Early pregnancy sex steroids and maternal risk of epithelial ovarian cancer

Helena Schock[1,2], Heljä-Marja Surcel[3], Anne Zeleniuch-Jacquotte[4,5], Kjell Grankvist[2], Hans-Åke Lakso[2], Renée Turzanski Fortner[1], Rudolf Kaaks[1], Eero Pukkala[6,7], Matti Lehtinen[7], Paolo Toniolo[4,8,9] and Eva Lundin[2,10]

[1]Division of Cancer Epidemiology, German Cancer Research Center, Im Neuenheimer Feld 280, Heidelberg 69120, Germany
[2]Department of Medical Biosciences, Umeå University, Umeå, Sweden
[3]Unit of Sexual and Reproductive Health, National Institute for Health and Welfare, Oulu, Finland
[4]Departments of Population Health and Environmental Medicine, New York University School of Medicine, New York, New York, USA
[5]New York University Cancer Institute, New York University School of Medicine, New York, New York, USA
[6]Finnish Cancer Registry, Institute for Statistical and Epidemiological Cancer Research, Helsinki, Finland
[7]School of Health Sciences, University of Tampere, Tampere, Finland
[8]Department of Obstetrics and Gynecology, New York University School of Medicine, New York, New York, USA
[9]Institute of Social and Preventive Medicine, Centre Hospitalier Universitaire Vaudois (CHUV), Lausanne, Switzerland
[10]Public Health and Clinical Medicine: Nutritional Research, Umeå University, Umeå, Sweden

Correspondence should be addressed to H Schock
Email
h.schock@dkfz.de

Abstract

Well-established associations between reproductive characteristics and epithelial ovarian cancer (EOC) support an involvement of sex steroid hormones in the etiology of EOC. Limited previous studies have evaluated circulating androgens and the risk of EOC, and estrogens and progesterone have been investigated in only one of the previous studies. Furthermore, there is little data on potential heterogeneity in the association between circulating hormones and EOC by histological subgroup. Therefore, we conducted a nested case–control study within the Finnish Maternity Cohort and the Northern Sweden Maternity Cohort to investigate the associations between circulating pre-diagnostic sex steroid concentrations and the histological subtypes of EOC. We identified 1052 EOC cases among cohort members diagnosed after recruitment (1975–2008) and before March 2011. Up to three controls were individually matched to each case ($n=2694$). Testosterone, androstenedione, 17-hydroxyprogesterone (17-OHP), progesterone, estradiol (E_2), and sex hormone-binding globulin levels were measured in serum samples collected during the last pregnancy before EOC diagnosis. We used conditional logistic regression to estimate odds ratios (ORs) and 95% CIs. Associations between hormones and EOC differed with respect to tumor histology and invasiveness. Sex steroid concentrations were not associated with invasive serous tumors; however, doubling of testosterone and 17-OHP concentration was associated with approximately 40% increased risk of borderline serous tumors. A doubling of androgen concentrations was associated with a 50% increased risk of mucinous tumors. The risk of endometrioid tumors increased with higher E_2 concentrations (OR: 1.89 (1.20–2.98)). This large prospective study in pregnant women supports a role of sex steroid hormones in the etiology of EOC arising in the ovaries.

Key Words
- prospective study
- case–control study
- pregnancy
- sex steroids
- ovarian neoplasms

Endocrine-Related Cancer (2014) **21**, 831–844

http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-14-0282

Published by Bioscientifica Ltd.

Introduction

Worldwide, more than 200 000 women are diagnosed with epithelial ovarian cancer (EOC) each year. It is the seventh most common cancer in women and the leading cause of gynecological cancer death among women with an overall 5-year survival rate of approximately 40% only in developed countries (Ferlay *et al*. 2014). EOC can be subdivided into invasive and borderline diseases; approximately 15% of EOC are borderline tumors with similar epidemiological risk factors to invasive tumors, but occurring at a younger age, presenting at an earlier stage, and with a more favorable prognosis (Trope *et al*. 2012).

Furthermore, EOC includes different histological subtypes, with the most common being serous (75%), endometrioid (10%), clear cell (10%), and mucinous (3%) (Prat 2012). Growing evidence indicates that EOC subgroups represent clinically, morphologically, and molecularly distinct diseases (Prat 2012).

EOC may develop from inclusion cysts by metaplasia of ovarian surface epithelium or implantation of other epithelia (e.g. tubal tissue; Auersperg *et al*. 2001, Kurman & Shih 2010). The pathogenic mechanisms involved in the development of ovarian cancer are poorly understood. The prevailing etiological hypotheses implicate long periods of ovulatory cycles (Fathalla 1971), retrograde menstrual flow (Cramer & Xu 1995), and endogenous, as well as exogenous, hormonal exposure (Risch 1998). Sex steroid hormones (androgens, progesterone, and estradiol (E_2)) are likely to be involved in the etiology of EOC, as there are well-established associations between reproductive characteristics affecting sex steroid concentrations and EOC. Examples include the protective effect of pregnancy and the use of oral contraceptives (OCs), and the increased risk associated with the current use of hormone replacement therapy (Morch *et al*. 2009, Tsilidis *et al*. 2011).

To date, studies directly relating pre-diagnostic endogenous hormone concentrations to the risk of EOC are confined to androgens and results are inconclusive (Helzlsouer *et al*. 1995, Lukanova *et al*. 2003, Rinaldi *et al*. 2007, Tworoger *et al*. 2008, Ose *et al*. 2014). Only one small study related progesterone and E_2 to the risk of EOC (Helzlsouer *et al*. 1995). This is most probably because concentrations of progesterone and E_2 show large intra-individual variations during the menstrual cycle among premenopausal women, making measurement difficult, and concentrations in postmenopausal women are low (Rannevik *et al*. 1995, Speroff & Marc 2005).

Given the sparse data on the relationship between sex steroid hormones and EOC, and the established association between parity and EOC, we investigated early pregnancy sex steroid hormones and the subsequent ovarian cancer risk in a case–control study nested in two prospective maternity cohorts. Furthermore, we present the first data, to our knowledge, on early pregnancy hormones and the risk of EOC on the basis of histological subtype and invasiveness. Androgens in early pregnancy are similar to preconception concentrations; therefore, our study characterizes premenopausal circulating androgen concentrations with a risk of EOC. On the other hand, progesterone and E_2 levels are elevated in early pregnancy and their concentrations increase further during pregnancy; thus, we are able to explore whether these hormones contribute to the protective effect conferred by pregnancy.

Materials and methods

Study population

A case–control study was nested within the Finnish Maternity Cohort (FMC) and the Northern Sweden Maternity Cohort (NSMC). These two bio-repositories store serum samples collected during the late weeks of the first, or early weeks of the second, trimester at −25 and −20 °C respectively. The FMC was established in 1983 and includes approximately two million specimens from almost 99% of pregnancies in the country (http://www.thl.fi/en/web/thlfi-en/research-and-expertwork/projects-and-programmes/projects/28232, accessed May 2014). The NSMC was established in 1975 and contains almost 120 000 samples from pregnancies in the three northernmost counties of Sweden (Pukkala 2011).

Study subjects were selected among members from the two cohorts with: i) no history of twin or multiple pregnancies; ii) a blood sample obtained during the first trimester of a pregnancy leading to childbirth; and iii) no history of invasive cancer (except for non-melanoma skin cancer) and/or borderline ovarian cancer before blood donation.

Identification of cases Cases diagnosed with invasive or borderline EOC after blood donation were identified through linkages with the Finnish Cancer Registry (founded in 1952) and the Swedish Cancer Registry (founded in 1958). Reporting of newly diagnosed cases is mandatory in both nationwide cancer registries, leading to very high (>95%) completeness for solid tumors (Teppo *et al*. 1994, Barlow *et al*. 2009). We identified 1105 incident EOC cases among FMC participants and

http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-14-0282

Published by Bioscientifica Ltd.

146 incident EOC cases among NSMC members; cases were diagnosed before December 2009 and March 2011 respectively. The serum sample from the last singleton pregnancy (or the most recent available for 68 cases) resulting in delivery of a neonate before diagnosis was selected for the study.

Selection of controls Selection of controls was carried out in two steps. First, up to 12 potentially eligible controls were selected for each case through linkages with the national population registries. Controls matching the case on study cohort, age at sampling (±6 months), date of sampling (±3 months), parity at sampling (1, 2, and >2 children), and parity at diagnosis (1, 2, and >2 children) were selected using incidence density sampling. In a second step, up to three controls were selected at random.

Cases with insufficient serum volume for laboratory measurements (FMC: $n=157$ and NSMC: $n=31$) and cases for whom no eligible controls could be identified (FMC: $n=5$ and NSMC: $n=6$) were excluded. Therefore, a total of 1052 EOC cases (FMC: $n=943$ and NSMC: $n=109$) and 2694 controls (FMC: $n=2383$ and NSMC: $n=311$) were included in the study.

Morphology codes were provided by the Finnish and Swedish cancer registries and recoded according to the World Health Organization guidelines (Lee *et al.* 2003) to histological subgroups of EOC as serous ($n=477$, 45%), mucinous ($n=356$, 34%), endometrioid ($n=102$, 10%), clear cell ($n=26$, 3%), and not otherwise specified (NOS) ($n=66$, 6%) tumors. Data on histology were not available for 25 cases (2%), and, thus, they were included only in overall analyses and analyses of tumor invasiveness, as were tumors diagnosed as NOS. Data on the stage of EOC at diagnosis were not available for cases from the NSMC, but were available for 87% of cases from the FMC. For cases from the FMC, stage I ($n=482$, 58%) was classified as localized, whereas stages II ($n=6$), III ($n=306$), and IV ($n=30$) were classified as advanced EOC ($n=342$, 42%).

Characteristics related to pregnancy (e.g. pregnancy length, smoking during pregnancy, maternal age at first birth, and number of incomplete pregnancies) and to the newborn (e.g. gender, and birth weight and length) were obtained through linkages from the country-specific birth registries. For 2200 members of the FMC ($n=629$ case–control sets, 60%) information on malignant cancers diagnosed among first-degree relatives was obtained through linkages with the Finnish population and Cancer registries.

This study was approved by the ethical committees of the National Institute for Health and Welfare, Finland, University of Umeå, Sweden, and German Cancer Research Center, Germany.

Laboratory analyses

All hormonal analyses were performed at the Clinical Chemistry Laboratory of Umeå University Hospital, Umeå, Sweden. Serum specimens of individually matched case and control subjects were always included in the same laboratory batch. The technicians performing the assays were blinded to the case, control, or quality control status of the specimens. In addition to routine laboratory quality controls, two pools of serum from each of the cohorts were created at the beginning of the study and three aliquots, undistinguishable from the test samples, were inserted in each laboratory run.

Concentrations of androgens (testosterone and androstenedione), 17-hydroxyprogesterone (17-OHP), progesterone, and E_2 were quantified by HPLC tandem mass spectrometry on an Applied Biosystems API4000 triple-stage quadrupole mass spectrometer. Inter- and intra-run coefficient of variation (CV) values based on the blinded pooled quality controls were <10 and <11%, respectively, for samples from the FMC and <11 and <9%, respectively, for samples from the NSMC for all sex steroids.

Sex hormone-binding globulin (SHBG) was quantified by solid-phase competitive chemiluminescence assays on an Immulite 2000 Siemens analyzer. Inter- and intra-run CV values based on the blinded pool quality controls were <10% in both cohorts.

Statistical analysis

All hormone values were $\log_2$-transformed to normalize their distributions and to allow for the estimation of the risk with a doubling of hormone concentration. Concentrations of progesterone, 17-OHP, E_2, and SHBG varied linearly with the gestational age ($r=0.49$, -0.26, 0.69, and 0.57, respectively; $P<0.0001$); thus, analyses including these hormones were limited to women with information on gestational age ($n=765$ case–control sets, 73%) and gestational age was included as a covariate in the statistical models. Pearson's partial correlation coefficients were used to assess correlations between individual hormone concentrations in control samples.

We used conditional logistic regression to assess differences between cases and controls and to calculate odds ratios (ORs) and the corresponding 95% CIs. For each of the hormones, ORs were calculated for tertiles of

http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-14-0282

Published by Bioscientifica Ltd.

hormone concentrations using the cohort-specific frequency distribution in controls. Likelihood ratio tests were used to assess linear trends across categories based on the median hormone values for the tertiles. In addition, ORs were calculated for a unit change of $\log_2$-transformed hormones.

The effect of potential confounders (maternal age at first birth, smoking during index pregnancy, family history of breast and/or ovarian cancers, child's sex, and birth length and weight) was evaluated. Missing values were assigned to a 'missing' category for categorical covariates, whereas, for continuous variables, missing values were assigned the individual cohort (NSMC or FMC) median value for that variable. Analyses that excluded subjects with missing values for any of these covariates were similar to those with values imputed as described. Among the available covariates, none changed point estimates by >10%. We repeated the analyses for each of the hormones mutually adjusting for the other hormones.

Stratified analyses were performed by histology, ages at sampling and diagnosis (histology-specific; below/above median), and the time between blood donation and diagnosis (i.e., 'lag-time'; below/above median). We also stratified by the stage (localized vs advanced disease), the number of children by diagnosis date of the matched case (1 vs >1), and the age at diagnosis (age of <51 vs ≥51 years). The cut-off for age at diagnosis was chosen as the average age at menopause is 51 years in Sweden and Finland (Thomas *et al*. 2001). Tests of heterogeneity between the ORs in different subgroups were based on χ^2 statistics, calculated as the deviations of logistic regression coefficients observed in each of the subgroups, relative to the overall regression coefficient (Whitehead & Whitehead 1991).

We conducted sensitivity analyses limited to women diagnosed at least 2 or 3 years after blood donation to assess whether the study results were influenced by the presence of undiagnosed, but hormonally active tumors. Additionally, we performed analyses limited to women with a full-term pregnancy or, for androgen analyses, with information on gestational age at blood sampling. Results adjusted for gestational age were similar to those for hormone concentrations for each study subject computed as the difference (residual) between the assay value and the estimated cohort-specific mean value determined for the day of gestation when the sample was drawn using local linear regression (Cleveland & Loader 1996; data not shown).

All statistical tests were two-sided with a significance level of 0.05. Statistical analyses were performed using the Statistical Analyses System (SAS), version 9.2 Software (SAS Institute, Inc., Cary, NC, USA). The figure was prepared using the R software (package 'rmeta', function 'forestplot) version 2.15.2 (R Core Team 2014).

Results

Key characteristics of the study subjects and their newborns by study cohort are presented in Table 1. The majority of the 1052 cases (n=943, FMC and n=109, NSMC) were diagnosed with invasive EOC (n=642, 61%). Out of them, 283 (44%) were serous, 150 (23%) mucinous, 102 (16%) endometrioid, and 26 (4%) clear cell. Among the borderline tumors (n=410, 39%), 194 (47%) were serous and 206 (50%) mucinous. The distribution by histological subtype and invasiveness was consistent between the two cohorts. Invasive tumors were predominantly diagnosed as advanced disease (69%), whereas almost all borderline tumors were diagnosed as localized disease (97%). Median age at EOC diagnosis was 43.9 years and on average 12.2 years elapsed between blood donation and diagnosis among all cases.

In the FMC, maternal and child characteristics were similar for both cases and controls except for median age at first birth (26.0 vs 26.9; P<0.0001), smoking during pregnancy (22 vs 14%; P<0.0001), and family history of breast and/or ovarian cancer (8 vs 5%; P=0.008). In the NSMC, only smoking during pregnancy was markedly different between cases and controls (38 vs 28%; P=0.06).

Cases with mucinous tumors (borderline and invasive) had the shortest lag-time between blood collection and cancer diagnosis (10.2 years) and the youngest median age at diagnosis (40.1 and 40.9 years respectively), whereas cases diagnosed with endometrioid or clear cell tumors had the longest lag-time (16.3 and 17 years respectively) and were the oldest at diagnosis (48.1 years) (Table 2).

Cases with borderline tumors were younger at first birth, those with borderline and invasive mucinous and invasive serous tumors were more likely to smoke, and those with invasive serous tumors were more likely to have a family history of breast and/or ovarian cancer relative to their matched controls (Table 2).

Case subjects from both cohorts had significantly higher geometric means of testosterone (FMC: 0.87 vs 0.79 ng/ml; P<0.0001 and NSMC: 0.83 vs 0.72 ng/ml; P=0.004) and androstenedione (FMC: 1.91 vs 1.73 ng/ml; P<0.0001 and NSMC: 1.98 vs 1.71; P=0.007) relative to control subjects. In the FMC, 17-OHP levels were also higher in cases compared with controls (2.37 vs 2.24 ng/ml; P=0.006).

http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-14-0282

Published by Bioscientifica Ltd.

Table 1 Distribution of characteristics of EOC cases and their matched controls, median (min and max) or *n* (%) from the Finnish Maternity Cohort (1983–2009) and the Northern Sweden Maternity Cohort (1975–2011)[a]

Characteristics	Missing (%)[b]	Finnish Maternity Cohort		*P*	Missing (%)[b]	Northern Sweden Maternity Cohort		*P*
		Cases (943)	Controls (2383)			Cases (109)	Controls (311)	
Age at blood donation (years)	–	31.8 (16.4–45.7)	31.7 (15.7–45.5)	–	–	29.0 (17.3–41.7)	28.8 (17.4–42.8)	–
Parity at index pregnancy	–			–	–			–
1 child		231 (24%)	597 (25%)			59 (54%)	170 (55%)	
2 children		386 (41%)	981 (41%)			27 (25%)	76 (24%)	
>2 children		326 (35%)	805 (34%)			23 (21%)	65 (21%)	
Gestational age (days)	25	75 (39–142)	73 (39–142)	0.50	–	75 (38–148)	73 (38–148)	0.16
Age at first birth (years)[c]	–	26.0 (14.8–46.2)	26.9 (14.6–45.9)	<0.0001	15	24.9 (16.1–40.8)	24.8 (16.8–40.0)	0.56
Pregnancy length (weeks)[c]	25			0.38	–			0.12
<37		35 (5%)	84 (5%)			1 (1%)	13 (4%)	
≥37		618 (95%)	1768 (95%)			108 (99%)	298 (96%)	
Child weight (g)[c]	25	3620 (460–5100)	3610 (580–5260)	0.50	–	3460 (1480–4500)	3500 (900–5920)	0.78
Child length (cm)[c]	25	51 (29–57)	50 (25–56)	0.95	–	50 (41–55)	50 (33–56)	0.42
Child gender	0.1			0.46	–			0.18
Male		481 (51%)	1255 (53%)			48 (44%)	160 (51%)	
Female		461 (49%)	1126 (47%)			61 (56%)	151 (49%)	
Smoking during pregnancy[c]	27	138 (22%)	245 (14%)	<0.0001	2	41 (38%)	85 (28%)	0.06
Family history of breast and/or ovarian cancer	34	51 (8%)	81 (5%)	0.008	100	–	–	–
Age at diagnosis (years)	–	43.3 (19.0–66.0)			–	47.8 (20.2–68.0)		
Years between blood draw and diagnosis	–	11.5 (0.1–25.6)			–	19.5 (1.3–31.7)		
Cancer type	–				23			
Borderline		367 (39%)				43 (39%)		
Serous		174 (47%)				20 (51%)		
Mucinous		189 (52%)				17 (44%)		
NOS		4 (1%)				2 (5%)		
Invasive		576 (61%)				66 (61%)		
Serous		263 (46%)				20 (44%)		
Mucinous		143 (25%)				7 (16%)		
Endometrioid		92 (16%)				10 (22%)		
Clear Cell		23 (4%)				3 (7%)		
NOS		55 (9%)				5 (11%)		
Disease spread[d]	13				100	–		
Localized (stage I)		482 (58%)						
Advanced (stage II–IV)		342 (42%)						
Hormones[e,f]								
Testosterone (ng/ml)	0.5	0.87 (0.84–0.90)	0.79 (0.78–0.81)	<0.0001	1	0.83 (0.76–0.90)	0.72 (0.68–0.75)	0.004
Androstenedione (ng/ml)	0.5	1.91 (1.85–1.97)	1.73 (1.71–1.77)	<0.0001	1	1.98 (1.81–2.16)	1.71 (1.63–1.81)	0.007
17–OHP (ng/ml)	25	2.37 (2.29–2.46)	2.24 (2.19–2.29)	0.006	1	2.21 (2.02–2.42)	2.03 (1.93–2.14)	0.16
Progesterone (ng/ml)	25	24.9 (24.3–25.5)	24.4 (24.1–24.8)	0.34	1	21.4 (19.9–23.0)	21.4 (20.5–22.3)	0.97
Estradiol (ng/ml)	25	1.97 (1.89–2.05)	1.89 (1.85–1.94)	0.09	1	1.96 (1.75–2.19)	1.84 (1.72–1.96)	0.35
SHBG (nmol/l)	27	211 (205–218)	205 (201–209)	0.11	5	122 (107–140)	131 (121–142)	0.33

NOS, not otherwise specified; 17-OHP, 17-hydroxyprogesterone; SHBG, sex hormone-binding globulin.
[a]Conditional logistic regression models were used to compare differences between cases and controls.
[b]Percentage of missing values, – indicates no missing values.
[c]Data from the Finnish Birth Registry are available since 1987.
[d]Percentage of missing values among cases.
[e]Geometric means and 10th–90th percentile of hormone concentrations (adjusted for study cohort and, except for androgens and gestational age).
[f]Conversion from ng/ml to nmol/l (SI units): testosterone×3.467, androstenedione×3.49, 17-OHP×3.025, progesterone×3.18, and estradiol×3.671.

Associations between early pregnancy hormones and EOC differed by tumor histology and, for the serous subtype, also by tumor invasiveness (Table 3 and Fig. 1). We did not observe any association between the evaluated hormones and the risk of invasive serous or clear cell tumors in our study population. High progesterone and SHBG were not associated with the risk of EOC overall or with the histological subgroups.

High testosterone was associated with a significantly increased risk of borderline serous tumors (3rd vs

http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-14-0282

Published by Bioscientifica Ltd.

http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-14-0282

Published by Bioscientifica Ltd.

Table 2 Distribution of characteristics of EOC cases by invasiveness and tumor histology, median (min and max) or *n* (%) from the Finnish Maternity Cohort (1983–2009) and the Northern Sweden Maternity Cohort (1975–2011)

		Serous		Mucinous				
	Invasive (642 cases)	Invasive (283 cases)	Borderline (194 cases)	Invasive (150 cases)	Borderline (206 cases)	Endometrioid (102 cases)	Clear cell (26 cases)	Controls (2694)
Tumor characteristics								
Age at diagnosis (years)	45.4 (20.2–68.0)	45.9 (25.9–68.0)	42.5 (23.9–62.9)	40.9 (25.4–57.8)	40.1 (19.0–66.0)	48.1 (27.2–60.8)	48.1 (32.8–60.2)	–
Lag time (years)	13.1 (0.2–30.8)	13.5 (0.2–30.7)	11.4 (0–28.7)	10.2 (0.2–27.6)	10.2 (0.2–28.7)	16.3 (0.6–29.7)	17.0 (0.4–30.8)	–
Spread[a]								–
Localized	150 (31%)	38 (16%)	158 (96%)	78 (71%)	171 (98%)	18 (22%)	6 (43%)	
Advanced	332 (69%)	196 (84%)	6 (4%)	32 (29%)	3 (2%)	63 (78%)	8 (57%)	
Unknown	160 (25%)	49 (17%)	30 (15%)	40 (27%)	32 (16%)	21 (21%)	12 (46%)	
Covariates								
Age at first birth (years)[b]	26.9 (14.8–46.2)	26.9 (14.8–41.6)	25.8 (16.1–39.5)[c]	27.2 (15.8–46.2)	23.9 (15.5–42.5)[c]	26.5 (16.9–38.4)	28.6 (20.4–41.1)	26.7 (14.6–45.9)
Smoking during pregnancy[d]	94 (21%)[c]	35 (18%)[c]	22 (16%)	35 (31%)[c]	62 (40%)[c]	6 (10%)	2 (12%)	330 (16%)
Family history of breast/ovarian cancer[e]	34 (9%)[c]	21 (12%)[c]	7 (6%)	3 (3%)	10 (8%)	5 (8%)	2 (22%)	81 (5%)
Hormones[f]								
Testosterone (ng/ml)	0.83 (0.79–0.87)[c]	0.76 (0.71–0.82)	0.79 (0.73–0.86)[c]	0.88 (0.78–1.00)[c]	0.88 (0.81–0.97)[c]	0.93 (0.82–1.04)	0.86 (0.67–1.10)	0.76 (0.74–0.78)
Androstenedione (ng/ml)	1.91 (1.83–2.00)[c]	1.77 (1.65–1.91)	1.80 (1.66–1.95)	2.15 (1.90–2.42)[c]	2.05 (1.88–2.23)[c]	2.00 (1.78–2.24)	2.11 (1.64–2.72)	1.73 (1.69–1.78)
17-OHP (ng/ml)	2.29 (2.18–2.40)[c]	2.28 (2.12–2.47)	2.22 (2.04–2.41)[c]	2.30 (2.04–2.60)	2.31 (2.11–2.52)	2.12 (1.88–2.39)	2.08 (1.62–2.68)	2.14 (2.08–2.19)
Progesterone (ng/ml)	23.4 (23.2–24.8)	24.9 (23.5–26.3)	22.2 (20.8–23.6)	24.0 (22.1–26.0)	22.5 (21.1–23.9)	23.7 (21.6–25.9)	22.5 (18.3–27.7)	22.8 (22.4–23.3)
Estradiol (ng/ml)	2.03 (1.92–2.14)	1.87 (1.71–2.04)	1.84 (1.68–2.01)	2.25 (1.97–2.58)	2.01 (1.81–2.24)	2.52 (2.17–2.93)[c]	1.79 (1.34–2.39)	1.88 (1.82–1.94)
SHBG (nmol/l)	169 (161–177)	171 (159–184)	153 (140–167)	145 (130–163)	168 (154–184)	173 (152–197)	167 (133–209)	163 (158–167)

17-OHP, 17-hydroxyprogesterone; SHBG, sex hormone-binding globulin.
[a]Tumor spread data are not available for cases from the Northern Sweden Maternity Cohort. Percentages for localized and advanced tumors are based on tumors with data, whereas percentages for unknown spread are based on histology subgroups.
[b]Data on age at first birth are missing for 15% of subjects from the NSMC.
[c]Indicates that cases and controls are significantly different ($P<0.05$) in the respective subgroup.
[d]Data on smoking during pregnancy are missing for 27% of subjects from the FMC and for 2% of subjects from the NSMC.
[e]Data on family history of breast and/or ovarian cancer are available for 629 cases and 1571 controls from the Finnish Maternity Cohort.
[f]Geometric means and 10th–90th percentile of hormone concentrations (adjusted for study cohort, except for androgens and gestational age).

Table 3 ORs (95% CI) by tumor invasiveness and histology across tertiles of circulating hormone concentrations from the Finnish Maternity Cohort (1983–2009) and the Northern Sweden Maternity Cohort (1975–2011)[a]

		Tertiles				
	Cases/controls	T_1	T_2	T_3	P_{trend}	P_{het}[b]
Testosterone						
All women	1047/2673	Ref.	1.27 (1.06–1.53)	1.56 (1.30–1.87)	<0.0001	
Invasive	640/1609	Ref.	1.18 (0.93–1.49)	1.37 (1.08–1.72)	0.008	0.08
Borderline	407/1064	Ref.	1.45 (1.07–1.96)	1.94 (1.43–2.63)	<0.0001	
Invasive serous	282/713	Ref.	1.11 (0.78–1.57)	0.99 (0.70–1.41)	0.97	0.03
Borderline serous	193/499	Ref.	1.62 (1.06–2.48)	1.87 (1.18–2.96)	0.008	
Invasive mucinous	150/388	Ref.	1.21 (0.74–1.98)	1.79 (1.10–2.90)	0.02	0.76
Borderline mucinous	204/537	Ref.	1.36 (0.88–2.10)	1.97 (1.30–2.99)	0.001	
Endometrioid	101/235	Ref.	1.41 (0.74–2.68)	1.83 (0.97–3.45)	0.06	
Clear cell	26/65	Ref.	0.52 (0.16–1.66)	0.62 (0.21–1.84)	0.36	
Androstenedione						
All women	1049/2677	Ref.	1.03 (0.85–1.24)	1.50 (1.24–1.80)	<0.0001	
Invasive	640/1609	Ref.	0.91 (0.71–1.16)	1.36 (1.08–1.72)	0.004	0.21
Borderline	409/1068	Ref.	1.25 (0.91–1.70)	1.74 (1.29–2.35)	0.0003	
Invasive serous	282/714	Ref.	0.98 (0.69–1.41)	1.25 (0.88–1.77)	0.19	0.72
Borderline serous	193/499	Ref.	1.37 (0.87–2.16)	1.38 (0.88–2.18)	0.18	
Invasive mucinous	150/387	Ref.	0.98 (0.58–1.64)	1.78 (1.09–2.92)	0.01	0.73
Borderline mucinous	206/541	Ref.	1.14 (0.74–1.77)	2.00 (1.32–3.02)	0.001	
Endometrioid	101/235	Ref.	0.71 (0.37–1.38)	0.98 (0.54–1.79)	0.85	
Clear cell	26/65	Ref.	0.90 (0.27–3.06)	1.00 (0.33–3.07)	0.99	
17-OHP						
All women	762/2138	Ref.	1.05 (0.85–1.30)	1.26 (1.01–1.56)	0.04	
Invasive	451/1251	Ref.	0.90 (0.68–1.18)	1.10 (0.83–1.46)	0.48	0.14
Borderline	311/887	Ref.	1.35 (0.96–1.88)	1.53 (1.09–2.15)	0.02	
Invasive serous	198/555	Ref.	0.96 (0.63–1.44)	1.09 (0.72–1.67)	0.69	0.11
Borderline serous	147/418	Ref.	1.56 (0.96–2.55)	1.85 (1.14–2.99)	0.02	
Invasive mucinous	114/321	Ref.	0.68 (0.38–1.21)	1.11 (0.65–1.91)	0.56	0.71
Borderline mucinous	156/445	Ref.	1.25 (0.78–2.00)	1.28 (0.78–2.08)	0.35	
Endometrioid	60/159	Ref.	1.12 (0.53–2.37)	0.84 (0.38–1.85)	0.69	
Clear cell	17/46	Ref.	0.61 (0.15–2.52)	0.52 (0.10–2.76)	0.42	
Progesterone						
All women	761/2136	Ref.	0.88 (0.71–1.08)	0.96 (0.76–1.21)	0.67	
Invasive	451/1252	Ref.	0.90 (0.68–1.19)	0.99 (0.73–1.35)	0.95	0.71
Borderline	310/884	Ref.	0.85 (0.62–1.18)	0.91 (0.63–1.31)	0.56	
Invasive serous	198/555	Ref.	0.98 (0.64–1.48)	1.03 (0.65–1.63)	0.93	0.96
Borderline serous	146/415	Ref.	0.86 (0.53–1.41)	1.05 (0.61–1.82)	0.92	
Invasive mucinous	114/322	Ref.	1.04 (0.59–1.85)	1.11 (0.60–2.06)	0.73	0.47
Borderline mucinous	156/445	Ref.	0.75 (0.48–1.18)	0.83 (0.50–1.37)	0.42	
Endometrioid	60/159	Ref.	0.61 (0.28–1.32)	0.81 (0.34–1.95)	0.59	
Clear cell	17/46	Ref.	0.24 (0.04–1.53)	0.47 (0.10–2.23)	0.43	
Estradiol						
All women	761/2136	Ref.	1.01 (0.81–1.26)	1.24 (0.94–1.62)	0.11	
Invasive	451/1252	Ref.	1.00 (0.75–1.33)	1.26 (0.88–1.79)	0.20	0.88
Borderline	310/884	Ref.	1.03 (0.73–1.46)	1.20 (0.79–1.84)	0.36	
Invasive serous	198/555	Ref.	0.79 (0.51–1.21)	0.98 (0.57–1.69)	0.89	0.78
Borderline serous	146/415	Ref.	0.82 (0.50–1.35)	0.87 (0.46–1.64)	0.64	
Invasive mucinous	114/322	Ref.	1.06 (0.59–1.89)	1.54 (0.77–3.07)	0.21	0.74
Borderline mucinous	156/445	Ref.	1.42 (0.85–2.35)	1.80 (1.00–3.22)	0.04	
Endometrioid	60/159	Ref.	1.41 (0.59–3.41)	2.76 (1.04–7.33)	0.03	
Clear cell	17/46	Ref.	1.93 (0.46–8.08)	1.58 (0.26–9.64)	0.53	
SHBG						
All women	735/2088	Ref.	1.01 (0.81–1.26)	1.14 (0.89–1.46)	0.40	
Invasive	429/1209	Ref.	1.00 (0.75–1.34)	1.11 (0.80–1.53)	0.64	0.79
Borderline	306/879	Ref.	1.03 (0.73–1.45)	1.19 (0.80–1.77)	0.45	
Invasive serous	185/528	Ref.	1.25 (0.81–1.92)	1.02 (0.63–1.67)	0.83	0.90
Borderline serous	143/413	Ref.	0.94 (0.58–1.54)	1.08 (0.59–1.96)	0.85	
Invasive mucinous	113/319	Ref.	0.73 (0.40–1.31)	1.37 (0.72–2.62)	0.56	0.99
Borderline mucinous	155/443	Ref.	1.20 (0.73–1.98)	1.38 (0.79–2.43)	0.27	
Endometrioid	55/150	Ref.	0.88 (0.37–2.10)	1.29 (0.51–3.26)	0.63	
Clear cell	15/43	Ref.	0.91 (0.20–4.24)	1.25 (0.24–6.53)	0.86	

17-OHP, 17-hydroxyprogesterone; OR, odds ratio; SHBG, sex hormone-binding globulin.
[a]Adjusted for gestational age (except for testosterone and androstenedione).
[b]Heterogeneity was tested between invasive and borderline tumors.

http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-14-0282

Published by Bioscientifica Ltd.

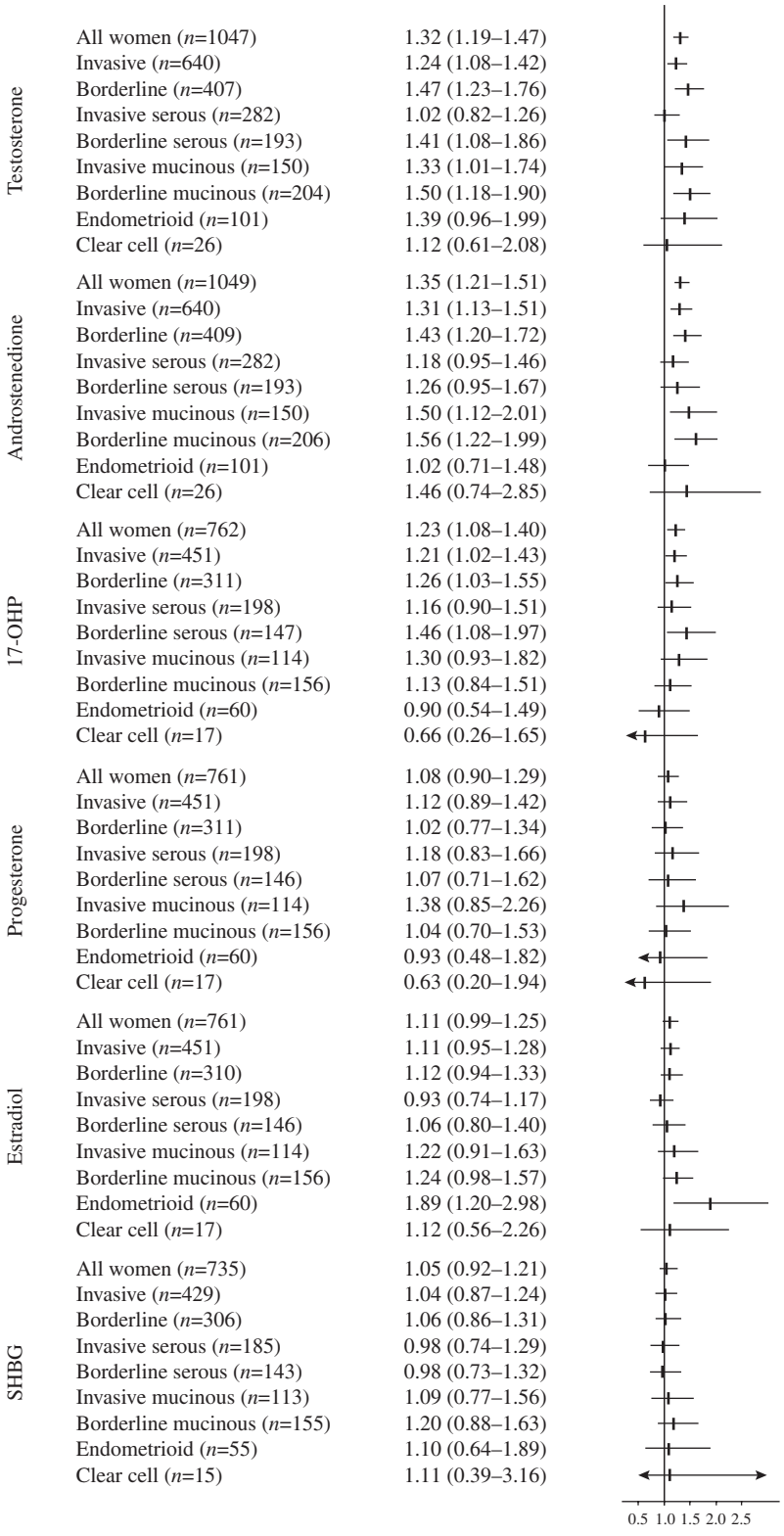

Figure 1

Risk (OR (95% CI)) by tumor histology of epithelial ovarian cancer per unit change in $\log_2$ concentrations of circulating testosterone, androstenedione, 17-hydroxyprogesterone (17-OHP), progesterone, estradiol, and sex hormone-binding globulin (SHBG), adjusted for gestational age (except for androstenedione and testosterone). The number of case–control sets is indicated in parentheses for each subgroup.

http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-14-0282

Published by Bioscientifica Ltd.

1st tertile: 1.87 (1.18–2.96); $P_{trend}=0.008$) and an almost twofold increased risk of invasive and borderline mucinous tumors (3rd vs 1st tertile: 1.79 (1.10–2.90); $P_{trend}=0.02$ and 1.97 (1.30–2.99); $P_{trend}=0.001$ respectively). High androstenedione concentrations were associated with an increased risk of invasive and borderline mucinous tumors (3rd vs 1st tertile: 1.78 (1.09–2.92); $P_{trend}=0.01$ and 2.00 (1.32–3.02); $P_{trend}=0.001$ respectively). High concentrations of 17-OHP were associated with an increased risk of borderline serous tumors (3rd vs 1st tertile: 1.85 (1.14–2.99); $P_{trend}=0.02$). A significantly increased risk was observed for high E_2 concentrations for endometrioid tumors (3rd vs 1st tertile: 2.76 (1.04–7.33); $P_{trend}=0.03$), as well as for borderline mucinous tumors (3rd vs 1st tertile: 1.80 (1.00–3.22); $P_{trend}=0.04$).

We then assessed the effects of mutually adjusting for the evaluated hormones (Supplementary Table 1, see section on supplementary data given at the end of this article). Including androgens or 17-OHP in models evaluating the association of a doubling of progesterone or E_2 concentrations (and *vice versa*) with the risk of EOC did not change the direction or the significance of the observed associations. After adjustment for androstenedione, the effect of a doubling of testosterone concentrations on invasive and borderline mucinous tumors was strongly attenuated and no longer significant (0.77 (0.43–1.36); $P=0.36$ and 1.12 (0.73–1.71); $P=0.60$), whereas the association with endometrioid tumors was strengthened (3.45 (1.62–7.35); $P=0.001$). A doubling of androstenedione was significantly associated with an increased risk of invasive serous tumors (1.67 (1.09–2.55); $P=0.02$) and a decreased risk of endometrioid tumors (0.33 (0.15–0.73); $P=0.006$), after adjusting for testosterone. After adjustment for androgens, a doubling of 17-OHP was no longer significantly associated with the risk of borderline serous tumors (from 46 to 23% increased risk for 17-OHP).

There was no evidence of heterogeneity ($p_{het}>0.05$) by age at blood donation (histology-specific; below/above median) or years between blood donation and diagnosis (histology-specific; below/above median), number of children, or study cohort. Heterogeneity by age at diagnosis (<51 vs ≥51 years, aproxy for menopausal status at diagnosis; Table 4) was observed in endometrioid tumors for a doubling of E_2 concentrations (<51 years: 1.36 (0.84–2.22) and ≥51 years: 14.11 (2.14–93.00); $P_{het}=0.02$), but as only 14 cases were diagnosed at or after the age of 51 years, this result should be regarded with caution. Although heterogeneity was not observed for invasive or invasive mucinous tumors, associations with androgens in women diagnosed at the age of <51 years were stronger than in older women. Heterogeneity by tumor stage at diagnosis (Table 5) was observed in invasive serous tumors for a doubling of circulating testosterone (localized: 1.91 (1.02–3.57) and advanced: 0.88 (0.68–1.14); $P_{het}=0.03$) and SHBG concentrations (localized: 2.38 (1.00–5.65) and advanced: 0.88 (0.61–1.27); $P_{het}=0.04$), but not for any other hormones or EOC subtypes.

Limiting the analyses of androgens to women with information on gestational age at blood sampling did not change risk estimates with the exception of a doubling of androstenedione which was significantly associated with an increased risk of borderline serous tumors (1.50 (1.10–2.05); $P=0.01$). Limiting the analysis to women with lag-time >2 or >3 years, or who provided a blood sample during a full-term pregnancy, did not change the risk estimates. The results did not materially change when only women who donated blood during the last pregnancy before diagnosis or selection as a control were included. Analyses limited to members of the FMC yielded similar results and analyses restricted to the NSMC ($n=109$ cases) were in the same direction but non-significant due to the lack of power.

Discussion

This is the first prospective case-control study, to our knowledge, examining sex steroid concentrations during pregnancy and the subsequent risk of EOC. We observed heterogeneity in the associations between sex steroid hormones with respect to EOC subtypes: higher androgen concentrations were associated with an increased risk of borderline serous and mucinous tumors, whereas higher E_2 was positively associated with the risk of endometrioid tumors. None of the studied hormones were associated with the risk of invasive serous tumors or clear cell tumors. Progesterone and SHBG were not associated with the risk of EOC, regardless of tumor histology and invasiveness.

There are substantial alterations in the secretion, metabolism, and concentrations of circulating hormones in maternal serum during gestation. First and early second trimester androgen concentrations are similar to those in non-pregnant women, with maternal testosterone concentration increasing gradually throughout pregnancy and androstenedione concentrations remaining relatively stable (O'Leary *et al.* 1991, Taylor & Lebovic 2004). During the very first weeks of pregnancy, the corpus luteum secretes progesterone, 17-OHP, and E_2 in increasing quantities. The major site of synthesis for progesterone and E_2 shifts

http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-14-0282

Published by Bioscientifica Ltd.

Table 4 ORs (95% CI) for doubling of circulating hormone concentrations by age at diagnosis from the Finnish Maternity Cohort (1983–2009) and the Northern Sweden Maternity Cohort (1975–2011)[a]

	Age at diagnosis <51 years		Age at diagnosis ≥51 years		
	Ca/Co	OR (95% CI)	Ca/Co	OR (95% CI)	P_{het}
Invasive tumors					
Testosterone	475/1228	1.32 (1.12–1.55)	165/381	1.05 (0.81–1.36)	0.15
Androstenedione	475/1227	1.40 (1.18–1.65)	165/382	1.10 (0.84–1.43)	0.13
17-OHP	357/1004	1.26 (1.04–1.53)	94/247	0.98 (0.66–1.45)	0.26
Progesterone	357/1005	1.14 (0.88–1.48)	94/247	1.04 (0.62–1.75)	0.76
Estradiol	357/1005	1.07 (0.91–1.27)	94/247	1.23 (0.88–1.72)	0.47
SHBG	350/993	1.04 (0.85–1.27)	79/216	1.07 (0.73–1.58)	0.88
Invasive serous tumors					
Testosterone	195/506	1.01 (0.78–1.31)	87/207	1.04 (0.72–1.53)	0.88
Androstenedione	195/506	1.23 (0.94–1.61)	87/208	1.10 (0.77–1.57)	0.63
17-OHP	145/413	1.27 (0.93–1.73)	53/142	0.91 (0.55–1.51)	0.27
Progesterone	145/413	1.25 (0.82–1.88)	53/142	1.01 (0.53–1.92)	0.59
Estradiol	145/413	0.91 (0.69–1.21)	53/142	0.95 (0.64–1.42)	0.86
SHBG	142/407	0.98 (0.71–1.35)	43/121	0.98 (0.53–1.78)	0.98
Invasive mucinous tumors					
Testosterone	128/339	1.46 (1.07–1.99)	22/49	0.94 (0.52–1.68)	0.19
Androstenedione	128/338	1.64 (1.18–2.28)	22/49	1.05 (0.55–2.02)	0.23
17-OHP	103/291	1.29 (0.90–1.84)	11/30	1.33 (0.49–3.64)	0.95
Progesterone	103/292	1.38 (0.82–2.31)	11/30	1.19 (0.24–5.78)	0.86
Estradiol	103/292	1.18 (0.87–1.59)	11/30	2.07 (0.47–9.16)	0.46
SHBG	102/290	1.10 (0.72–1.67)	11/29	1.11 (0.56–2.21)	0.97
Endometrioid tumors					
Testosterone	67/159	1.47 (0.95–2.27)	34/76	1.20 (0.62–2.32)	0.61
Androstenedione	67/159	1.06 (0.69–1.64)	34/76	0.92 (0.45–1.90)	0.74
17-OHP	41/111	0.87 (0.49–1.56)	19/48	1.00 (0.36–2.76)	0.82
Progesterone	41/111	0.86 (0.39–1.88)	19/48	1.17 (0.30–4.52)	0.69
Estradiol	41/111	1.36 (0.84–2.22)	19/48	14.11 (2.14–93.00)	0.02
SHBG	39/108	0.92 (0.49–1.73)	16/42	1.84 (0.61–5.56)	0.28

17-OHP, 17-hydroxyprogesterone; Ca, number of cases; Co, number of controls; OR, odds ratio; P_{het}, test for heterogeneity; SHBG, sex hormone-binding globulin.
[a]Adjusted for gestational age (except for testosterone and androstenedione).

to the placental trophoblasts as the corpus luteum declines (after the fifth week of gestation), and concentrations of these hormones increase further, whereas the concentration of 17-OHP decreases (Taylor & Lebovic 2004).

Support for an involvement of androgens in the pathogenesis of EOC comes from results of *in vitro* studies demonstrating increased cell proliferation of normal ovarian surface epithelial cells after androgen administration (Syed *et al*. 2001, Edmondson *et al*. 2002) and epidemiological studies showing a protective effect of OC use (which reduces circulating androgen levels and ovarian androgen synthesis (Lukanova & Kaaks 2005)) (Wiegratz *et al*. 1995). Women diagnosed with polycystic ovary syndrome, a hyper-androgenic disorder, might be at increased risk but available data are sparse and inconclusive (Schildkraut *et al*. 1996, Olsen *et al*. 2008, Bodmer *et al*. 2011).

However, results from the three largest prospective studies on androgens reported thus far (Rinaldi *et al*. 2007, Tworoger *et al*. 2008) did not show any association with the risk or report any inverse association of androstenedione for invasive serous tumors (Ose *et al*. 2014). In the current study, we observed an increased risk with both studied androgens for borderline serous tumors and mucinous tumors. Our study differs from previous investigations of the association between androgens and EOC in two important ways. First, this study is the first, to our knowledge, with the statistical power to study the risk associations with respect to tumor invasiveness and histology. Most of the previous reports included mainly invasive serous tumors, with negligible numbers of borderline and mucinous tumors. In the current study, we were able to investigate associations with the rarer subtypes, which are usually observed at a younger age (Chen *et al*. 2003). Secondly, blood samples used in our study were collected exclusively from pregnant women (by definition premenopausal); androgen concentrations are higher in premenopausal women when compared with

Published by Bioscientifica Ltd.

Table 5 ORs (95% CI) for doubling of circulating hormone concentrations in invasive tumors by tumor stage from the Finnish Maternity Cohort (1983–2009)[a]

	Local (stage I)		**Advanced** (stages II–IV)		
	Ca/Co	OR (95% CI)	Ca/Co	OR (95% CI)	P_{het}
Invasive tumors					
Testosterone	150/377	1.44 (1.10–1.90)	331/804	1.11 (0.91–1.34)	0.12
Androstenedione	150/377	1.44 (1.09–1.90)	331/805	1.17 (0.96–1.43)	0.24
17-OHP	104/292	1.15 (0.82–1.61)	213/580	1.24 (0.95–1.61)	0.74
Progesterone	104/292	1.18 (0.70–1.98)	213/580	1.27 (0.90–1.81)	0.81
Estradiol	104/292	1.19 (0.87–1.61)	213/580	1.09 (0.87–1.36)	0.66
SHBG	100/284	1.37 (0.90–2.08)	199/563	1.06 (0.79–1.42)	0.32
Invasive serous tumors					
Testosterone	38/96	1.91 (1.02–3.57)	196/481	0.88 (0.68–1.14)	0.03
Androstenedione	38/96	1.60 (0.91–2.80)	196/482	1.09 (0.84–1.40)	0.22
17-OHP	23/67	1.37 (0.75–2.51)	131/360	1.28 (0.90–1.81)	0.84
Progesterone	23/67	1.44 (0.60–3.41)	131/360	1.33 (0.84–2.09)	0.88
Estradiol	23/67	1.41 (0.75–2.67)	131/360	0.89 (0.67–1.18)	0.20
SHBG	23/67	2.38 (1.00–5.65)	120/341	0.88 (0.61–1.27)	0.04
Invasive mucinous tumors					
Testosterone	78/198	1.31 (0.90–1.90)	32/83	1.46 (0.78–2.71)	0.77
Androstenedione	78/198	1.45 (0.98–2.13)	32/83	1.79 (0.91–3.53)	0.59
17-OHP	57/161	1.15 (0.72–1.84)	22/63	1.93 (0.92–4.04)	0.24
Progesterone	57/161	1.73 (0.79–3.81)	22/63	2.96 (0.93–9.43)	0.45
Estradiol	57/161	1.23 (0.82–1.86)	22/63	1.42 (0.65–3.09)	0.75
SHBG	56/160	1.40 (0.77–2.55)	22/63	1.41 (0.51–3.89)	0.98
Endometrioid tumors					
Testosterone	18/45	1.12 (0.49–2.56)	62/139	1.53 (0.97–2.42)	0.52
Androstenedione	18/45	1.05 (0.45–2.46)	62/139	1.05 (0.66–1.65)	0.99
17-OHP	13/35	0.87 (0.32–2.34)	33/85	0.75 (0.38–1.48)	0.82
Progesterone	13/35	0.43 (0.07–2.72)	33/85	0.86 (0.37–2.04)	0.51
Estradiol	13/35	0.71 (0.27–1.86)	33/85	2.15 (1.06–4.36)	0.07
SHBG	11/30	0.65 (0.20–2.07)	31/85	1.39 (0.67–2.85)	0.28

17-OHP, 17-hydroxyprogesterone; Ca, number of cases; Co, number of controls; OR, odds ratio; P_{het}, test for heterogeneity; SHBG, sex hormone-binding globulin.
[a]Adjusted for gestational age (except for testosterone and androstenedione).

postmenopausal women. In previous prospective studies, only 20% (Ose *et al*. 2014) to 42% (Helzlsouer *et al*. 1995) of cases were premenopausal.

It has been proposed that EOC originates via two main pathways of carcinogenesis and can be divided into two subtypes (type I and type II tumors; Kurman & Shih 2011, Lim & Oliva 2013). Type I tumors include low-grade serous and endometrioid carcinoma, clear cell, mucinous, and malignant Brenner tumors that develop slowly and are often diagnosed at an early stage. These tumors are typically confined to one ovary and progress from benign, with increasing degrees of atypia, to non-invasive and then invasive tumors. Thus, borderline ovarian tumors might be the precursors of type I tumors.

Type II tumors include high-grade serous and endometrioid carcinoma, undifferentiated, malignant mixed mesodermal, and transitional cell tumors that are highly aggressive and usually present at an advanced stage. It has been suggested that type II tumors mainly originate from the epithelium outside the ovary and invade the ovary secondarily (Kurman & Shih 2011, Lim & Oliva 2013).

We observed significant positive associations between androgen concentrations and borderline/invasive mucinous, as well as borderline serous subtypes, indicating that androgens may be involved in the development of the slowly growing tumors in the ovaries, whereas they do not appear to influence the risk of the most aggressive cancers, which are probably of extraovarian origin. This hypothesis is also supported by the observation in our study that risk increases for a doubling of testosterone concentrations in invasive serous tumors diagnosed as localized (proxy for type I: 1.91 (1.02–3.57)), but not advanced disease (proxy for type II: 0.88 (0.68–1.14); $P_{het}=0.03$).

Results from experimental studies and a number of indirect observations indicate that elevated progesterone concentrations may be inversely associated with the risk of EOC. Progesterone has been shown to have a potent apoptotic effect on the surface epithelium

Published by Bioscientifica Ltd.

(Rodriguez *et al*. 1998) and to induce cellular senescence of ovarian cancer cells through FOXO1 (Diep *et al*. 2013). Results from epidemiological studies consistently indicate an inverse association of full-term pregnancies, increasing parity, and the use of OCs with EOC (Tsilidis *et al*. 2011, Merritt *et al*. 2013). Owing to its potent apoptotic effect and elevated production during pregnancy, progesterone is the most plausible candidate to mediate a 'washout effect', i.e., elimination from the ovary of cells that have undergone malignant transformation, a hypothesis proposed to explain the greater protective effect of pregnancies completed at an older age (Adami *et al*. 1994). In addition, results from observational studies have indicated that incomplete pregnancies confer less protection than a pregnancy conducted to term (Whittemore *et al*. 1992, Riman *et al*. 2002) or are not associated with the risk (Risch *et al*. 1994).

However, we observed no association between early pregnancy progesterone and the risk of EOC. One explanation for the lack of association could be that only the very high progesterone concentrations as observed during the third trimester are etiologically important, whereas the substantially lower hormone concentrations during the first trimester are not. Results obtained using cell culture models indicate that growth inhibition and apoptosis occur only at very high concentrations ($\geq 10^{-6}$ M; Edmondson *et al*. 2002). In addition, during multiple pregnancies, progesterone concentrations are higher compared with singleton pregnancies and women with a history of multiple births are at a reduced risk to develop non-mucinous EOC (Whiteman *et al*. 2000).

Our finding of a positive association between estrogen concentrations with the risk of endometrioid tumors is novel. During pregnancy, E_2 is synthesized by aromatization of androgens, mainly in the placenta, with androgens derived from the ovary, the maternal adrenal, and also *de novo* synthesis in placental syncytiotrophoblasts (Escobar *et al*. 2011). Elevated estrogen concentrations are consistently associated with an increased risk of endometrial cancer in postmenopausal women (Lukanova *et al*. 2004, Allen *et al*. 2008). Furthermore, endometrial cancer and EOC share similar risk factor profiles, as well as several common genes and pathways that are involved in their molecular pathogenesis (Merritt & Cramer 2010). It has also been reported that patients with endometriosis, which is associated with molecular aberrations that favor increased local production of E_2 (Worley *et al*. 2013), are at an increased risk of developing endometrioid EOC (Pearce *et al*. 2012). Although clear cell tumors may originate in part from endometriosis, we did not find any association between E_2 and clear cell tumors. This finding might be due to the small number of cases in our study ($n=26$), but it is consistent with the hypothesis that clear cell tumors may arise from endometriosis through mechanisms independent of hormonal signaling (Conklin & Gilks 2013).

Our study has a number of strengths. We have presented data from two unique Maternity Cohorts in Finland and Northern Sweden and have conducted the largest prospective study on pre-diagnostic sex steroids and EOC to date (1052 vs 565 cases in the largest previous study by Ose *et al*. (2014)) with detailed analyses by tumor histology. As this study was conducted in a pregnant population, it was easier to measure the progesterone levels, because early pregnancy concentrations do not cycle as observed in non-pregnant premenopausal women. As the population was relatively young (median age at blood draw = 31.5 years), we had sufficient statistical power to assess invasive and borderline tumors. Case and control subjects were tightly matched for age and date at sampling, as well as parity at the index pregnancy, thus controlling for several sources of potential confounding factors (e.g. hormone concentrations differing by parity).

A major limitation of this study is the lack of information on grade and the incomplete data on stage (available for 824 (78%) cases). However, analyses by stage for invasive serous tumors showed the expected heterogeneity. Another limitation is the lack of data on OC use before pregnancy. However, former OC use is unlikely to influence hormone concentrations during pregnancy as steroid levels suppressed during OC use return to normal levels within one cycle after cessation (Mall-Haefeli *et al*. 1983). Although study samples had been stored for a long time (median 21 years) at relatively high temperatures (−25/−20 °C), hormone levels were uncorrelated with time in storage, as has been reported previously (Holl *et al*. 2008), and case and control samples were stored under the same conditions. Another limitation is that we carried out multiple statistical tests to analyze associations with histological subtypes and thus some of our findings could be due to chance.

In summary, we provide evidence that associations of steroids and the risk of EOC vary with respect to tumor histology and invasiveness as we observed i) positive associations of androgens with borderline serous, invasive, and borderline mucinous tumors; ii) positive associations of E_2 with endometrioid, and to a lesser extent with borderline tumors; and iii) no associations with invasive serous tumors. These results support a role of sex steroid hormones in the etiology of EOC arising in the ovaries, but

http://erc.endocrinology-journals.org
DOI: 10.1530/ERC-14-0282

Published by Bioscientifica Ltd.

do not support an association between sex steroids and invasive serous tumors, which are presumed to originate in the fallopian tubes and metastasize to the ovaries. Our findings provide additional evidence that EOC is a heterogeneous disease and indicate that further research on sex steroids and the rarer histological subtypes (endometrioid, clear cell, and mucinous) are necessary.

Supplementary data

This is linked to the online version of the paper at http://dx.doi.org/10.1530/ERC-14-0282.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

This work was supported by the National Cancer Institute at the National Institutes of Health (Grant R01 CA120061) and the Lion's Cancer Foundation at Umeå University, Sweden.

References

Adami HO, Hsieh CC, Lambe M, Trichopoulos D, Leon D, Persson I, Ekbom A & Janson PO 1994 Parity, age at first childbirth, and risk of ovarian cancer. *Lancet* **344** 1250–1254. (doi:10.1016/S0140-6736(94)90749-8)

Allen NE, Key TJ, Dossus L, Rinaldi S, Cust A, Lukanova A, Peeters PH, Onland-Moret NC, Lahmann PH, Berrino F *et al.* 2008 Endogenous sex hormones and endometrial cancer risk in women in the European Prospective Investigation into Cancer and Nutrition (EPIC). *Endocrine-Related Cancer* **15** 485–497. (doi:10.1677/ERC-07-0064)

Auersperg N, Wong AS, Choi KC, Kang SK & Leung PC 2001 Ovarian surface epithelium: biology, endocrinology, and pathology. *Endocrine Reviews* **22** 255–288. (doi:10.1210/edrv.22.2.0422)

Barlow L, Westergren K, Holmberg L & Talback M 2009 The completeness of the Swedish Cancer Register: a sample survey for year 1998. *Acta Oncologica* **48** 27–33. (doi:10.1080/02841860802247664)

Bodmer M, Becker C, Meier C, Jick SS & Meier CR 2011 Use of metformin and the risk of ovarian cancer: a case–control analysis. *Gynecologic Oncology* **123** 200–204. (doi:10.1016/j.ygyno.2011.06.038)

Chen VW, Ruiz B, Killeen JL, Cote TR, Wu XC & Correa CN 2003 Pathology and classification of ovarian tumors. *Cancer* **97** 2631–2642. (doi:10.1002/cncr.11345)

Cleveland WS & Loader C 1996 Smoothing by local regression: principles and methods. In *Statistical Theory and Computational Aspects of Smoothing*, pp 10–49. Eds W Haerdle & MG Schimek. Heidelberg: Physica-Verlag.

Conklin CM & Gilks CB 2013 Differential diagnosis and clinical relevance of ovarian carcinoma subtypes. *Expert Review of Obstetrics & Gynecology* **8** 67–82. (doi:10.1586/eog.12.72)

Cramer DW & Xu H 1995 Epidemiologic evidence for uterine growth factors in the pathogenesis of ovarian cancer. *Annals of Epidemiology* **5** 310–314. (doi:10.1016/1047-2797(94)00098-E)

Diep CH, Charles NJ, Gilks CB, Kalloger SE, Argenta PA & Lange CA 2013 Progesterone receptors induce FOXO1-dependent senescence in ovarian cancer cells. *Cell Cycle* **12** 1433–1449. (doi:10.4161/cc.24550)

Edmondson RJ, Monaghan JM & Davies BR 2002 The human ovarian surface epithelium is an androgen responsive tissue. *British Journal of Cancer* **86** 879–885. (doi:10.1038/sj.bjc.6600154)

Escobar JC, Patel SS, Beshay VE, Suzuki T & Carr BR 2011 The human placenta expresses CYP17 and generates androgens *de novo*. *Journal of Clinical Endocrinology and Metabolism* **96** 1385–1392. (doi:10.1210/jc.2010-2504)

Fathalla MF 1971 Incessant ovulation – a factor in ovarian neoplasia? *Lancet* **2** 163. (doi:10.1016/S0140-6736(71)92335-X)

Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F 2014. Globocan 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11.

Helzlsouer KJ, Alberg AJ, Gordon GB, Longcope C, Bush TL, Hoffman SC & Comstock GW 1995 Serum gonadotropins and steroid hormones and the development of ovarian cancer. *Journal of the American Medical Association* **274** 1926–1930. (doi:10.1001/jama.1995.03530240036037)

Holl K, Lundin E, Kaasila M, Grankvist K, Afanasyeva Y, Hallmans G, Lehtinen M, Pukkala E, Surcel HM, Toniolo P *et al.* 2008 Effect of long-term storage on hormone measurements in samples from pregnant women: the experience of the Finnish Maternity Cohort. *Acta Oncologica* **47** 406–412. (doi:10.1080/02841860701592400)

Kurman RJ & Shih IM 2010 The origin and pathogenesis of epithelial ovarian cancer: a proposed unifying theory. *American Journal of Surgical Pathology* **34** 433–443. (doi:10.1097/PAS.0b013e3181cf3d79)

Kurman RJ & Shih IM 2011 Molecular pathogenesis and extraovarian origin of epithelial ovarian cancer – shifting the paradigm. *Human Pathology* **42** 918–931. (doi:10.1016/j.humpath.2011.03.003)

Lee KR, Tavassoli FA, Prat J, Dietel M, Gersell DJ, Karseladze AI, Hauptmann S, Rutgers J, Russel P, Buckley CH *et al.* 2003 Tumors of the ovary and peritoneum. In *Pathology and Genetics Tumours of the Breast and Female Genital Organs*, pp 113–202. Eds FA Tavassoli & P Devilee. Lyon: IARC Press.

Lim D & Oliva E 2013 Precursors and pathogenesis of ovarian carcinoma. *Pathology* **45** 229–242. (doi:10.1097/PAT.0b013e32835f2264)

Lukanova A & Kaaks R 2005 Endogenous hormones and ovarian cancer: epidemiology and current hypotheses. *Cancer Epidemiology, Biomarkers & Prevention* **14** 98–107.

Lukanova A, Lundin E, Akhmedkhanov A, Micheli A, Rinaldi S, Zeleniuch-Jacquotte A, Lenner P, Muti P, Biessy C, Krogh V *et al.* 2003 Circulating levels of sex steroid hormones and risk of ovarian cancer. *International Journal of Cancer* **104** 636–642. (doi:10.1002/ijc.10990)

Lukanova A, Lundin E, Micheli A, Arslan A, Ferrari P, Rinaldi S, Krogh V, Lenner P, Shore RE, Biessy C *et al.* 2004 Circulating levels of sex steroid hormones and risk of endometrial cancer in postmenopausal women. *International Journal of Cancer* **108** 425–432. (doi:10.1002/ijc.11529)

Mall-Haefeli M, Werner-Zodrow I, Huber PR, Darragh A & Lambe R 1983 Effects of various combined oral contraceptives on sex steroids, gonadotropins and SHBG. *Irish Medical Journal* **76** 266–272.

Merritt MA & Cramer DW 2010 Molecular pathogenesis of endometrial and ovarian cancer. *Cancer Biomarkers* **9** 287–305. (doi:10.3233/CBM-2011-0167)

Merritt MA, De Pari M, Vitonis AF, Titus LJ, Cramer DW & Terry KL 2013 Reproductive characteristics in relation to ovarian cancer risk by histologic pathways. *Human Reproduction* **28** 1406–1417. (doi:10.1093/humrep/des466)

Morch LS, Lokkegaard E, Andreasen AH, Kruger-Kjaer S & Lidegaard O 2009 Hormone therapy and ovarian cancer. *Journal of the American Medical Association* **302** 298–305. (doi:10.1001/jama.2009.1052)

O'Leary P, Boyne P, Flett P, Beilby J & James I 1991 Longitudinal assessment of changes in reproductive hormones during normal pregnancy. *Clinical Chemistry* **37** 667–672.

Olsen CM, Green AC, Nagle CM, Jordan SJ, Whiteman DC, Bain CJ, Webb PM & Australian Cancer Study G & the Australian Ovarian Cancer Study G 2008 Epithelial ovarian cancer: testing the 'androgens hypothesis'. *Endocrine-Related Cancer* **15** 1061–1068. (doi:10.1677/ERC-08-0075)

Published by Bioscientifica Ltd.

Ose J, Fortner RT, Rinaldi S, Schock H, Overvad K, Tjonneland A, Hansen L, Dossus L, Fournier A, Baglietto L *et al.* 2014 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* [in press]. (doi:10.1002/ijc.29000)

Pearce CL, Templeman C, Rossing MA, Lee A, Near AM, Webb PM, Nagle CM, Doherty JA, Cushing-Haugen KL, Wicklund KG *et al.* 2012 Association between endometriosis and risk of histological subtypes of ovarian cancer: a pooled analysis of case–control studies. *Lancet Oncology* **13** 385–394. (doi:10.1016/S1470-2045(11)70404-1)

Prat J 2012 Ovarian carcinomas: five distinct diseases with different origins, genetic alterations, and clinicopathological features. *Virchows Archiv* **460** 237–249. (doi:10.1007/s00428-012-1203-5)

Pukkala E 2011 Nordic biological specimen bank cohorts as basis for studies of cancer causes and control: quality control tools for study cohorts with more than two million sample donors and 130,000 prospective cancers. In *Methods in Biobanking*, pp 61–112. Ed J Dillner. New York, NY: Humana Press.

R Core Team 2014 *Team R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing.

Rannevik G, Jeppsson S, Johnell O, Bjerre B, Laurell-Borulf Y & Svanberg L 1995 A longitudinal study of the perimenopausal transition: altered profiles of steroid and pituitary hormones, SHBG and bone mineral density. *Maturitas* **21** 103–113. (doi:10.1016/0378-5122(94)00869-9)

Riman T, Dickman PW, Nilsson S, Correia N, Nordlinder H, Magnusson CM & Persson IR 2002 Risk factors for invasive epithelial ovarian cancer: results from a Swedish case–control study. *American Journal of Epidemiology* **156** 363–373. (doi:10.1093/aje/kwf048)

Rinaldi S, Dossus L, Lukanova A, Peeters PH, Allen NE, Key T, Bingham S, Khaw KT, Trichopoulos D, Trichopoulou A *et al.* 2007 Endogenous androgens and risk of epithelial ovarian cancer: results from the European Prospective Investigation into Cancer and Nutrition (EPIC). *Cancer Epidemiology, Biomarkers & Prevention* **16** 23–29. (doi:10.1158/1055-9965.EPI-06-0755)

Risch HA 1998 Hormonal etiology of epithelial ovarian cancer, with a hypothesis concerning the role of androgens and progesterone. *Journal of the National Cancer Institute* **90** 1774–1786. (doi:10.1093/jnci/90.23.1774)

Risch HA, Marrett LD & Howe GR 1994 Parity, contraception, infertility, and the risk of epithelial ovarian cancer. *American Journal of Epidemiology* **140** 585–597.

Rodriguez GC, Walmer DK, Cline M, Krigman H, Lessey BA, Whitaker RS, Dodge R & Hughes CL 1998 Effect of progestin on the ovarian epithelium of macaques: cancer prevention through apoptosis? *Journal of the Society for Gynecologic Investigation* **5** 271–276. (doi:10.1016/S1071-5576(98)00017-3)

Schildkraut JM, Schwingl PJ, Bastos E, Evanoff A & Hughes C 1996 Epithelial ovarian cancer risk among women with polycystic ovary syndrome. *Obstetrics and Gynecology* **88** 554–559. (doi:10.1016/0029-7844(96)00226-8)

Speroff L & Marc AF 2005 Regulation of the menstrual cycle. In *Clinical Gynecologic Endocrinology and Infertility*, pp 187–231. Eds L Speroff & AF Marc. Baltimore, MD: Lippincott Williams & Wilkins.

Syed V, Ulinski G, Mok SC, Yiu GK & Ho SM 2001 Expression of gonadotropin receptor and growth responses to key reproductive hormones in normal and malignant human ovarian surface epithelial cells. *Cancer Research* **61** 6768–6776.

Taylor RN & Lebovic DI 2004 The endocrinology of pregnancy. In *Basic & Clinical Endocrinology*, pp 637–657. Eds FS Greenspan & DG Gardner. New York, NY: Lange Medical Books/McGraw-Hill.

Teppo L, Pukkala E & Lehtonen M 1994 Data quality and quality control of a population-based cancer registry. Experience in Finland. *Acta Oncologica* **33** 365–369. (doi:10.3109/02841869409098430)

Thomas F, Renaud F, Benefice E, de Meeus T & Guegan JF 2001 International variability of ages at menarche and menopause: patterns and main determinants. *Human Biology* **73** 271–290. (doi:10.1353/hub.2001.0029)

Trope CG, Kaern J & Davidson B 2012 Borderline ovarian tumours. *Best Practice & Research. Clinical Obstetrics & Gynaecology* **26** 325–336. (doi:10.1016/j.bpobgyn.2011.12.006)

Tsilidis KK, Allen NE, Key TJ, Dossus L, Lukanova A, Bakken K, Lund E, Fournier A, Overvad K, Hansen L *et al.* 2011 Oral contraceptive use and reproductive factors and risk of ovarian cancer in the European Prospective Investigation into Cancer and Nutrition. *British Journal of Cancer* **105** 1436–1442. (doi:10.1038/bjc.2011.371)

Tworoger SS, Lee IM, Buring JE & Hankinson SE 2008 Plasma androgen concentrations and risk of incident ovarian cancer. *American Journal of Epidemiology* **167** 211–218. (doi:10.1093/aje/kwm278)

Whitehead A & Whitehead J 1991 A general parametric approach to the meta-analysis of randomized clinical trials. *Statistics in Medicine* **10** 1665–1677. (doi:10.1002/sim.4780101105)

Whiteman DC, Murphy MF, Cook LS, Cramer DW, Hartge P, Marchbanks PA, Nasca PC, Ness RB, Purdie DM & Risch HA 2000 Multiple births and risk of epithelial ovarian cancer. *Journal of the National Cancer Institute* **92** 1172–1177. (doi:10.1093/jnci/92.14.1172)

Whittemore AS, Harris R & Itnyre J 1992 Characteristics relating to ovarian cancer risk: collaborative analysis of 12 US case–control studies. II. Invasive epithelial ovarian cancers in white women. Collaborative Ovarian Cancer Group. *American Journal of Epidemiology* **136** 1184–1203.

Wiegratz I, Jung-Hoffmann C & Kuhl H 1995 Effect of two oral contraceptives containing ethinylestradiol and gestodene or norgestimate upon androgen parameters and serum binding proteins. *Contraception* **51** 341–346. (doi:10.1016/0010-7824(95)00098-U)

Worley MJ, Welch WR, Berkowitz RS & Ng SW 2013 Endometriosis-associated ovarian cancer: a review of pathogenesis. *International Journal of Molecular Sciences* **14** 5367–5379. (doi:10.3390/ijms14035367)

Received 25 August 2014
Accepted 1 September 2014

Published by Bioscientifica Ltd.