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

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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 (AMH), follicle-stimulating hormone (FSH), and inhibin B with ovarian cancer risk.

Methods: Our study included 370 women from the Janus Serum Bank, including 54 type I and 82 type II invasive epithelial ovarian cancers, 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 (types I and II).

Results: Inhibin B was associated with increased risk of ovarian cancer overall [OR, 1.97; 95% confidence interval (CI), 1.14–3.39; $P_{trend} = 0.05$] and with type I 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 II 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 premenopausal markers of ovarian reserve and gonadotropin signaling. Heterogeneity of inhibin B and FSH effects in different tumor types may be informative of tumor etiology.

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 postmenopausal 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 (LOC) 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 5-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). 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 premenopausal 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 postmenopausal women with ovarian granulosa cell tumors and most mucinous epithelial tumors, but it is not clear whether premenopausal inhibins are associated with ovarian cancer (24, 27, 30). Inconsistent results have been reported for the

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Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

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Cancer Epidemiol Biomarkers Prev 2020;29:636–42

doi: 10.1158/1055-9965.EPI-19-0675

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.

Materials and 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 previously (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, Norway and surrounding areas (33). The collection period was from 1972 to 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 (Oslo, Norway; refs. 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 premenopausal 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 postmenopausal women; therefore, women between 35–45 years old without a history of cancer (other than nonmelanoma skin cancer) were included. Women with rare nonepithelial 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.; refs. 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). 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 ($n = 100$) versus nonserous ($n = 36$) separately. The main analysis used the dichotomous type 1 versus 2 classification that includes morphology and grade as described previously (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 on the basis of 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 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; ref. 37). Sensitivity analyses included histologic subtype (serous vs. nonserous) and time between blood draw and diagnosis (<10 or ≥ 10 years). In addition, 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 (CV) and intraclass correlation coefficients (ICC) 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

Table 1. Characteristics of cases and controls for invasive and borderline ovarian cancer.

Characteristics	Invasive Cases ($n = 136$) N	Mean ± SD[a] (%)	Matched controls ($n = 136$) N	Mean ± SD (%)	Borderline Cases ($n = 49$) N	Mean ± SD[a]	Matched controls ($n = 49$) N	Mean ± SD
AMH (pg/mL)	136	1,398.4 ± 1,459.1	136	1,305.3 ± 1,713.01	49	1,118.7 ± 1,297.8	49	1,039.1 ± 1,198.3
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	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	—	—

[a]Means were produced with data from cases and controls with nonmissing data.

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 S1). 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 S2.

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 with those with undetectable values (below the LOD) [detectable vs. undetectable: OR 1.97, 95% confidence interval (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 I versus type II cancers

The associations between FSH and inhibin B and ovarian cancer risk differed across subtypes. Inhibin B was associated with increased risk of type I ovarian cancer in women with detectable values compared with those with undetectable values (detectable vs. undetectable: OR 3.10, 95% CI: 1.04–9.23). ORs 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 II ovarian cancers ($P_{het} = 0.41$). ORs for the association between FSH and ovarian cancer risk were elevated in the type II cancers in all quartiles compared with 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 I cancers, where ORs 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 I ovarian cancers and increased levels of FSH and type II 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 I 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 II tumors (> median vs. < median: OR 2.78; 95% CI: 1.05–7.38; **Table 4**). Higher FSH remained associated with type II ovarian cancer after exclusion of tumors with unknown grade (> median vs.

Table 2. Overall associations of AMH, FSH, and inhibin B with ovarian cancer in cases and controls.

	Cases (%) $n = 136$	Controls (%) $n = 136$	Conditional-adjusted OR[a] (95% CI)
AMH (pg/mL)[b]			
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}[c]			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[d] <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)

Abbreviations: LOD, level of detection; MV, multivariable; Q, quartile; ref, reference group.

[a]Adjusted for parity; conditional on matching variables (age at blood draw and birth year).

[b]All analytes were log transformed.

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

[d]Median values for inhibin B reflect the median split of detectable values above the LOD.

< median: OR 2.74; 95% CI: 1.04–7.21). No tumors of unknown grade were removed from the type I stratum, as all nonserous tumors are considered type I, regardless of grade, and the association between inhibin B and type I 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 S3. Joint effects were not observed between FSH and inhibin B, indicating that no interaction is present between these analytes in our data (Supplementary Table S4).

Sensitivity analyses

In sensitivity analyses, ORs for the association between inhibin B and ovarian cancer risk were elevated in nonserous ovarian cancer compared with serous tumors for those with detectable values compared with 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 S5]. 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 versus those diagnosed more than 10 years after blood draw (Supplementary Table S6).

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 versus below the median split of detectable values for inhibin B (Supplementary Table S7).

Discussion

In a population of 370 premenopausal 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 II ovarian tumors, and higher inhibin B was associated with increased risk of type I 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 premenopausal 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 premenopausal AMH. Previous analyses of FSH (31) showed a protective effect of FSH with increasing quartile, but women were both pre- and postmenopausal, and FSH levels are known to differ between pre-and postmenopausal women (40). We found that higher FSH may be associated with increased risk of type II ovarian cancer but inversely associated with type I 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 with our analysis. Studies of inhibin also included both pre- and postmenopausal 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 premenopausal 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 premenopausal 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

Table 3. Associations of AMH, FSH, and inhibin B with ovarian cancer by tumor characteristics.

	Type I				Type II			
	Cases (%) $n = 54$	Control (%) $n = 54$	Conditional adjusted OR[a] (95% CI)		Cases (%) $n = 82$	Control (%) $n = 82$	Conditional adjusted OR[a] (95% CI)	P_{het}
AMH (pg/mL)[b]								
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}[c]			0.37	P_{trend}[c]			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 (mIU/L)								
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}[c]			0.97	P_{trend}[c]			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 (pg/mL)								
Group								
Undetectable <1.11	25 (46)	32 (59)	1.0 (ref)	Undetectable <1.11	32 (39)	43 (52)	1.0 (ref)	
Below median[d] <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}[c]			0.06	P_{trend}[c]			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)	

Abbreviations: LOD, level of detection; MV, multivariable; Q, quartile; ref, reference group.
[a]Conditional on matching variables (age at blood draw and birth year) and adjusted for parity.
[b]All analytes were log transformed.
[c]Test of trend using the Wald statistic from ordinal regression over quartile/group.
[d]Median values for inhibin B reflect the median split of detectable values above the LOD.

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 II cancers composed primarily of serous tumors, and inhibin B in nonserous 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 I tumors, or inhibin B in the type II 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 premenopausal 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 CVs, 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 toward the null. In addition, 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

Table 4. Associations between FSH and inhibin B with risk of ovarian cancer excluding rare subtypes tumors of unknown grade.

	Type I			Type II			
Excluding subtypes designated "other"							
FSH[a]	**Cases ($n = 53$)**	**Control ($n = 53$)**	**Conditional adjusted OR[b] (95% CI)**		**Cases ($n = 50$)**	**Control ($n = 50$)**	**Conditional adjusted OR[b] (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 ($n = 54$)[c]**	**Control ($n = 54$)**	**Conditional adjusted OR[b] (95% CI)**		**Cases ($n = 60$)**	**Control ($n = 60$)**	**Conditional adjusted OR[b] (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)

Abbreviations: LOD, level of detection; MV, multivariable; Q, quartile; ref, reference group.
[a]All analytes were log transformed.
[b]Conditional on matching variables (age at blood draw and birth year) and adjusted for parity.
[c]All endometrioid, mucinous, and clear cell tumors are categorized as type I independent of grade.

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 1980s and early 1990s 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.

Disclosure of Potential Conflicts of Interest

F.Z. Stanczyk is a consultant for TherapeuticsMD, Dr. Reddy's Laboratories, and Mithra Pharmaceuticals. No potential conflicts of interest were disclosed by the other authors.

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.

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Acknowledgments

This work was funded by the Intramural Research Program of the NCI. We would like to thank the Janus Serum Bank of Norway for the privilege to analyze its 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-year surveys 1985–1999, and the Medial Birth Registry of Norway for providing parity data for our sample.

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Received June 12, 2019; revised September 5, 2019; accepted December 18, 2019; published first January 13, 2020.

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