
MV model <sup>e</sup>	1.00 (ref)	0.86 (0.36-2.03)	1.26 (0.50-3.14)	0.69 (0.25-1.93)	0.79	

≥10 yrs

N cases/controls	42/42	26/32	32/39	38/41		
MV model <sup>e</sup>	1.00 (ref)	0.90 (0.43-1.86)	1.07 (0.49-2.33)	1.00 (0.48-2.11)	0.93	

Abbreviations: AMH; anti-Müllerian hormone; MV, multivariable

<sup>a</sup> Study-specific quartiles of AMH concentration, defined among controls, were used.

<sup>b</sup> Test for trend was conducted using the Wald statistic of the ordinal value of study-specific quartiles of AMH concentration, modeled as a continuous term.

<sup>c</sup> Test for heterogeneity by type of cancer was conducted using the contrast test, comparing the risk estimates of the quartile ordinal trend term observed with each specific cancer type

<sup>d</sup> Cancer is defined as type II if cases were documented as high grade serous or serous, nos with grade≥2 (for Shanghai Women’s Health Study, because grade information was not available, high grade serous tumor was defined as cases documented as high grade serous or serous, nos and died of ovarian cancer), otherwise all other tumors, except those documented as carcinoma, nos and sarcoma, were defined as type I.

<sup>e</sup> Conditional logistic regression model was conditioned on case/control matching factors and adjusted for age at blood draw (continuous, yrs), year of blood draw (quintiles, yrs), oral contraceptive use (never, ever) and age at menarche (11-<12yrs, 12-<13yrs, 13-<14yrs, ≥ 14yrs).

<sup>f</sup> Cancer is defined as early stage if stage at diagnosis is equal to 1, otherwise defined as late stage cancer.

<sup>g</sup> Cancer is defined as low grade if grade at diagnosis is equal to 1, otherwise defined as high grade cancer.