HHS Public Access

Author manuscript

Cancer Epidemiol Biomarkers Prev. Author manuscript; available in PMC 2020 September 01.

Published in final edited form as:

Cancer Epidemiol Biomarkers Prev. 2020 March ; 29(3): 636–642. doi:10.1158/1055-9965.EPI-19-0675.

Association of Anti-Mullerian Hormone, Follicle-Stimulating Hormone and Inhibin B with Risk of Ovarian Cancer in the Janus Serum Bank

Sarah R. Irvin[1], **Elisabete Weiderpass**[2], **Frank Z. Stanczyk**[4], **Louise A. Brinton**[1], **Britton Trabert**[1], **Hilde Langseth**[3], **Nicolas Wentzensen**[1]

[1]Division of Cancer Epidemiology and Genetics, National Cancer Institute, Rockville, Maryland
[2]International Agency for Research on Cancer, World Health Organization, Lyon, France
[3]Department of Research, Cancer Registry of Norway, Oslo, Norway [4]University of Southern California Keck School of Medicine, Los Angeles, California, United States of America

Abstract

Background—Reproductive factors, including parity, breastfeeding and contraceptive use affect lifetime ovulatory cycles and cumulative exposure to gonadotropins and are associated with ovarian cancer. To understand the role of ovulation-regulating hormones in the etiology of ovarian cancer, we prospectively analyzed the association of Anti-Mullerian Hormone, Follicle Stimulating Hormone and Inhibin B with ovarian cancer risk.

Methods—Our study included 370 women from the Janus Serum Bank, including 54 Type 1 and 82 Type 2 invasive epithelial ovarian cancers and 49 borderline tumors and 185 age-matched controls. We used conditional logistic regression to assess the relationship between hormones and risk of ovarian cancer overall and by subtype (Type 1 and 2).

Results—Inhibin B was associated with increased risk of ovarian cancer overall (OR 1.97 95% CI 1.14–3.39, p_{trend} 0.05) and with type 1 ovarian (OR 3.10, 95% CI 1.04–9.23, p_{trend} 0.06). FSH was not associated with ovarian cancer risk overall but higher FSH was associated with type 2 ovarian cancers (OR 2.78, 95% CI 1.05–7.38). AMH was not associated with ovarian cancer risk.

Conclusions—FSH and Inhibin B may be associated with increased risk in different ovarian cancer subtypes, suggesting that gonadotropin exposure may influence risk of ovarian cancer differently across subtypes.

Impact—Associations between prospectively collected AMH, FSH and inhibin B levels with risk of ovarian cancer provide novel insight on the influence of pre-menopausal markers of ovarian

Corresponding author: Sarah R. Irvin, 9609 Medical Center Drive, Rockville, MD 20850, Phone: 240-276-5773, Fax: 240-276-7838, sarah.irvin@nih.gov.

Conflicts of interest: The authors declare no potential conflicts of interest

Disclaimer: The Norwegian Public Health Institute and Medical Birth Registry of Norway are not responsible for carrying out the analyses and obtaining the results, or the interpretation of the results of this work. Where authors are identified as personnel of the International Agency for Research on Cancer / World Health Organization, the authors alone are responsible for the views expressed in this article and they do not necessarily represent the decisions, policy or views of the International Agency for Research on Cancer / World Health Organization.

reserve and gonadotropin signaling. Heterogeneity of inhibin B and FSH effects in different tumor types may be informative of tumor etiology.

Keywords

ovarian cancer risk; anti-mullerian hormone; follicle-stimulating hormone; inhibin B; case-control study

INTRODUCTION

Ovarian cancer is the deadliest gynecologic malignancy, causing over 150,000 deaths worldwide every year (1,2). It is typically asymptomatic at early stages and difficult to detect, with > 70% of cases identified in post-menopausal women as late stage disease (3). Large screening trials based on CA-125 and transvaginal ultrasound have not shown a meaningful improvement of mortality (4,5). An understanding of molecular factors that may differ between ovarian cancer cases and healthy women could help to elucidate important changes in early carcinogenic processes.

The etiology of ovarian cancers is unclear and heterogenous across subtypes. A prevailing theory of ovarian carcinogenesis relates to incessant ovulation which causes repeated disruption of the ovarian epithelium (6–8). This hypothesis is supported by consistent evidence that a higher quantity of lifetime ovulatory cycles (LOCs) increases risk of epithelial ovarian cancer (9–12). A second theory, the gonadotropin hypothesis, posits that carcinogenesis occurs due to high exposure of the ovarian epithelium to gonadotropins produced in the pituitary gland (13–15). Multiple reproductive factors that affect LOCs and ovarian exposure to gonadotropins, like early menarche and late menopause, are associated with risk that varies by ovarian cancer subtype. A five year increase in menopause age is associated with serous, endometrioid and clear cell tumors, while later age at menarche is associated with a decreased risk of clear cell tumors (16). Higher parity, breastfeeding and oral contraceptive use are associated with decreased risk that also varies by subtype (16,17).

Anti-Mullerian Hormone (AMH), follicle stimulating hormone (FSH) and Inhibin B are protein hormones that regulate ovulatory cycles (18–20). AMH is an indicator of ovarian reserve and is produced by ovarian granulosa cells. AMH regulates the number of primordial follicles selected to transition to primary follicles and can predict the quantity of follicles remaining in the ovaries (21,22). Follicle-stimulating hormone (FSH) and Inhibin B (also produced by granulosa cells) control growth of follicles and are regulated by the hypothalamus-pituitary-gonadal (HPA) axis (19,23). There is evidence for an association between elevated levels of AMH and inhibin B with ovarian granulosa cell tumors, a rare malignancy composing <5% of ovarian cancers (24–27), but the association between AMH, inhibin B and FSH and epithelial ovarian cancers is not well understood.

Two previous analyses, one in a population of pregnant women (28) and one across nine cohorts (29), explored the association between pre-menopausal AMH levels and ovarian cancer. Both found no association between AMH and risk of ovarian cancer. Neither study evaluated FSH or Inhibin B. Total serum inhibins (A and B dimers) are elevated in post-menopausal women with ovarian granulosa cell tumors and most mucinous epithelial

tumors, but it is not clear whether pre-menopausal inhibins are associated with ovarian cancer (24,27,30). Inconsistent results have been reported for the association between FSH and ovarian cancer, but some studies demonstrated a reduced risk of ovarian cancer for higher FSH levels (31). We analyzed associations between the three analytes and ovarian cancer in a population of Norwegian women.

METHODS

Study population

Study subjects were selected from the Janus Serum Bank of Norway. The cohort recruitment, specimen collection and participant characteristics have been described (32). In brief, blood specimens (n=318,628 individuals) came from two main sources: participants in the Norwegian Regional Health Studies (~90%) and Red Cross blood donors (~10%) from Oslo and surrounding areas (33). The collection period was from 1972–2004 with age of blood draw ranging from 18 to 65 years (mean = 41). The serum samples were stored at −25°C (34). Janus participants gave broad written informed consent for their blood specimens to be used for medical research. The serum bank was linked to the Cancer Registry of Norway (CRN) by using a unique personal identification number system implemented in Norway in 1964 to obtain information on cancer diagnoses. Covariate information on lifestyle and anthropometric factors was collected at the time of blood collection and parity was linked from the Norwegian Institute of Public Health (32,33).

From a total of 1,300 ovarian cancer cases in the Janus Serum Bank, we included 199 ovarian cancer cases and 199 matched control subjects that met our inclusion criteria. Cases were required to have had a pre-menopausal blood draw between the ages of 35–45 after the year 1985. Cases were matched to controls by age at time of blood collection (± 1 year) and birthyear (± 1 year) to account for the strong association of AMH levels with increasing age. Control subjects were required to be free of cancer prior to the date of their matched case's diagnosis date. A set of 40 quality control (QC) samples from women between the ages of 35–45 drawn after 1985 were chosen to evaluate assay reproducibility and were blinded to the laboratory analyst. AMH is undetectable in post-menopausal women, therefore, women between 35–45 years old without a history of cancer (other than nonmelanoma skin cancer) were included. Women with rare non-epithelial subtypes and unknown histologies (n=11) and missing analyte values (n=3), left 185 sets (n=370 women) with complete data on all three analytes for the main analysis (n=136 invasive epithelial, and n=49 borderline). This study was approved by the regional committees for medical and health research ethics, Oslo, Norway (2013/583).

Hormone measurements

Serum values of AMH, Inhibin B and FSH were measured at the University of Southern California Keck School of Medicine, Los Angeles, CA by a sensitive and specific assay (Beckman-Coulter Diagnostics Systems Laboratories). AMH was quantified by an enzymatically amplified two-site enzyme linked assay (ELISA, Beckman Coulter, Inc. Brea, CA) (35,36). The limit of detection (LOD) was 0.07 ng/mL. Inhibin B was measured by a similar ELISA. The limit of detection was 5 pg/mL. FSH was measured by a solid-phase,

enzyme-labeled chemiluminescent immunormetric assay on the Immulite 1000 analyzer (Siemens Healthcare Corporation, Deerfield IL). This assay LOD was 0.1 mIU/mL.

Tumor classification

Epithelial ovarian cancers were confirmed in the CRN. The ICD-7 code 175 and ICD-10 codes C56.1–2, 9 and C57.0–4 identified the cases. We evaluated the associations between each analyte and all epithelial tumors combined (n=136). We also divided the epithelial cancers into the four histologic subtypes (serous, endometrioid, mucinous and clear cell) and evaluated serous (n= 100) vs non-serous (n= 36) separately. The main analysis used the dichotomous type 1 vs. 2 classification that includes morphology and grade as previously described (37). Briefly, the type 1 stratum (n= 54) included low grade serous, endometrioid, and mucinous tumors. Type 2 tumors (n= 82) were 50 high grade serous tumors and 32 tumors with other morphologies usually classified as type 2 (38). Tumors without grade information were categorized based on known histology characteristics and previously published literature (38). Undifferentiated and mixed cell carcinomas were included with the type 2 cases, as were ungraded serous carcinomas.

Statistical Analyses

Analyte values for AMH were converted to pg/mL and all three analytes were log transformed to normalize their distributions. Log AMH and FSH were divided into quartiles based on the distribution in controls. Because >50% of controls for inhibin B fell below the LOD, we created three groups for inhibin B in each analysis, with the reference group comprising all values below LOD (undetectable), and a median split of values above the LOD (39). Conditional logistic regression models were used to generate odds ratios (ORs) and 95% confidence intervals for individual analyte associations with ovarian cancer risk. The lowest quartile of AMH and FSH was the reference for those analytes. For inhibin B, the reference group included subjects that fell below the assay limit of detection (n=75). In addition to the matching factors (age at blood draw and birthyear), body mass index (BMI), parity, smoking, weight, and height were evaluated as potential confounders. Parity was included in the final models because it is the strongest reproductive risk factor, and more cases were missing parity than controls. A missing indicator was used in the models that included parity. In the analysis of borderline tumors, models were additionally adjusted for smoking status because adjustment for smoking status changed the effect estimate by >10%. Tests for trend were conducted using the Wald p value for trend across quartiles for AMH and FSH, and across the three inhibin B categories. Tests for heterogeneity were conducted by comparing nested models via likelihood ratio tests that allowed for effect modification by subtype.

The association of each hormone with ovarian cancer risk was explored in the overall sample and by subtype (type 1 vs type 2) (37). Sensitivity analyses included histologic subtype (serous vs. non-serous) and time between blood draw and diagnosis (<10 or ≥ 10 years). Additionally, we explored associations among those with reported grade information and by removal of rare histologies.

Assay reproducibility was analyzed using the serum collected from the QC samples (n=40). Coefficients of variation (CVs) and Intraclass correlation coefficients (ICCs) were generated by utilizing a nested components of variance model to assess consistency of the analyte measurements within each batch and between batches. ICCs were >95% for all three analytes. CVs were <7% for FSH and AMH, and 16% for inhibin B. No differences between batch measurements were observed.

RESULTS

Characteristics of the subjects, including mean concentrations of AMH, FSH and Inhibin B, are displayed in Table 1. Nulliparity was more common in controls. All other lifestyle and reproductive factors were balanced between the cases and controls. The mean age at blood collection was 41.5 ± 1.3 for cases and 41.4 ± 1.2 for controls. Most (94%) cases were 40–43 years old at time of blood collection. Mean age at diagnosis for the invasive epithelial cases (n=136) was 54.6 ± 6.4. All invasive cases had histology information and 75% (n=102) had grade information (Supplementary Table 1). FSH and AMH were inversely correlated in both parametric and nonparametric analyses ($\rho=-0.15$ and $\rho=-0.37$, respectively), while inhibin B an AMH were positively correlated ($\rho=0.35$ and $\rho=0.23$). FSH and inhibin B correlations were inconsistent between parametric and nonparametric analyses ($\rho=-0.12$ and $\rho=0.34$). Correlations of analytes are displayed in Supplementary Table 2.

Associations of AMH, FSH and Inhibin B in the overall sample

The associations of AMH, FSH and inhibin B with ovarian cancer risk were evaluated in the overall sample of 136 cases and matched controls. Inhibin B was associated with ovarian cancer in women with detectable levels (above the LOD) compared to those with undetectable values (below the LOD) (detectable vs. undetectable: OR 1.97, 95% CI: 1.14–3.39). AMH and FSH were not associated with ovarian cancer risk overall (FSH: Q4 vs. Q1 OR 1.53, 95% CI: 0.69–3.38, p_{trend} 0.26; AMH: Q4 vs. Q1 OR 1.37, 95% CI: 0.67–2.81) (Table 2).

Associations of FSH and Inhibin B with Type 1 vs. Type 2 cancers

The associations between FSH and inhibin B and ovarian cancer risk differed across subtypes. Inhibin B was associated with increased risk of type 1 ovarian cancer in women with detectable values compared to those with undetectable values (detectable vs. undetectable: OR 3.10, 95% CI: 1.04–9.23). Odds ratios for the association between Inhibin B and ovarian cancer risk were elevated in both groups of women above and below the median split of detectable values (> median split vs. undetectable: OR 2.92, 95% CI: 0.80–10.74 and < median split vs. undetectable OR 3.25, 95% CI: 0.93–11.34, p_{trend} 0.06), but did not reach statistical significance. Higher Inhibin B was not associated with risk of type 2 ovarian cancers (p-het=0.41). Odds ratios for the association between FSH and ovarian cancer risk were elevated in the type 2 cancers in all quartiles compared to the reference group (Q1: OR 2.20, 95% CI: 0.78–6.18; Q2: OR 2.32, 95% CI: 0.83–6.48; Q3: OR 2.43, 95% CI: 0.86–6.88). This contrasted with the association of FSH with type 1 cancers, where odds ratios for each quartile with the reference group were below 1. The association between

FSH and risk of ovarian cancer showed borderline heterogeneity across type (p-het=0.06) (Table 3).

The associations between increased levels of inhibin B and type 1 ovarian cancers and increased levels of FSH and type 2 cancers were also observed in two additional analyses removing 32 tumors with rare histologies and 22 tumors of unknown grade (Table 4). Inhibin B was associated with increased risk of type 1 tumors for women with detectable values (detectable vs. undetectable OR 3.06, 95% CI: 1.03–9.07). FSH above the median was associated with increased risk of type 2 tumors (> median vs. < median: OR 2.78, 95% CI: 1.05–7.38) (Table 4). Higher FSH remained associated with type 2 ovarian cancer after exclusion of tumors with unknown grade (> median vs. < median: OR 2.74, 95% CI: 1.04–7.21). No tumors of unknown grade were removed from the type 1 stratum, as all nonserous tumors are considered type 1, regardless of grade, and the association between inhibin B and type 1 ovarian cancer remained (detectable vs. undetectable: OR 3.10, 95% CI: 1.04–9.23) (Table 4). Designations of each case to the subtype strata are presented in Supplementary Table 3. Joint effects were not observed between FSH and inhibin B, indicating that no interaction is present between these analytes in our data (Supplementary Table 4).

Sensitivity Analyses

In sensitivity analyses, odds ratios for the association between inhibin B and ovarian cancer risk were elevated in nonserous ovarian cancer compared to serous tumors for those with detectable values compared to undetectable values (nonserous detectable vs. undetectable: OR 4.06, 95% CI: 0.97–16.94) vs (serous detectable vs. undetectable: OR 1.66, 95% CI: 0.89–3.09) (Supplementary Table 5). Among tumors diagnosed within 10 years of blood collection, inhibin B above the median split of detectable values was associated with ovarian cancer risk (> median split vs. undetectable OR 4.02, 95% CI 1.03–15.66). Both AMH and FSH were not heterogenous across serous and nonserous strata or strata of women diagnosed within 10 years vs. those diagnosed more than 10 years after blood draw (Supplementary Table 6).

Borderline ovarian cancer

The associations between AMH, FSH and inhibin B were evaluated in the subset of 49 borderline tumors, but no analyte was associated with an increased risk of borderline ovarian cancer in either the quartile analysis or for those above vs. below the median split of detectable values for inhibin B (Supplementary Table 7).

DISCUSSION

In a population of 370 pre-menopausal Norwegian women, we analyzed the association between three hormones that regulate follicle formation and ovulation with risk of epithelial ovarian cancer. Increased levels of Inhibin B, but not AMH or FSH were associated with an increased risk of ovarian cancer overall. Although subgroups had limited sample size, we observed that higher FSH was associated with increased risk of type 2 ovarian tumors, and higher inhibin B was associated with increased risk of Type 1 tumors and was associated

with ovarian cancer risk those cases with blood collection less than 10 years prior to diagnosis.

This work represents the only study, to date, that evaluated ovarian cancer risk in relation to all three analytes (AMH, FSH and Inhibin B) in a population of pre-menopausal women. Results obtained in this study for AMH are consistent with previous publications. We found no evidence of increased risk of ovarian cancer women with increasing quartile of pre-menopausal AMH. Previous analyses of FSH (31) showed a protective effect of FSH with increasing quartile, but women were both pre- and post-menopausal, and FSH levels are known to differ between pre-and post-menopausal women (40). We found that higher FSH may be associated with increased risk of Type 2 ovarian cancer but inversely associated with Type 1 cancers. Results from a study evaluating the association of inhibin with epithelial ovarian cancers grouped both inhibin dimers (A and B), rather than evaluating inhibin B alone (30). This study compared subtype specific inhibin levels to levels to postmenopausal controls, a time of life when inhibin B is drastically reduced, and thus it was not comparable to our analysis. Studies of inhibin also included both pre- and post-menopausal women or focused mainly on serous and high-grade tumors (41). We evaluated inhibin B as it is the dimer primarily active during folliculogenesis in pre-menopausal women and is a marker of ovarian reserve (42). Our study is unique as it was able to capture subtype-specific differences from women who were pre-menopausal at blood draw.

FSH, a gonadotropin, and Inhibin B, a negative feedback glycoprotein regulator of FSH have different origins in the female body. FSH is produced in the pituitary gland and acts distantly on FSH receptors in the granulosa cells of the ovary. Inhibin B is produced in the granulosa cells and acts within the pituitary gland, blocking activins and FSH secretion, making Inhibin B an antagonist of FSH secretion (41). The mechanism of action of gonadotropins responsible for potential ovarian carcinogenesis has been suggested by some mechanistic studies and posits that inclusion cysts that form after ovulation are susceptible to exposure to gonadotropins that causes an uptick in cellular replication (31,43). We demonstrated that higher values of FSH and Inhibin B may be associated with ovarian cancer risk in distinct subgroups and that these associations were not accompanied by associations between low values of the complementary analyte and ovarian cancer.

Recent etiologic work has suggested that epithelial ovarian cancer is a heterogenous disease, with risk factor associations and molecular profiles that vary by histology (16,44). It is important to incorporate this understanding of etiologic heterogeneity into ongoing studies of ovarian cancer. We saw no association of FSH or AMH with ovarian cancer overall but found inhibin B associated with increased risk in overall ovarian cancer. We also saw associations for FSH and Inhibin B in different tumor subtypes (FSH in type 2 cancers composed primarily of serous tumors, and Inhibin B in non-serous cancers). Our results demonstrate the complexity of exposure associations within ovarian cancer subtypes (serous, nonserous). We did not see inverse associations for FSH in type 1 tumors, or Inhibin B in the type 2 tumors. This is surprising, given the antagonistic property of Inhibin B on FSH secretion, and further indicates the importance of studying ovarian cancer subtypes in etiologic studies. Our findings support the gonadotropin hypothesis of ovarian cancer

carcinogenesis (13,14), because FSH and Inhibin B both influence the cumulative lifetime exposure of the ovarian epithelium to gonadotropins.

Our study has several strengths. We conducted our analysis in a homogenous population of pre-menopausal women with prospectively collected and banked serum samples that have been shown to remain stable over time. Most women in this analysis had sufficient information that allowed for robust subtype analyses. High quality serum analysis, exampled by the low CV's, and high ICC produced robust data for our analysis. Samples from the Janus Serum Bank have been shown to be stable up to 30 years after collection (34).

We note that our study has some limitations. Our study was adequately powered to detect associations in the overall sample, and although we observed subtype-specific differences, most of our heterogeneity statistics were not significant, indicating that larger studies are needed to confirm our results. We did not have data on the day of each woman's menstrual cycle when the serum sample was collected. However, we expect that this variation would be random with respect to cycle phase, and our outcome measures would be biased only towards the null. Additionally, we did not have data on use of oral contraceptives, which have strong associations with ovarian cancer. The mechanism of action of many oral contraceptives is to block follicle development, and thus suppresses the secretion of FSH from the pituitary gland (45), thus making OCs strongly related to FSH. Although we were not able to adjust for OC use in our study, the birth year range of women in our study was (1941–1951), and The Norway Fertility and Family Survey reports that <5% of women in this birth cohort would have taken oral contraception when surveyed at approximately 40 years old. Therefore, as our study samples were collected and banked in the late 1980's and early 1990's when the women were between the ages of 40–43, we expect the effect of OC use on FSH and/or Inhibin B to have been minimal and balanced between cases and controls.

To summarize, our analysis represents the only study, to our knowledge, that analyzed prospectively collected serum data for three analytes (AMH, FSH and Inhibin B) with respect to ovarian cancer. We showed that higher inhibin B and FSH may be associated with distinct cancer subtypes, but future work should confirm these associations in larger populations. Our work informs future studies of ovarian carcinogenesis as it pertains to gonadotropin action in the ovary.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENTS

We would like to thank the Janus Serum Bank of Norway for the privilege to analyze their samples and to all persons who donated blood to this biobank. We also acknowledge the Norwegian Cancer Society for use of valuable demographic information for the Janus samples. We would like to thank the Norwegian Public Health Institute for providing the data on Health Examinations in the Norwegian Counties 1974–1985 and 40-years surveys 1985–1999, and the Medial Birth Registry of Norway for providing parity data for our sample.

FINANCIAL SUPPORT

This work was funded by the Intramural Research Program of the National Cancer Institute.

Abbreviations

AMH	Anti-Mullerian Hormone
FSH	Follicle Stimulating Hormone
ICC	Intraclass Correlation Coefficient
CV	Coefficient of Variation
OR	odds ratio
LOC	lifetime ovulatory cycles
QC	Quality control
BMI	body mass index
ICD	International Classification of Disease
LOC	limit of detection
CI	confidence interval

REFERENCES

1. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 2015;136(5):E359–86 doi 10.1002/ijc.29210. [PubMed: 25220842]
2. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin 2015;65(2):87–108 doi 10.3322/caac.21262. [PubMed: 25651787]
3. Burges A, Schmalfeldt B. Ovarian cancer: diagnosis and treatment. Dtsch Arztebl Int 2011;108(38):635–41 doi 10.3238/arztebl.2011.0635. [PubMed: 22025930]
4. Buys SS, Partridge E, Black A, Johnson CC, Lamerato L, Isaacs C, et al. Effect of screening on ovarian cancer mortality: the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Randomized Controlled Trial. JAMA 2011;305(22):2295–303 doi 10.1001/jama.2011.766. [PubMed: 21642681]
5. Jacobs IJ, Menon U, Ryan A, Gentry-Maharaj A, Burnell M, Kalsi JK, et al. Ovarian cancer screening and mortality in the UK Collaborative Trial of Ovarian Cancer Screening (UKCTOCS): a randomised controlled trial. Lancet 2016;387(10022):945–56 doi 10.1016/S0140-6736(15)01224-6. [PubMed: 26707054]
6. Ness RB, Grisso JA, Cottreau C, Klapper J, Vergona R, Wheeler JE, et al. Factors related to inflammation of the ovarian epithelium and risk of ovarian cancer. Epidemiology 2000;11(2):111–7. [PubMed: 11021606]
7. Fleming JS, Beaugie CR, Haviv I, Chenevix-Trench G, Tan OL. Incessant ovulation, inflammation and epithelial ovarian carcinogenesis: revisiting old hypotheses. Mol Cell Endocrinol 2006;247(1–2):4–21 doi 10.1016/j.mce.2005.09.014. [PubMed: 16297528]
8. Fathalla MF. Incessant ovulation--a factor in ovarian neoplasia? Lancet 1971;2(7716):163. [PubMed: 4104488]
9. Yang HP, Murphy KR, Pfeiffer RM, George N, Garcia-Closas M, Lissowska J, et al. Lifetime Number of Ovulatory Cycles and Risks of Ovarian and Endometrial Cancer Among Postmenopausal Women. Am J Epidemiol 2016;183(9):800–14 doi 10.1093/aje/kwv308. [PubMed: 27190045]

10. Gates MA, Rosner BA, Hecht JL, Tworoger SS. Risk factors for epithelial ovarian cancer by histologic subtype. Am J Epidemiol 2010;171(1):45–53 doi 10.1093/aje/kwp314. [PubMed: 19910378]
11. Purdie DM, Bain CJ, Siskind V, Webb PM, Green AC. Ovulation and risk of epithelial ovarian cancer. Int J Cancer 2003;104(2):228–32 doi 10.1002/ijc.10927. [PubMed: 12569579]
12. Pelucchi C, Galeone C, Talamini R, Bosetti C, Montella M, Negri E, et al. Lifetime ovulatory cycles and ovarian cancer risk in 2 Italian case-control studies. Am J Obstet Gynecol 2007;196(1):83 e1–7 doi 10.1016/j.ajog.2006.06.088. [PubMed: 17240246]
13. Rice MS, Hankinson SE, Tworoger SS. Tubal ligation, hysterectomy, unilateral oophorectomy, and risk of ovarian cancer in the Nurses' Health Studies. Fertil Steril 2014;102(1):192–8 e3 doi 10.1016/j.fertnstert.2014.03.041. [PubMed: 24825424]
14. Lee AW, Tyrer JP, Doherty JA, Stram DA, Kupryjanczyk J, Dansonka-Mieszkowska A, et al. Evaluating the ovarian cancer gonadotropin hypothesis: a candidate gene study. Gynecol Oncol 2015;136(3):542–8 doi 10.1016/j.ygyno.2014.12.017. [PubMed: 25528498]
15. Choi JH, Wong AS, Huang HF, Leung PC. Gonadotropins and ovarian cancer. Endocr Rev 2007;28(4):440–61 doi 10.1210/er.2006-0036. [PubMed: 17463396]
16. Wentzensen N, Poole EM, Trabert B, White E, Arslan AA, Patel AV, et al. Ovarian Cancer Risk Factors by Histologic Subtype: An Analysis From the Ovarian Cancer Cohort Consortium. J Clin Oncol 2016;34(24):2888–98 doi 10.1200/JCO.2016.66.8178. [PubMed: 27325851]
17. Yang HP, Trabert B, Murphy MA, Sherman ME, Sampson JN, Brinton LA, et al. Ovarian cancer risk factors by histologic subtypes in the NIH-AARP Diet and Health Study. Int J Cancer 2012;131(4):938–48 doi 10.1002/ijc.26469. [PubMed: 21960414]
18. Luisi S, Florio P, Reis FM, Petraglia F. Inhibins in female and male reproductive physiology: role in gametogenesis, conception, implantation and early pregnancy. Hum Reprod Update 2005;11(2):123–35 doi 10.1093/humupd/dmh057. [PubMed: 15618291]
19. Visser JA, Schipper I, Laven JS, Themmen AP. Anti-Mullerian hormone: an ovarian reserve marker in primary ovarian insufficiency. Nat Rev Endocrinol 2012;8(6):331–41 doi 10.1038/nrendo.2011.224. [PubMed: 22231848]
20. Simoni M, Gromoll J, Nieschlag E. The follicle-stimulating hormone receptor: biochemistry, molecular biology, physiology, and pathophysiology. Endocr Rev 1997;18(6):739–73 doi 10.1210/edrv.18.6.0320. [PubMed: 9408742]
21. Shaw CM, Stanczyk FZ, Egleston BL, Kahle LL, Spittle CS, Godwin AK, et al. Serum antimullerian hormone in healthy premenopausal women. Fertil Steril 2011;95(8):2718–21 doi 10.1016/j.fertnstert.2011.05.051. [PubMed: 21704216]
22. Dewailly D, Andersen CY, Balen A, Broekmans F, Dilaver N, Fanchin R, et al. The physiology and clinical utility of anti-Mullerian hormone in women. Hum Reprod Update 2014;20(3):370–85 doi 10.1093/humupd/dmt062. [PubMed: 24430863]
23. Durlinger AL, Visser JA, Themmen AP. Regulation of ovarian function: the role of anti-Mullerian hormone. Reproduction 2002;124(5):601–9. [PubMed: 12416998]
24. Mom CH, Engelen MJ, Willemse PH, Gietema JA, ten Hoor KA, de Vries EG, et al. Granulosa cell tumors of the ovary: the clinical value of serum inhibin A and B levels in a large single center cohort. Gynecol Oncol 2007;105(2):365–72 doi 10.1016/j.ygyno.2006.12.034. [PubMed: 17306349]
25. La Marca A, Volpe A. The Anti-Mullerian hormone and ovarian cancer. Hum Reprod Update 2007;13(3):265–73 doi 10.1093/humupd/dml060. [PubMed: 17213257]
26. Robertson DM, Pruysers E, Jobling T. Inhibin as a diagnostic marker for ovarian cancer. Cancer Lett 2007;249(1):14–7 doi 10.1016/j.canlet.2006.12.017. [PubMed: 17320281]
27. Healy DL, Burger HG, Mamers P, Jobling T, Bangah M, Quinn M, et al. Elevated serum inhibin concentrations in postmenopausal women with ovarian tumors. N Engl J Med 1993;329(21):1539–42 doi 10.1056/NEJM199311183292104. [PubMed: 8413476]
28. Schock H, Lundin E, Vaarasmaki M, Grankvist K, Fry A, Dorgan JF, et al. Anti-Mullerian hormone and risk of invasive serous ovarian cancer. Cancer Causes Control 2014;25(5):583–9 doi 10.1007/s10552-014-0363-9. [PubMed: 24562905]

29. Jung S, Allen N, Arslan AA, Baglietto L, Barricarte A, Brinton LA, et al. Anti-Mullerian hormone and risk of ovarian cancer in nine cohorts. Int J Cancer 2018;142(2):262–70 doi 10.1002/ijc.31058. [PubMed: 28921520]
30. Tsigkou A, Marrelli D, Reis FM, Luisi S, Silva-Filho AL, Roviello F, et al. Total inhibin is a potential serum marker for epithelial ovarian cancer. J Clin Endocrinol Metab 2007;92(7):2526–31 doi 10.1210/jc.2007-0235. [PubMed: 17473066]
31. McSorley MA, Alberg AJ, Allen DS, Allen NE, Brinton LA, Dorgan JF, et al. Prediagnostic circulating follicle stimulating hormone concentrations and ovarian cancer risk. Int J Cancer 2009;125(3):674–9 doi 10.1002/ijc.24406. [PubMed: 19444906]
32. Langseth H, Gislefoss RE, Martinsen JI, Dillner J, Ursin G. Cohort Profile: The Janus Serum Bank Cohort in Norway. Int J Epidemiol 2017;46(2):403–4g doi 10.1093/ije/dyw027. [PubMed: 27063606]
33. Hjerkind KV, Gislefoss RE, Tretli S, Nystad W, Bjorge T, Engeland A, et al. Cohort Profile Update: The Janus Serum Bank Cohort in Norway. Int J Epidemiol 2017;46(4):1101–2f doi 10.1093/ije/dyw302. [PubMed: 28087783]
34. Gislefoss RE, Grimsrud TK, Morkrid L. Stability of selected serum hormones and lipids after long-term storage in the Janus Serum Bank. Clin Biochem 2015;48(6):364–9 doi 10.1016/j.clinbiochem.2014.12.006. [PubMed: 25523301]
35. Dorgan JF, Spittle CS, Egleston BL, Shaw CM, Kahle LL, Brinton LA. Assay reproducibility and within-person variation of Mullerian inhibiting substance. Fertil Steril 2010;94(1):301–4 doi 10.1016/j.fertnstert.2009.03.032. [PubMed: 19409547]
36. Dorgan JF, Stanczyk FZ, Egleston BL, Kahle LL, Shaw CM, Spittle CS, et al. Prospective case-control study of serum mullerian inhibiting substance and breast cancer risk. J Natl Cancer Inst 2009;101(21):1501–9 doi 10.1093/jnci/djp331. [PubMed: 19820206]
37. Kurman RJ, Shih Ie M. The Dualistic Model of Ovarian Carcinogenesis: Revisited, Revised, and Expanded. Am J Pathol 2016;186(4):733–47 doi 10.1016/j.ajpath.2015.11.011. [PubMed: 27012190]
38. Matz M, Coleman MP, Sant M, Chirlaque MD, Visser O, Gore M, et al. The histology of ovarian cancer: worldwide distribution and implications for international survival comparisons (CONCORD-2). Gynecol Oncol 2017;144(2):405–13 doi 10.1016/j.ygyno.2016.10.019. [PubMed: 27931752]
39. Trabert B, Eldridge RC, Pfeiffer RM, Shiels MS, Kemp TJ, Guillemette C, et al. Prediagnostic circulating inflammation markers and endometrial cancer risk in the prostate, lung, colorectal and ovarian cancer (PLCO) screening trial. Int J Cancer 2017;140(3):600–10 doi 10.1002/ijc.30478. [PubMed: 27770434]
40. Su HI, Freeman EW. Hormone changes associated with the menopausal transition. Minerva Ginecol 2009;61(6):483–9. [PubMed: 19942836]
41. Walentowicz P, Krintus M, Sadlecki P, Grabiec M, Mankowska-Cyl A, Sokup A, et al. Serum inhibin A and inhibin B levels in epithelial ovarian cancer patients. PLoS One 2014;9(3):e90575 doi 10.1371/journal.pone.0090575. [PubMed: 24599287]
42. Roudebush WE, Kivens WJ, Mattke JM. Biomarkers of Ovarian Reserve. Biomark Insights 2008;3:259–68. [PubMed: 19578510]
43. Cramer DW, Welch WR. Determinants of ovarian cancer risk. II. Inferences regarding pathogenesis. J Natl Cancer Inst 1983;71(4):717–21. [PubMed: 6578367]
44. Steffensen KD, Waldstrom M, Grove A, Lund B, Pallisgard N, Jakobsen A. Improved classification of epithelial ovarian cancer: results of 3 danish cohorts. Int J Gynecol Cancer 2011;21(9):1592–600 doi 10.1097/IGC.0b013e31822a0f6b. [PubMed: 21926912]
45. Sondheimer SJ. Oral contraceptives: mechanism of action, dosing, safety, and efficacy. Cutis 2008;81(1 Suppl):19–22. [PubMed: 18338654]

Table 1.

Characteristics of cases and controls for Invasive and Borderline Ovarian Cancer

	Invasive				Borderline			
Characteristics	**N**	**Cases (n=136) Mean ± SD[†](%)**	**N**	**Matched Controls (n=136) Mean ± Sd(%)**	**N**	**Cases (n=49) Mean ± SD[†]**	**N**	**Matched Controls (n=49)Mean ± SD**
AMH (pg/mL)	136	1398.4 ± 1459.1	136	1305.3 ± 1713.01	49	1118.7 ± 1297.8	49	1039.1 ± 1198.3
FSH (miU/L)	136	8.0 ± 10.3	136	8.5 ± 16.0	49	8.5 ± 13.0	49	10.6 ± 17.7
Inhibin B	136	40.3 ± 32.4	136	36.8 ± 41.6	49	41.0 ± 45.4	49	35.1 ± 33.2
Age at blood draw	136	41.5 ± 1.3	136	41.4 ± 1.2	49	41.6 ± 1.1	49	41.5 ± 1.1
Year of birth	136	1947.1 ± 2.16	136	1947.1 ± 2.1	49	1947 ± 1.7	49	1947 ± 1.8
Weight (kg)	130	67.1 ± 13.0	130	65.7 ± 13.0	48	65.3 ± 10.2	48	66.1 ± 10.6
Height (cm)	130	164.8 ± 6.0	130	165.6 ± 5.9	48	164.0 ± 5.7	48	165.8 ± 6.0
Parity	110	1.40 ± 0.9	128	1.39 ± 1.0				
0	14	(12.7)	19	(14.8)	5	10%	3	6%
1	56	(50.9)	59	(46.1)	20	41%	26	53%
2	25	(22.7)	36	(28.1)	12	24%	13	27%
3+	15	(13.6)	14	(10.9)	6	12%	4	8%
Missing	26	-	8	-	6	12%	3	6%
BMI[b]	130	24.7 ± 4.8	130	24.0 ± 4.6				
< 18.5 (Underweight)	2	(1.5)	2	(1.5)	1	2%	0	0%
18.5 – 25 (Normal)	83	(63.4)	93	(71.5)	29	59%	31	63%
25 – 30 (Overweight)	28	(21.5)	29	(22.3)	14	29%	13	27%
30+ (Obese)	17	(13.1)	6	(4.6)	4	8%	4	8%
Missing	6	-	6	-	1	2%	1	2%
Smoking status	129	-	130	-				
Current	48	(37.2)	59	(45.4)	17	35%	25	51%
Former	24	(18.6)	22	(16.9)	6	12%	9	18%
Never	57	(44.2)	49	(37.7)	25	51%	14	29%
Missing	7	-	8	-	1	2%	1	2%
Age at diagnosis	136	54.6 ± 6.4	-	-	49	53.0 ± 5.7	-	-
Time blood draw to diagnosis (years)	136	13.59 ± 6.24	-	-	49	11.9 ± 5.8	-	-

Abbreviation: BMI, body mass index; SD, standard deviation.

† Means were produced with data from cases and controls with non-missing data.

Table 2:

Overall associations of AMH, FSH and Inhibin B with ovarian cancer in cases and controls

	Cases (%)	Controls(%)	Conditional-Adjusted OR[†] (95% CI)
AMH pg/mL[§]	**n=136**	**n=136**	
Quartiles			
Q1 <2.47	33 (24.3)	35 (25.7)	1.0 ref
Q2 <2.88	22 (16.2)	34 (25.0)	0.75 (0.36–1.55)
Q3 <3.22	38 (27.9)	35 (25.7)	1.30 (0.65–2.59)
Q4 <4.10	43 (31.6)	32 (23.5)	1.37 (0.67–2.81)
p trend[‡]			*0.17*
Median			
Below median <2.88	55 (40.4)	69 (50.7)	1.0 ref
Above median >2.88	81 (59.6)	67 (49.3)	1.55 (0.94–2.56)
FSH mIU/L			
Quartiles			
Q1 <0.55	28 (20.6)	37 (27.2)	1.0 ref
Q2 <0.74	33 (24.3)	31 (22.8)	1.32 (0.61–2.84)
Q3 <0.93	38 (27.9)	35 (25.7)	1.49 (0.69–3.21)
Q4 <2.20	37 (27.2)	33 (24.3)	1.53 (0.69–3.38)
p trend			*0.26*
Median			
Below median < 0.74	62 (45.6)	70 (51.5)	1.0 ref
Above median > 0.74	74 (54.4)	66 (48.5)	1.29 (0.76–2.19)
Inhibin B (pg/mL)			
Group			
Undetectable <1.11	57 (41.9)	75 (55.2)	1.0 ref
Below median[‖] <1.76	40 (29.4)	28 (20.6)	1.87 (0.98 –3.56)
Above median <2.60	39 (28.7)	33 (24.3)	1.84 (0.93–3.62)
p trend			*0.05*
LOD Split			
Undetectable <1.11	57 (41.9)	75 (55.2)	1.0 ref
Detectable >1.11	79 (58.1)	61 (44.9)	1.97 (1.14–3.39)

Abbreviation: SD, standard deviation; OR, odds ratio; Q, quartile; ref, reference group; MV, multivariable; LOD, level of detection

[*] Conditional on matching variables (age at blood draw and birthyear)

[†] Adjusted for parity

[‡] Test of trend using the Wald statistic from ordinal regression over quartile/group

[§] All analytes were log transformed

// Median values for inhibin B reflect the median split of detectable values above the LOD

Table 3:

Associations of AMH, FSH and Inhibin B with ovarian cancer by tumor characteristic

	Type 1			Type 2			
	Cases(%) n=54	**Control (%) n=54**	**Conditional Adjusted OR* (95% CI)**		**Cases (%) n=82**	**Control (%) n=82**	**Conditional Adjusted OR* (95% CI)**	**P-het**
AMH§								
Quartiles								
Q1 <2.34	13 (24)	13 (24)	1.0 (ref)	*Q1<2.55*	22 (57)	21 (26)	1.0 (ref)	
Q2 <2.84	6 (11)	14 (26)	0.48 (0.13–1.78)	*Q2<2.91*	15 (18)	25 (30)	0.70 (0.28–1.78)	
Q3 <3.18	18 (33)	13 (24)	1.54 (0.53–4.43)	*Q3<3.23*	19 (23)	18 (22)	1.16 (0.43–3.10)	
Q4 <3.94	17 (32)	14 (26)	1.22 (0.39–3.87)	*Q4 <4.00*	26 (32)	18 (22)	1.44 (0.54–3.83)	
p trend			*0.37*	*p trend*			*0.28*	
Median								0.32
Below median <2.83	19 (35)	27 (50)	1.0 (ref)	Below median <2.91	37 (45)	46 (56)	1.0 (ref)	
Above median >2.83	35 (65)	27 (50)	1.86 (0.82–4.20)	Above median >2.91	45 (55)	36 (44)	1.61 (0.82–3.13)	
FSH								
Quartiles								
Q1 <0.54	15 (28)	14 (26)	1.0 (ref)	*Q1<0.54*	13 (16)	22 (27)	1.0 (ref)	
Q2 <0.76	11 (20)	13 (24)	0.32 (0.07–1.38)	*Q2<0.73*	22 (27)	21 (26)	2.20 (0.78–6.18)	
Q3 <0.97	14 (26)	14 (26)	0.59 (0.17–2.08)	*Q3<0.88*	21 (26)	18 (22)	2.32 (0.83–6.48)	
Q4 <2.18	14 (26)	13 (24)	0.79 (0.23–2.68)	*Q4 <1.49*	26 (32)	21 (26)	2.43 (0.86–6.88)	
p trend			*0.97*	*p trend*			*0.11*	
Median								0.06
Below median <0.76	26 (48)	27 (50)	1.0 (ref)	Below median <0.73	35 (43)	43 (52)	1.0 (ref)	
Above median >0.76	28 (52)	27 (50)	1.12 (0.47–2.71)	Above median >0.73	47 (57)	39 (48)	1.51 (0.76–3.00)	
Inhibin B								
Group								
Undetectable <1.11	25 (46)	32 (59)	1.0 (ref)	*Undetectable <1.11*	32 (39)	43 (52)	1.0 (ref)	

	Type 1			Type 2				
	Cases(%) n=54	**Control (%) n=54**	**Conditional Adjusted OR* (95% CI)**		**Cases (%) n=82**	**Control (%) n=82**	**Conditional Adjusted OR* (95% CI)**	**P-het**
AMH§								
Below median// <1.62	11 (20)	8 (15)	2.92 (0.80–10.74)	*Below median <1.76*	25 (30)	19 (23)	1.57 (0.72–3.43)	
Above median <2.08	18 (33)	14 (26)	3.25 (0.93–11.34)	*Above median <2.52*	25 (30)	20 (24)	1.68 (0.70–4.00)	
p trend			*0.06*	*p trend*			*0.13*	
LOD Split								0.41
Undetectable < 1.11	25 (46)	32 (59)	1.0 (ref)	Undetectable <1.11	32 (39)	43 (52)	1.0 (ref)	
Detectable >1.11	29 (54)	22 (41)	3.10 (1.04–9.23)	Detectable >1.11	50 (61)	39 (48)	1.61 (0.83–3.13)	

Abbreviation: OR, odds ratio; Q, quartile; ref, reference group; MV, multivariable; LOD, level of detection

*conditional on matching variables (age at blood draw and birthyear) and adjusted for parity

‡Test of trend using the Wald statistic from ordinal regression over quartile/group

§All analytes were log transformed

//Median values for inhibin B reflect the median split of detectable values above the LOD

Table 4:

Associations of AMH, FSH and Inhibin B with ovarian cancer by tumor characteristic

	Type 1				Type 2		
Excluding subtypes designated “other”							
FSH[§]	Cases (N=53)	Control (N=53)	Conditional Adjusted OR[*] (95% CI)		Cases (n=50)	Control (N= 50)	Conditional Adjusted OR[*] (95% CI)
Median							
Below median < 0.77	26 (49)	26 (49)	1.0 (ref)	Below median < 0.76	21 (42)	30 (60)	1.0 (ref)
Above median > 0.77	27 (51)	27 (51)	1.09 (0.45–2.65)	Above median > 0.76	29 (58)	20 (40)	2.78 (1.05–7.38)
Inhibin B							
LOD split							
Undetectable < 1.11	25 (47)	32 (60)	1.0 (ref)	Undetectable < 1.32	22 (44)	28 (56)	1.0 (ref)
Detectable > 1.11	28 (53)	21 (40)	3.06 (1.03–9.07)	Detectable > 1.32	28 (56)	22 (44)	1.80 (0.75–4.37)
Excluding tumors of unknown grade							
FSH	Cases (N=54)[≠]	Control (N=54)	Conditional Adjusted OR[*] (95% CI)		Cases (n=60)	Control (N= 60)	Conditional Adjusted OR[*] (95% CI)
Median							
Below median < 0.76	26 (48)	27 (50)	1.0 (ref)	Below median < 0.76	20 (33)	30 (50)	1.0 (ref)
Above median > 0.76	28 (52)	27 (50)	1.12 (0.47–2.71)	Above median > 0.76	40 (67)	30 (50)	2.74 (1.04–7.21)
Inhibin B							
LOD Split							
Undetectable < 1.11	25 (46)	32 (60)	1.0 (ref)	Undetectable < 1.11	22 (37)	33 (66)	1.0 (ref)
Detectable > 1.11	29 (54)	22 (40)	3.10 (1.04–9.23)	Detectable > 1.11	38 (63)	27 (54)	1.89 (0.87–4.12)

Abbreviation: OR, odds ratio; Q, quartile; ref, reference group; MV, multivariable; LOD, level of detection

[*] Conditional on matching variables (age at blood draw and birthyear) and adjusted for parity

[†] Test of trend using the Wald statistic from ordinal regression over quartile/group

[≠] All endometrioid, mucinous and clear cell tumors are categorized as Type 1 independent of grade.

$\S$All analytes were log transformed