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Reproductive characteristics in relation to ovarian cancer risk by histologic pathways

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STUDY QUESTION: Do reproductive risk factor associations differ across subgroups of invasive epithelial ovarian cancer (EOC) defined by the dualistic model (type I/II) or a histologic pathway-based classification?

SUMMARY ANSWER: Associations with parity, history of endometriosis, tubal ligation and hysterectomy were found to differ in the context of the type I/II and the histologic pathways classification of ovarian cancer.

WHAT IS KNOWN ALREADY: Shared molecular alterations and candidate precursor lesions suggest that tumor histology and grade may be used to classify ovarian tumors into likely etiologic pathways.

DESIGN: This case–control study included 1571 women diagnosed with invasive EOC and 2100 population-based controls that were enrolled from 1992 to 2008. Reproductive risk factors as well as other putative risk factors for ovarian cancer were assessed through in-person interviews.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Eligible cases were diagnosed with incident ovarian cancer, were aged 18 and above and resided in eastern Massachusetts or New Hampshire, USA. Controls were identified through random digit dialing, drivers' license and town resident lists and were frequency matched with the cases based on age and study center.

MAIN RESULTS AND THE ROLE OF CHANCE: We used polytomous logistic regression to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for type I/II EOC or using a pathway-based grouping of histologic subtypes. In multivariate analyses, we observed that having a history of endometriosis (OR = 1.92, 95% CI: 1.36–2.71) increased the risk for a type I tumor. Factors that were strongly inversely associated with risk for a type I tumor included parity (≥3 versus 0 children, OR = 0.15, 95% CI: 0.11–0.21), having a previous tubal ligation (OR = 0.40, 95% CI: 0.26–0.60) and more weakly hysterectomy (OR = 0.71, 95% CI: 0.45–1.13). In analyses of histologic pathways, parity (≥3 versus 0 children, OR = 0.13, 95% CI: 0.10–0.18) and having a previous tubal ligation (OR = 0.41, 95% CI: 0.28–0.60) or hysterectomy (OR = 0.54, 95% CI: 0.34–0.86) were inversely associated with risk of endometrioid/clear cell tumors. Having a history of endometriosis strongly increased the risk for endometrioid/clear cell tumors (OR = 2.41, 95% CI: 1.78–3.26). We did not observe significant differences in the risk associations across these tumor classifications for age at menarche, menstrual cycle length or infertility.

LIMITATIONS, REASONS FOR CAUTION: A potential limitation of this study is that dividing the cases into subgroups may limit the power of these analyses, particularly for the less common tumor types. Since cases were enrolled after their diagnosis, it is possible that the most aggressive cases were not included in the study.

WIDER IMPLICATIONS OF THE FINDINGS: This study provides insights about the role of reproductive factors in relation to risk of pathway-based subgroups of ovarian cancer that with further confirmation may assist with the development of improved strategies for the prevention of these different tumor types.

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Introduction

In epidemiologic studies, important insights about etiologic factors that modify risk for epithelial ovarian cancer (EOC) may be missed if ovarian cancer heterogeneity is not appreciated. EOC includes four major histologic subtypes (serous, endometrioid, clear cell, mucinous) classified based on their morphologic features. Invasive tumors are frequently given a histologic grade that indicates how closely the tumor resembles the normal tissue and may range from well (G1) or moderately differentiated (G2) to poorly differentiated (G3) or undifferentiated (G4). A dualistic model has been proposed which encompasses this variety in histologic subtype and grade as well as variability in molecular alterations and emerging evidence from studies of putative precursor lesions (Shih and Kurman, 2004; Kurman and Shih, 2011). Type I tumors (low-grade serous, low-grade endometrioid, clear cell and mucinous carcinomas) are thought to arise in a stepwise manner from a precursor lesion, generally exhibit lower rates of cell proliferation and a gradual increase in chromosomal instability, tend to have a better prognosis and harbor a variety of somatic mutations [including *KRAS* and *BRAF* (Ichikawa *et al.*, 1994; Cuatrecasas *et al.*, 1998; Gemignani *et al.*, 2003; Singer *et al.*, 2003), *PTEN* (Obata *et al.*, 1998), *CTNNB1* (Moreno-Bueno *et al.*, 2001; Wu *et al.*, 2001), *PIK3CA* (Campbell *et al.*, 2004) and *ARID1A* (Jones *et al.*, 2010; Wiegand *et al.*, 2010)]. In contrast, type II tumors (high-grade serous carcinoma, high-grade endometrioid as well as malignant, mixed mesodermal tumors and undifferentiated carcinomas) tend to develop rapidly, metastasize early and are usually associated with a poor prognosis (Kurman and Shih, 2011). The most common type II tumor (high-grade serous) exhibits nearly ubiquitous p53 mutation and widespread chromosomal instability (Ahmed *et al.*, 2010, The Cancer Genome Atlas Research Network, 2011).

A complementary model subdivides invasive EOC into three histologic 'pathways' (adapted from Jarboe *et al.*, 2008): (i) low-grade serous and mucinous carcinomas with a high frequency of *KRAS* mutations and a presumed origin in the ovarian surface epithelium and its Mullerian inclusion cysts, (ii) endometrioid and clear cell carcinomas with a high prevalence of *ARID1A* mutation (Jones *et al.*, 2010; Wiegand *et al.*, 2010) that are thought to arise from the ovarian surface epithelium and/or endometriosis implanted on the ovary (Somigliana *et al.*, 2006) and (iii) high-grade serous and other/undifferentiated tumors with p53 mutations and multiple candidate cells of origin, including the ovarian surface epithelium/Mullerian inclusion cysts, endometriosis and the distal Fallopian tube epithelium (Jarboe *et al.*, 2008).

Ovarian tumors also can be classified by histology into their likely etiologic pathways. Previous epidemiologic studies have evaluated all of the major histologic subtypes separately (Modugno *et al.*, 2001; Tung *et al.*, 2003; Kurian *et al.*, 2005; Chiaffarino *et al.*, 2007; Gates *et al.*, 2010; Yang *et al.*, 2011) or have highlighted differences between specific histologic subtypes versus all others (particularly mucinous versus non-mucinous tumors) (Risch *et al.*, 1996; Wittenberg *et al.*, 1999; Purdie *et al.*, 2001; Soegaard *et al.*, 2007). To our knowledge, no studies have evaluated risk factor associations in the context of the dualistic model or a pathway-based grouping of histologic subtypes.

We hypothesized that evaluating key reproductive risk factor associations by these subgroups would further characterize these current models of ovarian carcinogenesis and also could identify new mechanisms through which these risk factors influence disease development. Our study objective was to utilize epidemiologic data from three phases of a New England-based case–control (NECC) study of ovarian cancer to evaluate reproductive risk factor associations between subgroups of EOC defined as type I/II or using a histologic pathway-based classification of ovarian cancer.

Materials and Methods

Study population

Details regarding case and control enrollment in the NECC study were described previously (Terry *et al.*, 2005; Harris *et al.*, 2012). Briefly, 3957 women residing in eastern Massachusetts or New Hampshire, USA, with a diagnosis of incident ovarian cancer were identified through hospital tumor boards and statewide cancer registries. Of these, 3083 (78%) cases met eligibility criteria and 2203 (71%) were enrolled. This analysis was restricted to 2076 cases with epithelial tumors of ovarian, primary peritoneal and Fallopian tube origin. Pathology reports were obtained for all cases from the treating hospitals, and details were abstracted by the study pathologist, including histologic subtype and behavior (invasive versus borderline), tumor grade and stage. We examined the four major histologic subtypes of ovarian cancer (serous, mucinous, endometrioid and clear cell). Mixed tumors that were described as predominantly one epithelial type while containing a focal area of another type were classified as the predominant type. We included transitional cell tumors or mixed serous/transitional cell tumors in the serous category (McCluggage, 2008) and mixed endometrioid/clear cell tumors were classified as endometrioid. Other mixed epithelial tumors ($n = 70$), malignant Brenner tumors ($n = 4$), unspecified epithelial ($n = 25$) and undifferentiated tumors ($n = 41$) were classified as 'other/undifferentiated'. Exclusions from this analysis were borderline tumors ($n = 426$), serous tumors of unknown grade ($n = 32$), other/undifferentiated G1 tumors ($n = 12$) and carcinosarcomas (mixed mesodermal Mullerian tumors, $n = 35$) (carcinosarcomas could not be easily categorized into an etiologic pathway) leaving 1571 cases for the current study. In analyses of the type I/II classification, we evaluated 358 type I tumors (49 low-grade serous, 100 low-grade endometrioid/mixed, 95 mucinous and 114 clear cell tumors) and 1108 type II tumors (846 high-grade serous, 221 high-grade endometrioid/mixed and 41 undifferentiated tumors). An additional 105 tumors were excluded from the analyses of typeI/II; these included 6 endometrioid tumors with missing grade, 25 unspecified epithelial, 4 malignant Brenner and 70 tumors classified as other/mixed epithelial.

Controls were identified through random digit dialing, drivers' license lists and town resident lists. Between 1992 and 1997, 420 (72%) and 102 (51%) of the eligible controls identified through random digit dialing and town resident lists, respectively, agreed to participate. From 1998 to 2008, 4366 potential controls were identified of whom 2940 (67%) were eligible; 1362 (46%) declined to participate by phone or by mail via an 'opt-out' postcard and 1578 (54%) were enrolled ($n = 2100$ total). Controls were frequency matched with cases based on age and state of residence. Study participants were interviewed in-person at the time of enrollment about known and putative ovarian cancer risk factors that occurred at least 1 year before diagnosis (for cases) or enrollment (for controls).

Exposure assessment

Reproductive characteristics were evaluated including parity, duration of breastfeeding, age at menarche and natural menopause, menstrual cycle length, use of oral contraceptive (OC) pills and intrauterine devices (IUDs) and having a previous hysterectomy or tubal ligation. The age at

natural menopause is the age at the last menstrual period due to natural causes. We also estimated the lifetime number of ovulatory cycles, calculated as the current age (if premenopausal) or age at last menstrual period minus the age at menarche and the time spent pregnant, breastfeeding or using OCs (Terry et al., 2007). To investigate infertility, participants were asked if they had tried to become pregnant without success, or if they had seen a doctor about having difficulties in getting pregnant or carrying a pregnancy to term. The causes of infertility were assessed and classified as male (defined as a problem with the husband) or female (problem with the participant or problem with both the husband and the participant). Participants were asked whether they had a history of endometriosis, and they were asked to indicate how much pain they usually had with their periods (ranging from none to severe cramps with medication and bed rest required) as a proxy for undiagnosed endometriosis. Family history of ovarian or breast cancer was defined as having a history of the disease in a first-degree relative (mother or sister).

Statistical methods

Polytomous logistic regression (PLR) was used to simultaneously estimate separate odds ratios (ORs) and 95% confidence intervals (95% CIs) for the main effects of the above-defined reproductive factors across (i) type I/II and (ii) histologic pathways of invasive ovarian cancer. The likelihood ratio test was used to calculate a *P*-value for heterogeneity comparing the PLR model in which all of the associations were held constant between the case subgroups to a model that allowed only the association of interest to differ between the case subgroups (Glynn and Rosner, 2004). In other words, the P_{het} indicates whether there were statistically significant differences in the association of interest between the different outcome categories. All multivariable analyses were adjusted for age (continuous), study center (Massachusetts and New Hampshire), study phase (1992–1997, 1998–2003, 2003–2008), number of pregnancies (0, 1, 2, >2), OC use (0, 3 months to <5 years, ≥5 years), family history of ovarian cancer, family history of breast cancer and history of tubal ligation (yes/no). Tests for linear trend were based on the Wald statistic using continuous variables. A *P*-value <0.05 was considered to be statistically significant. Analyses were performed using SAS v9.2 (SAS Institute, Cary, NC, USA) and PLR analyses were performed in Stata (StataCorp LP, College Station, TX, USA).

Ethical approval

Institutional review boards at the Brigham and Women's Hospital and the Dartmouth Medical School approved the study and all participants provided written informed consent.

Results

The study population included 1571 women with invasive EOC and 2100 controls. Cases with high-grade serous/other tumors tended to be older than the other case groups. All cases reported having fewer children, a shorter duration of breastfeeding and OC use and were more likely to report a family history of ovarian or breast cancer when compared with controls (Table I).

Reproductive risk factor associations for type I/II ovarian cancer

We evaluated whether associations with reproductive factors varied in relation to risk of tumors classified as type I (low-grade serous, low-grade endometrioid, clear cell and mucinous tumors) or type II (high-grade serous, high-grade endometrioid and undifferentiated tumors). We identified significant differences in the risk associations for parity, duration of breastfeeding, age at menopause, history of endometriosis or painful periods and history of tubal ligation and hysterectomy between type I and type II tumors ($P_{het} \leq 0.04$, Table II). Parity was most strongly protective for type I tumors and the protective effects increased with additional children (≥3 versus 0 children, OR = 0.15, 95% CI: 0.11–0.21). In contrast, having one child was significantly protective for type II tumors (1 versus 0 children, OR = 0.64, 95% CI: 0.49–0.83) but having additional children (≥3 versus 0 children, OR = 0.44, 95% CI: 0.35–0.55) did not confer the same degree of protection as was seen with type I cases. Breastfeeding was protective for all tumors and a significant trend of increasing protection with a longer lifetime duration of breastfeeding was observed for type II tumors (>19 versus 0 months, OR = 0.47, 95% CI: 0.34–0.64, $P_{trend} = 0.001$) while comparisons within the type I group were limited by the smaller number of cases. In a composite variable that estimates the total number of ovulatory cycles, having a high number of ovulatory cycles increased the risk for all tumors; however, the increased risk was most striking in type II tumors (>431 versus ≤272 cycles, type II, OR = 5.88, 95% CI: 4.33–7.99; type I, OR = 1.83, 95% CI: 1.28–2.62; $P_{het} < 0.001$) (data not shown). Participants who reported a history of endometriosis had an increased risk to develop a type I but not a type II tumor (type I, OR = 1.92, 95% CI: 1.36–2.71). Reports of moderate/severe painful periods were more strongly associated with type I tumors (OR = 1.55, 95% CI: 1.22–1.95) although an increased risk also was observed for type II tumors (OR = 1.20, 95% CI: 1.03–1.41). We observed a suggestive increase in risk of type I tumors with female infertility (OR = 1.35, 95% CI: 0.92–1.97) but not for type II tumors. We observed that tubal ligation reduced the risk of type I tumors (OR = 0.40, 95% CI: 0.26–0.60) but not type II tumors, and in a similar way, there was a non-significant inverse association for participants reporting a previous hysterectomy only for type I tumors (OR = 0.71, 95% CI: 0.45–1.13). In analyses of contraceptive use, IUD use was suggestively protective only for type I tumors (OR = 0.71, 95% CI: 0.49–1.01). OC use was protective for all tumors but the protective effects appeared stronger among type II tumors (≥60 months versus <3 OC use, type II, OR = 0.43, 95% CI: 0.35–0.53; type I, OR = 0.66, 95% CI: 0.49–0.89; $P_{het} = 0.06$). We did not observe any statistically significant differences in the risk associations for age at menarche, menstrual cycle length and infertility (all categories) in comparisons of type I/II tumors.

Reproductive risk associations by histologic pathways

We investigated reproductive characteristics in relation to risk of invasive EOC classified into three histologic pathways: (i) low-grade serous and mucinous carcinomas, (ii) endometrioid and clear cell carcinomas and (iii) high-grade serous and other/undifferentiated tumors. We noted significant heterogeneity between these histologic categories for the same risk associations as in the comparison of type I/II tumors (*P*-values for heterogeneity, $P_{het} \leq 0.04$, Table III) with the exception of breastfeeding, which was no longer statistically significant. Parity was most strongly inversely associated with risk of endometrioid/clear cell tumors (≥3 versus 0 children, OR = 0.13, 95% CI: 0.10–0.18) and low-grade serous/mucinous tumors (≥3 versus 0

Table I Descriptive characteristics of invasive ovarian cancer cases classified by histologic pathways and controls in the NECC study.

Population characteristics	Controls	G1 serous and all mucinous	Endometrioid and clear cell	G2/3 serous, other/ undifferentiated
Participants, *N*	2100	144	441	986
Mean (SD)				
Age (years)[a]	52.3 (12.6)	50.7 (13.4)	50.5 (11.0)	56.6 (10.2)
Parity among parous women	2.7 (1.4)	2.5 (1.3)	2.2 (1.1)	2.6 (1.3)
Duration of breastfeeding (months)[b]	8.6 (14.2)	7.2 (15.6)	5.3 (9.7)	5.6 (11.7)
Duration of OC pill use (years)[c]	5.7 (5.0)	4.9 (4.8)	4.7 (4.6)	4.3 (4.4)
n (%)				
Family history of ovarian cancer	54 (2.6)	5 (3.5)	17 (3.9)	55 (5.6)
Family history of breast cancer	279 (13.3)	20 (13.9)	62 (14.1)	174 (17.7)
Tumor stage[d]				
I	—	94 (65.7)	287 (65.4)	118 (12.0)
II	—	13 (9.1)	62 (14.1)	101 (10.3)
III	—	35 (24.5)	86 (19.6)	713 (72.4)
IV	—	1 (0.7)	4 (0.9)	53 (5.4)
Tumor grade				
1	—	104 (72.2)	101 (22.9)	—
2	—	29 (20.1)	145 (32.9)	139 (14.1)
3	—	7 (4.9)	143 (32.4)	805 (81.6)
Ungraded	—	4 (2.8)	46 (10.4)	34 (3.5)
Missing	—	—	6 (1.4)	8 (0.8)
Tumor histology				
Serous invasive	—	49 (34.0)	—	846 (85.8)
Mucinous	—	95 (66.0)	—	—
Endometrioid/mixed	—	—	327 (74.2)	—
Clear cell	—	—	114 (25.9)	—
Other/undifferentiated	—	—	—	140 (14.2)

[a]Cases and controls were frequency-matched on age.
[b]Total duration of breastfeeding among parous women.
[c]Duration of OC use among ever users (used OCs for ≥3 months).
[d]Numbers may not add up to total due to missing data.

children, OR = 0.28, 95% CI: 0.18–0.44). In contrast, having one child was significantly protective for the high-grade serous/other category (1 child versus 0, OR = 0.68, 95% CI: 0.51–0.90) but having additional children did not confer the same degree of additional protection (≥3 versus 0 children, OR = 0.54, 95% CI: 0.43–0.68) as was seen with the other case groups. Having a high number of ovulatory cycles strongly increased the risk for the high-grade serous/other tumors (>431 versus ≤272 cycles, OR = 6.22, 95% CI: 4.52–8.54) and for endometrioid/clear cell tumors (OR = 3.16, 95% CI: 2.19–4.56) (data not shown). We additionally evaluated the association with the number of ovulatory cycles among 10-year age groups using age-specific quartiles and observed similar results.

Participants who reported a history of endometriosis or painful periods had a strong increased risk for endometrioid/clear cell tumors (endometriosis, OR = 2.41, 95% CI: 1.78–3.26; painful periods, OR = 1.66, 95% CI: 1.34–2.05). We also observed that female infertility was associated with increased risk of endometrioid/clear cell tumors (OR = 1.54, 95% CI: 1.09–2.16) but not any of the other histologic pathways. Reporting a previous tubal ligation or hysterectomy was strongly inversely associated with risk of developing an endometrioid/clear cell tumor (tubal ligation, OR = 0.41, 95% CI: 0.28–0.60; hysterectomy, OR = 0.54, 95% CI: 0.34–0.86). We observed no difference in the risk associations for the duration of breastfeeding, age at menarche, menstrual cycle length, duration of OC use, IUD use and infertility (all categories) across the histologic pathway-based groups ($P_{het} \geq 0.13$). However, due to the small sample size for some of the outcome categories, such as the G1 serous/mucinous, this may have limited the power to detect significant differences in the risk associations across these subgroups.

Reproductive risk factor associations in endometrioid and clear cell tumors

In the dualistic model, endometrioid tumors were separated into low-grade and high-grade categories. We hypothesized that the distinction between low- and high-grade endometrioid and clear cell tumors also

Table II Adjusted ORs to estimate risk of type I/II invasive ovarian cancer for reproductive factors.

Reproductive factors	Controls (*N* = 2100)	Type I (*N* = 358)		Type II (*N* = 1108)		P_{het}
	n (%)	*n* (%)	OR[a] (95% CI)	*n* (%)	OR[a] (95% CI)	
Parity						
Nulliparous	378 (18.0)	160 (44.7)	1.00 (Ref)	287 (25.9)	1.00 (Ref)	<0.001[b]
1	267 (12.7)	53 (14.8)	0.44 (0.31–0.63)	137 (12.4)	0.64 (0.49–0.83)	
2	667 (31.8)	80 (22.4)	0.27 (0.20–0.36)	337 (30.4)	0.63 (0.50–0.78)	
≥3	788 (37.5)	65 (18.2)	0.15 (0.11–0.21)	347 (31.3)	0.44 (0.35–0.55)	
Breastfeeding (parous)						
0 months	699 (40.6)	104 (52.5)	1.00 (Ref)	447 (54.5)	1.00 (Ref)	0.006[b]
>0 to ≤4 months	290 (16.8)	42 (21.2)	1.03 (0.70–1.51)	122 (14.9)	0.69 (0.54–0.89)	
>4 to ≤10 months	242 (14.1)	15 (7.6)	0.43 (0.24–0.76)	98 (11.9)	0.65 (0.50–0.86)	
>10 to ≤19 months	244 (14.2)	13 (6.6)	0.38 (0.21–0.69)	93 (11.3)	0.63 (0.48–0.83)	
>19 months	247 (14.3)	24 (12.1)	0.79 (0.49–1.27)	61 (7.4)	0.47 (0.34–0.64)	
			$P^c_{trend} = 0.09$		$P^c_{trend} = 0.001$	0.85[d]
Age at menarche						
<12 years	423 (20.1)	72 (20.1)	0.99 (0.71–1.38)	246 (22.2)	1.16 (0.94–1.45)	0.65
12 years	572 (27.2)	100 (27.9)	1.00 (Ref)	290 (26.2)	1.00 (Ref)	
>12 years	1105 (52.6)	186 (52.0)	0.98 (0.75–1.28)	572 (51.6)	1.04 (0.87–1.25)	
			$P^c_{trend} = 0.67$		$P^c_{trend} = 0.03$	0.32[d]
Menstrual cycle length[e]						
<28 days	245 (11.7)	47 (13.4)	1.00 (0.70–1.42)	142 (12.9)	1.06 (0.83–1.34)	0.42[b]
28 days	1025 (49.1)	196 (55.7)	1.00 (Ref)	561 (51.0)	1.00 (Ref)	
>28 to ≤30 days	497 (23.8)	69 (19.6)	0.73 (0.54–0.99)	267 (24.3)	0.99 (0.82–1.20)	
>30 days	170 (8.2)	19 (5.4)	0.68 (0.41–1.13)	66 (6.0)	0.83 (0.61–1.13)	
Irregular cycles	149 (7.1)	21 (6.0)	0.76 (0.47–1.24)	65 (5.9)	0.82 (0.60–1.13)	
			$P^c_{trend} = 0.05$		$P^c_{trend} = 0.15$	0.32[d]
Age at natural menopause[f]						
<45 years	32 (2.9)	9 (5.7)	1.76 (0.77–4.00)	21 (3.0)	0.96 (0.51–1.81)	0.02[b]
45 to <50 years	58 (5.2)	11 (6.9)	1.25 (0.60–2.61)	49 (7.0)	1.30 (0.80–2.13)	
50 to <53 years	991 (88.7)	129 (81.1)	1.00 (Ref)	600 (85.6)	1.00 (Ref)	
≥53 years	36 (3.2)	10 (6.3)	1.99 (0.91–4.36)	31 (4.4)	1.44 (0.81–2.56)	
			$P^c_{trend} = 0.67$		$P^c_{trend} = 0.43$	0.007[d]
OC pill use						
Never or <3 months	766 (36.5)	161 (45.0)	1.00 (Ref)	581 (52.4)	1.00 (Ref)	0.06[b]
3 to <12 months	161 (7.7)	33 (9.2)	1.14 (0.75–1.73)	95 (8.6)	0.91 (0.68–1.21)	
12 to <24 months	164 (7.8)	25 (7.0)	0.81 (0.51–1.28)	92 (8.3)	0.82 (0.62–1.10)	
24 to <60 months	378 (18.0)	58 (16.2)	0.85 (0.61–1.18)	149 (13.5)	0.60 (0.48–0.76)	
≥60 months	631 (30.1)	81 (22.6)	0.66 (0.49–0.89)	191 (17.2)	0.43 (0.35–0.53)	
Including non-users			$P^c_{trend} = 0.007$		$P^c_{trend} = <0.001$	0.003[d]
IUD use	353 (16.8)	39 (10.9)	0.71 (0.49–1.01)	164 (14.8)	1.00 (0.81–1.24)	0.06[b]
Infertility						
None	1664 (79.2)	274 (76.5)	1.00 (Ref)	879 (79.3)	1.00 (Ref)	0.53[b]
Male	41 (2.0)	10 (2.8)	1.24 (0.60–2.55)	22 (2.0)	0.85 (0.49–1.47)	
Female	161 (7.7)	38 (10.6)	1.35 (0.92–1.97)	95 (8.6)	1.05 (0.80–1.38)	
Cause not found	234 (11.1)	36 (10.1)	0.89 (0.61–1.30)	112 (10.1)	0.87 (0.67–1.11)	
Endometriosis	165 (7.9)	51 (14.3)	1.92 (1.36–2.71)	95 (8.6)	1.09 (0.83–1.43)	0.003[b]
Painful periods[e]	677 (32.4)	153 (43.3)	1.55 (1.22–1.95)	413 (37.3)	1.20 (1.03–1.41)	0.04[b]
Hysterectomy[g]	183 (8.7)	23 (6.4)	0.71 (0.45–1.13)	111 (10.0)	1.16 (0.89–1.51)	0.03[b]

Continued

Table II *Continued*

Reproductive factors	Controls (*N* = 2100)	Type I (*N* = 358)		Type II (*N* = 1108)		P_{het}
	n (%)	*n* (%)	OR[a] (95% CI)	*n* (%)	OR[a] (95% CI)	
Tubal ligation	419 (20.0)	26 (7.3)	0.40 (0.26–0.60)	168 (15.2)	0.91 (0.74–1.12)	<0.001[b]

[a]Models were adjusted for age (continuous), study center (Massachusetts, New Hampshire), study phase (1992–1997, 1998–2003, 2003–2008), parity (0, 1, 2, >2), OC pill use (0, 3 months to <5 years, ≥ 5 years), family history of ovarian cancer (yes/no), family history of breast cancer (yes/no) and tubal ligation (yes/no) unless noted otherwise.
[b]The *P*-value for heterogeneity (P_{het}) is from the likelihood-ratio test that compares a model with the same estimate for the association with the exposure of interest (e.g. categories of parity) across type I/II categories to a model, which allows the association of interest to vary across type I/II categories. It indicates if there were statistically significant differences in the association of interest between the different outcome categories.
[c]The P_{trend} is from the Wald statistic using a continuous variable.
[d]The P_{het} is from the likelihood-ratio test that compares a model with the same estimate for the association with the exposure of interest (trend variable) across type I/II categories to a model that allows the exposure of interest to vary across type I/II categories.
[e]Numbers may not add up to total due to missing data.
[f]Analyses were restricted to post-menopausal women and were additionally adjusted for an indicator if a participant had not had a natural menopause.
[g]Models were additionally adjusted for ever use of post-menopausal hormones (yes/no).

might be paralleled by differences in reproductive risk factors. Indeed, we observed statistically significant differences in the risk associations for parity and tubal ligation across these categories of endometrioid and clear cell tumors ($P_{het} \leq 0.03$, Table IV). Having at least one child conferred strong protection for clear cell tumors (1 versus 0 children, OR = 0.18, 95% CI: 0.08–0.38) and these protective effects persisted with additional children. Parity also was inversely associated with risk for both low-grade and high-grade endometrioid tumors and having more children further increased the protective effects (≥3 versus 0 children, low-grade endometrioid, OR = 0.10, 95% CI: 0.05–0.20; high-grade endometrioid, OR = 0.23, 95% CI: 0.15–0.35). We evaluated whether additional adjustment for female infertility altered the association with parity; however, this did not substantially change the risk estimates (data not shown). Having a higher number of ovulatory cycles increased the risk for all tumors; however, we observed a striking increased risk for high-grade endometrioid (>431 versus ≤272 cycles, OR = 12.7, 95% CI: 6.9–23.3) and clear cell tumors (OR = 8.1, 95% CI: 4.3–15.4) (data not shown). Tubal ligation was protective for all endometrioid/clear cell tumors; however, the differences between the subgroups were difficult to evaluate due to the small numbers. As expected, we observed that a history of endometriosis increased the risk for all endometrioid/clear cell tumor groups with the greatest increase in risk observed for the clear cell tumors (OR = 3.54, 95% CI: 2.20–5.70). We did not observe statistically significant differences in the risk associations for breastfeeding, age at menarche, menstrual cycle length, OC pill or IUD use, infertility, endometriosis, painful periods or hysterectomy but these analyses were limited by the smaller number of cases for these comparisons.

Discussion

EOC is an extremely heterogeneous disease that exhibits differences in tumor histology and grade, molecular alterations and putative cells of origin; hence, the type I/II classification (Kurman and Shih, 2011) and a pathway-based grouping of histologic subtypes (Jarboe *et al.*, 2008) have been proposed to better understand ovarian tumor heterogeneity. We hypothesized that important differences in risk factor associations could be missed if these different models of ovarian carcinogenesis are not considered. We utilized epidemiologic data from three phases of a NECC study (including 1571 cases and 2100 controls) to evaluate reproductive risk factor associations and found differences in the associations for parity, number of ovulatory cycles, history of endometriosis and tubal ligation and hysterectomy across these different classifications of ovarian cancer.

Parity is an established protective factor for ovarian cancer with the greatest reduction in risk with the first pregnancy and additional risk reduction with each subsequent pregnancy (Whittemore *et al.*, 1992). In the current study, we made similar observations for endometrioid tumors, low-grade serous and mucinous invasive tumors, while in other subgroups (type II/high-grade serous and clear cell tumors) having just one child conferred strong protection while having additional children did not substantially alter the risk estimates. The protection conferred by pregnancy has been attributed to various mechanisms including anovulation (Fathalla, 1971), reduced gonadotrophin secretion (Cramer and Welch, 1983) and higher levels of progesterone (Risch, 1998). However, we hypothesize that the strong protective effect for the first pregnancy, particularly for type II/high-grade serous and clear cell tumors, is consistent with the mechanism where pregnancy may clear away cells that have accumulated somatic mutations over time and/or have already undergone malignant transformation (Adami *et al.*, 1994), possibly acting through hormonal changes that occur during pregnancy such as increased levels of progesterone, which may induce apoptosis (Risch, 1998; Rodriguez *et al.*, 1998; Lambe *et al.*, 1999; Riman *et al.*, 2004; Lukanova and Kaaks, 2005; Baik *et al.*, 2007). In contrast, each subsequent pregnancy would be expected to induce a similar cell clearance; however, the cell population remaining after the first pregnancy would have comparatively less time to accumulate mutations. On the other hand, we observed an additional risk reduction with each subsequent pregnancy for all endometrioid tumors and the low-grade serous/mucinous subgroup. In regards to the cell clearance hypothesis, Adami *et al.* (1994) suggested that elimination of the initiated cell should have an effect that diminishes with time. However, in the current study, we did not observe a change in the risk estimates when evaluating the time since the last birth (data not shown). Nevertheless, based on the reported results related to the number of pregnancies, it remains to be determined how pregnancy and/or its associated hormones may have an additive effect on reducing ovarian cancer risk for the endometrioid, low-grade serous and mucinous invasive tumors.

Table III ORs to estimate risk of invasive ovarian cancer by histologic pathways for reproductive factors.

Reproductive factors	Controls (*N* = 2100)	G1 serous/mucinous (*N* = 144)		Endometrioid/clear cell (*N* = 441)		G2/3 serous, other/ undifferentiated (*N* = 986)		P_{het}
	n (%)	*n* (%)	OR[a] (95% CI)	*n* (%)	OR[a] (95% CI)	*n* (%)	OR[a] (95% CI)	
Parity								
Nulliparous	378 (18.0)	48 (33.3)	1.00 (Ref)	198 (44.9)	1.00 (Ref)	229 (23.2)	1.00 (Ref)	<0.001[b]
1	267 (12.7)	23 (16.0)	0.64 (0.38–1.09)	65 (14.7)	0.44 (0.32–0.61)	116 (11.8)	0.68 (0.51–0.90)	
2	667 (31.8)	36 (25.0)	0.40 (0.25–0.63)	106 (24.0)	0.28 (0.22–0.37)	299 (30.3)	0.69 (0.55–0.87)	
≥3	788 (37.5)	37 (25.7)	0.28 (0.18–0.44)	72 (16.3)	0.13 (0.10–0.18)	342 (34.7)	0.54 (0.43–0.68)	
Breastfeeding (parous)								
0 months	699 (40.6)	43 (44.8)	1.00 (Ref)	135 (55.6)	1.00 (Ref)	418 (55.2)	1.00 (Ref)	0.13[b]
>0 to ≤4 months	290 (16.8)	24 (25.0)	1.41 (0.84–2.38)	37 (15.2)	0.69 (0.47–1.03)	114 (15.1)	0.69 (0.53–0.89)	
>4 to ≤10 months	242 (14.0)	8 (8.3)	0.55 (0.26–1.20)	22 (9.1)	0.48 (0.30–0.78)	94 (12.4)	0.67 (0.51–0.88)	
>10 to ≤19 months	244 (14.2)	9 (9.4)	0.64 (0.30–1.33)	28 (11.5)	0.63 (0.41–0.98)	76 (10.0)	0.55 (0.41–0.74)	
>19 months	247 (14.3)	12 (12.5)	0.95 (0.49–1.83)	21 (8.6)	0.53 (0.32–0.86)	55 (7.3)	0.45 (0.32–0.62)	
			P^c_{trend} = 0.70		P^c_{trend} = 0.009		P_{trend}<0.001	0.34[d]
Age at menarche								
<12 years	423 (20.1)	24 (16.7)	0.89 (0.52–1.51)	99 (22.5)	1.06 (0.79–1.42)	224 (22.7)	1.20 (0.95–1.50)	0.27[b]
12 years	572 (27.2)	37 (25.7)	1.00 (Ref)	128 (29.0)	1.00 (Ref)	256 (26.0)	1.00 (Ref)	
>12 years	1105 (52.6)	83 (57.6)	1.18 (0.79–1.76)	214 (48.5)	0.88 (0.69–1.12)	506 (51.3)	1.04 (0.86–1.25)	
			P^c_{trend} = 0.24		P^c_{trend} = 0.03		P^c_{trend} = 0.02	0.06[d]
Menstrual cycle length[e]								
<28 days	245 (11.7)	18 (12.7)	0.95 (0.56–1.63)	70 (16.1)	1.28 (0.94–1.75)	113 (11.5)	0.92 (0.72–1.19)	0.25[b]
28 days	1025 (49.1)	79 (55.6)	1.00 (Ref)	228 (52.3)	1.00 (Ref)	511 (52.2)	1.00 (Ref)	
>28 to ≤30 days	497 (23.8)	30 (21.1)	0.79 (0.51–1.22)	85 (19.5)	0.77 (0.59–1.02)	238 (24.3)	0.97 (0.79–1.17)	
>30 days	170 (8.2)	5 (3.5)	0.44 (0.18–1.11)	27 (6.2)	0.83 (0.54–1.28)	60 (6.1)	0.82 (0.60–1.13)	
Irregular cycles	149 (7.1)	10 (7.0)	0.91 (0.46–1.80)	26 (6.0)	0.82 (0.52–1.28)	57 (5.8)	0.80 (0.58–1.12)	
			P^c_{trend} = 0.10		P^c_{trend} = 0.007		P^c_{trend} = 0.49	0.07[d]
Age at menopause[f]								
<45 years	32 (2.9)	3 (4.2)	1.43 (0.41–4.99)	6 (3.1)	0.93 (0.37–2.38)	22 (3.3)	1.11 (0.59–2.09)	0.045[b]
45 to <50 years	58 (5.2)	7 (9.9)	1.93 (0.80–4.65)	13 (6.7)	1.17 (0.59–2.33)	42 (6.4)	1.23 (0.74–2.04)	
50 to <53 years	991 (88.7)	56 (78.9)	1.00 (Ref)	169 (86.7)	1.00 (Ref)	567 (85.8)	1.00 (Ref)	
≥53 years	36 (3.2)	5 (7.0)	2.40 (0.87–6.66)	7 (3.6)	1.10 (0.46–2.65)	30 (4.5)	1.54 (0.86–2.74)	
			P^c_{trend} = 0.68		P^c_{trend} = 0.49		P^c_{trend} = 0.59	0.74[d]
OC pill use								
Never or <3 months	766 (36.5)	63 (43.8)	1.00 (Ref)	210 (47.6)	1.00 (Ref)	525 (53.3)	1.00 (Ref)	0.16[b]
3 to <12 months	161 (7.7)	15 (10.4)	1.33 (0.73–2.40)	39 (8.8)	1.03 (0.70–1.53)	81 (8.2)	0.86 (0.64–1.16)	
12 to <24 months	164 (7.8)	12 (8.3)	0.99 (0.52–1.89)	30 (6.8)	0.74 (0.49–1.14)	86 (8.7)	0.85 (0.64–1.15)	
24 to <60 months	378 (18.0)	25 (17.4)	0.93 (0.57–1.51)	68 (15.4)	0.76 (0.56–1.03)	132 (13.4)	0.59 (0.46–0.75)	
≥60 months	631 (30.1)	29 (20.1)	0.60 (0.38–0.96)	94 (21.3)	0.59 (0.45–0.77)	162 (16.4)	0.40 (0.33–0.50)	
			P^c_{trend} = 0.05		P^c_{trend}<0.001		P^c_{trend} < 0.001	0.02[d]
IUD use	353 (16.8)	19 (13.2)	0.88 (0.53–1.45)	53 (12.0)	0.79 (0.58–1.08)	146 (14.8)	1.00 (0.81–1.25)	0.35[b]
Infertility								
None	1664 (79.2)	115 (79.9)	1.00 (Ref)	335 (76.0)	1.00 (Ref)	785 (79.6)	1.00 (Ref)	0.13[b]
Male	41 (2.0)	6 (4.2)	1.79 (0.74–4.36)	6 (1.4)	0.61 (0.26–1.48)	22 (2.2)	0.96 (0.56–1.66)	
Female	161 (7.7)	11 (7.6)	0.93 (0.49–1.77)	53 (12.0)	1.54 (1.09–2.16)	80 (8.1)	0.99 (0.74–1.32)	
Cause not found	234 (11.1)	12 (8.3)	0.71 (0.39–1.31)	47 (10.7)	0.96 (0.68–1.35)	99 (10.0)	0.86 (0.66–1.11)	
Endometriosis	165 (7.9)	8 (5.6)	0.68 (0.33–1.42)	76 (17.2)	2.41 (1.78–3.26)	72 (7.3)	0.91 (0.68–1.23)	<0.001[b]

Continued

Table III *Continued*

Reproductive factors	Controls (*N* = 2100)	G1 serous/mucinous (*N* = 144)		Endometrioid/clear cell (*N* = 441)		G2/3 serous, other/ undifferentiated (*N* = 986)		P_{het}
	n (%)	*n* (%)	OR[a] (95% CI)	*n* (%)	OR[a] (95% CI)	*n* (%)	OR[a] (95% CI)	
Painful periods[e]	677 (32.4)	57 (40.4)	1.37 (0.96–1.95)	198 (45.1)	1.66 (1.34–2.05)	357 (36.2)	1.15 (0.97–1.35)	0.007[b]
Hysterectomy[g]	183 (8.7)	9 (6.3)	0.69 (0.34–1.38)	22 (5.0)	0.54 (0.34–0.86)	113 (11.5)	1.33 (1.03–1.73)	0.0001[b]
Tubal ligation	419 (20.0)	14 (9.7)	0.55 (0.31–0.96)	33 (7.5)	0.41 (0.28–0.60)	163 (16.5)	1.00 (0.81–1.24)	<0.001[b]

[a]Models were adjusted for age (continuous), study center (Massachusetts, New Hampshire, USA), study phase (1992–1997, 1998–2003, 2003–2008), parity (0, 1, 2, >2), OC pill use (0, 3 months to <5 years, ≥5 years), family history of ovarian cancer (yes/no), family history of breast cancer (yes/no) and tubal ligation (yes/no).
[b]The *P*-value for heterogeneity (P_{het}) is from the likelihood-ratio test that compares a model with the same estimate for the association with the exposure of interest (e.g. categories of parity) across the histologic categories to a model that allows the association of interest to vary across the histologic categories. It indicates if there were statistically significant differences in the association of interest between the different outcome categories.
[c]The P_{trend} is based on the Wald statistic using a continuous variable.
[d]The P_{het} is from the likelihood-ratio test that compares a model with the same estimate for the association with the exposure of interest (trend variable) across the histologic categories to a model that allows the exposure of interest to vary across the histologic categories.
[e]Numbers may not add up to total due to missing data.
[f]Analyses were restricted to post-menopausal women and were additionally adjusted for an indicator if a participant had not had a natural menopause.
[g]Models were additionally adjusted for ever use of post-menopausal hormones (yes/no).

We and others have found that a higher number of ovulatory cycles is associated with an increasing risk for ovarian cancer (Moorman *et al.*, 2002; Purdie *et al.*, 2003; Tung *et al.*, 2005; Terry *et al.*, 2007). In the current study, we identified a strong increased risk for the type II/high-grade serous tumors. The incessant ovulation hypothesis proposed that ovarian cancer originated in the ovarian surface epithelium through repeated ovulation and the associated repair of the ovulatory wound (Fathalla, 1971). However, in the case of high-grade serous carcinoma, there is strong evidence that a subset of these tumors may originate in the Fallopian tube epithelium. Such evidence includes the observation of dysplastic changes in normal Fallopian tubes from women predisposed to ovarian cancer (Piek *et al.*, 2001), the subsequent identification of putative cancer precursor lesions with mutated p53 in the Fallopian tube fimbria epithelium even among patients without a family history (Lee *et al.*, 2007) and findings of conserved p53 mutations in candidate tubal precursor lesions and matched tumors from the same patients (Kindelberger *et al.*, 2007). It is therefore necessary to extend the incessant ovulation hypothesis to understand the influence of ovulation on non-ovarian tissues. A recent study investigated how processes linked to ovulation influenced tubal cells from mice and baboons, and they found that ovulation appeared to affect the Fallopian tube epithelium by inducing DNA damage and stimulating macrophage infiltration (King *et al.*, 2011). Further studies are needed to evaluate the influence of processes linked to ovulation on human tubal epithelium.

In the case of endometrioid and clear cell ovarian carcinomas, it is thought that these tumors could arise in ectopic uterine endometrium (endometriosis) implanted on the ovary based on the observations of shared mutations in *ARID1A* between the tumor and contiguous atypical endometriosis (Wiegand *et al.*, 2010; Yamamoto *et al.*, 2012). However, not all endometrioid and clear cell tumors show loss of *ARID1A*, and the precise cell of origin of endometriosis is controversial (Bulun, 2009). Nevertheless, in the current study and in a recent pooled analysis of 13 case–control studies (including the NECC study), it was shown that endometriosis increases the risk of endometrioid and clear cell tumors (Pearce *et al.*, 2012). We also observed a striking increased risk for clear cell and high-grade endometrioid tumors with a higher number of ovulatory cycles. In this example, it may be most relevant to equate 'incessant ovulation' with 'incessant menstruation' involving the repeated disruption and regrowth of the uterine lining (Merritt and Cramer, 2010). Thus, factors that accompany ovulation (DNA damage, inflammation) may be involved in the earliest stages of ovarian carcinogenesis. Alternatively, since ovulatory years is a composite variable and it has been shown that pregnancy and OC use have stronger protective effects for ovarian cancer than other anovulatory factors (e.g. see Pelucchi *et al.*, 2007), it is also possible that pregnancy and OC use, rather than the process of ovulation itself, may be driving this association.

We evaluated prior gynecologic surgeries (tubal ligation and hysterectomy) in relation to ovarian cancer risk and found that having a tubal ligation was protective for type I tumors, and specifically for the low-grade serous/mucinous and endometrioid/clear cell histologic pathways, while hysterectomy was inversely associated with risk of endometrioid/clear cell tumors. The strong inverse association for tubal ligation with risk of endometrioid tumors is consistent with two meta-analyses (both included the NECC study) (Cibula *et al.*, 2011; Rice *et al.*, 2012). In contrast, we found no evidence that tubal ligation or hysterectomy were protective for type II/high-grade serous tumors, although this result is not supported by the finding of a 27% risk reduction with tubal ligation for serous invasive cancers (Cibula *et al.*, 2011). Fewer studies have investigated hysterectomy in relation to risk of specific histologic subtypes of EOC. Consistent with our findings, the Nurses' Health Study found that hysterectomy was more strongly inversely associated with endometrioid cancers (Gates *et al.*, 2010). However, in an Australian case–control study, hysterectomy did not reduce risk for serous invasive (Jordan *et al.*, 2008), endometrioid or clear cell tumors (Nagle *et al.*, 2008) and a case–control study in the Delaware Valley, USA, did not observe any significant differences in the risk associations with hysterectomy across the histologic subtypes (Modugno *et al.*, 2001). Findings that

Table IV ORs to estimate risk of G1 or G2/3 endometrioid and clear cell ovarian cancer for reproductive factors.

Reproductive factors	Controls (*N* = 2100)	G1 endometrioid (*N* = 100)		G2/3 endometrioid (*N* = 221)		Clear cell (*N* = 114)		P_{het}
	n (%)	*n* (%)	OR[a] (95% CI)	*n* (%)	OR[a] (95% CI)	*n* (%)	OR[a] (95% CI)	
Parity								
Nulliparous	378 (18.0)	48 (48.0)	1.00 (Ref)	85 (38.5)	1.00 (Ref)	64 (56.1)	1.00 (Ref)	0.003[b]
1	267 (12.7)	22 (22.0)	0.65 (0.38–1.11)	33 (14.9)	0.55 (0.36–0.86)	8 (7.0)	0.18 (0.08–0.38)	
2	667 (31.8)	20 (20.0)	0.26 (0.15–0.44)	62 (28.1)	0.45 (0.31–0.65)	24 (21.1)	0.23 (0.14–0.38)	
≥3	788 (37.5)	10 (10.0)	0.10 (0.05–0.20)	41 (18.6)	0.23 (0.15–0.35)	18 (15.8)	0.13 (0.08–0.23)	
Breastfeeding (parous)								
None	699 (40.6)	30 (57.7)	1.00 (Ref)	70 (51.5)	1.00 (Ref)	31 (62.0)	1.00 (Ref)	0.66[b]
<12 months	567 (32.9)	14 (26.9)	0.50 (0.26–0.97)	36 (26.5)	0.56 (0.36–0.86)	11 (22.0)	0.38 (0.19–0.78)	
≥12 months	456 (26.5)	8 (15.4)	0.40 (0.18–0.89)	30 (22.1)	0.64 (0.40–1.02)	8 (16.0)	0.39 (0.17–0.86)	
			$P^c_{trend} = 0.11$		$P^c_{trend} = 0.057$		$P^c_{trend} = 0.04$	0.52[d]
Age at menarche								
<12 years	423 (20.1)	22 (22.0)	0.99 (0.56–1.75)	50 (22.6)	1.11 (0.74–1.66)	26 (22.8)	1.13 (0.66–1.95)	0.99[b]
12 years	572 (27.2)	31 (31.0)	1.00 (Ref)	63 (28.5)	1.00 (Ref)	32 (28.1)	1.00 (Ref)	
>12 years	1105 (52.6)	47 (47.0)	0.84 (0.53–1.35)	108 (48.87)	0.95 (0.68–1.34)	56 (49.1)	0.97 (0.62–1.53)	
			$P^c_{trend} = 0.30$		$P^c_{trend} = 0.21$		$P^c_{trend} = 0.48$	0.96[d]
Menstrual cycle length[e]								
<28 days	245 (11.7)	10 (10.3)	0.76 (0.38–1.52)	41 (18.6)	1.53 (1.03–2.29)	19 (16.8)	1.23 (0.72–2.12)	0.45[b]
28 days	1025 (49.1)	54 (55.7)	1.00 (Ref)	109 (49.3)	1.00 (Ref)	63 (55.8)	1.00 (Ref)	
>28 days	667 (32.0)	26 (26.8)	0.83 (0.51–1.34)	57 (25.8)	0.90 (0.63–1.27)	27 (23.9)	0.73 (0.46–1.18)	
Irregular cycles	149 (7.1)	7 (7.2)	1.01 (0.44–2.28)	14 (6.3)	1.00 (0.55–1.82)	4 (3.5)	0.49 (0.17–1.39)	
			$P^c_{trend} = 0.99$		$P^c_{trend} = 0.02$		$P^c_{trend} = 0.17$	0.34[d]
OC pill use								
Never or <3 months	766 (36.5)	41 (41.0)	1.00 (Ref)	108 (48.9)	1.00 (Ref)	57 (50.0)	1.00 (Ref)	0.32[b]
3 to <12 months	161 (7.7)	6 (6.0)	0.76 (0.31–1.85)	20 (9.1)	0.96 (0.57–1.63)	12 (10.5)	1.10 (0.57–2.12)	
12 to <24 months	164 (7.8)	7 (7.0)	0.77 (0.34–1.77)	16 (7.2)	0.67 (0.38–1.18)	6 (5.3)	0.47 (0.20–1.13)	
24 to <60 months	378 (18.0)	14 (14.0)	0.70 (0.37–1.33)	35 (15.8)	0.67 (0.44–1.02)	19 (16.7)	0.69 (0.40–1.19)	
≥60 months	631 (30.1)	32 (32.0)	0.85 (0.52–1.39)	42 (19.0)	0.42 (0.29–0.63)	20 (17.5)	0.38 (0.22–0.65)	
Including non-users			$P^c_{trend} = 0.44$		$P^c_{trend} < 0.001$		$P^c_{trend} = 0.001$	0.03[d]
IUD use	353 (16.8)	11 (11.0)	0.83 (0.43–1.58)	32 (14.5)	1.14 (0.76–1.71)	9 (7.9)	0.58 (0.29–1.16)	0.19[b]
Infertility								
None	1664 (79.2)	70 (70.0)	1.00 (Ref)	171 (77.4)	1.00 (Ref)	89 (78.1)	1.00 (Ref)	0.79[b]
Male	41 (2.0)	2 (2.0)	0.83 (0.19–3.60)	2 (0.9)	0.34 (0.08–1.46)	2 (1.8)	0.66 (0.15–2.82)	
Female	161 (7.7)	14 (14.0)	1.77 (0.96–3.27)	25 (11.3)	1.30 (0.81–2.07)	13 (11.4)	1.29 (0.70–2.40)	
Cause not found	234 (11.1)	14 (14.0)	1.32 (0.72–2.41)	23 (10.4)	0.89 (0.55–1.42)	10 (8.8)	0.74 (0.38–1.46)	
Endometriosis	165 (7.9)	16 (16.0)	2.17 (1.23–3.85)	32 (14.5)	1.93 (1.26–2.96)	27 (23.7)	3.54 (2.20–5.70)	0.11[b]
Painful periods[e]	677 (32.4)	40 (40.8)	1.21 (0.79–1.84)	101 (45.7)	1.48 (1.10–1.97)	56 (49.1)	1.69 (1.15–2.49)	0.48[b]
Hysterectomy[f]	183 (8.7)	10 (10.0)	1.45 (0.73–2.88)	8 (3.6)	0.49 (0.23–1.02)	4 (3.5)	0.47 (0.17–1.32)	0.06[b]
Tubal ligation	419 (20.0)	2 (2.0)	0.12 (0.03–0.49)	20 (9.1)	0.58 (0.36–0.95)	10 (8.8)	0.56 (0.29–1.10)	0.03[b]

[a]Models were adjusted for age (continuous), study center (Massachusetts, New Hampshire, USA), study phase (1992–1997, 1998–2003, 2003–2008), parity (0, 1, 2, >2), OC pill use (0, 3 months to <5 years, ≥5 years), family history of ovarian cancer (yes/no), family history of breast cancer (yes/no) and tubal ligation (yes/no) unless noted otherwise.
[b]The *P*-value for heterogeneity (P_{het}) is from the likelihood-ratio test that compares a model with the same estimate for the association with the exposure of interest (e.g. categories of parity) across the histologic categories to a model that allows the exposure of interest to vary across the histologic categories.
[c]The P_{trend} is based on the Wald statistic using a continuous variable.
[d]The P_{het} is from the likelihood-ratio test that compares a model with the same estimate for the association with the exposure of interest (trend variable) across the tumor subgroups to a model that allows the association of interest to vary across the subgroups. It indicates if there were statistically significant differences in the association of interest between the different outcome categories.
[e]Numbers may not add up to total due to missing data.
[f]Models were additionally adjusted for ever use of post-menopausal hormones (yes/no).

tubal ligation and hysterectomy are protective for endometrioid ovarian cancer support the hypothesis that retrograde passage of the endometrium or endometrial fluids through patent Fallopian tubes may lead to the development of endometrioid ovarian carcinoma. If confirmed in future studies, tubal ligation may be a useful measure to prevent the development of endometrioid ovarian

carcinoma, particularly among women with a history of endometriosis who are at higher risk to develop this type of disease. Further studies are needed to evaluate the association of hysterectomy in relation to risk of endometrioid and clear cell tumors.

Previous studies have noted differences in risk factor associations for mucinous and non-mucinous tumors (Risch *et al.*, 1996; Wittenberg *et al.*, 1999; Purdie *et al.*, 2001; Soegaard *et al.*, 2007). In the current study, we did not evaluate mucinous tumors separately because the dualistic and histologic pathway models combined mucinous tumors with other histologic subtypes in the type I and the mucinous/low-grade serous histologic pathways. Consistent with these prior findings, in a separate analysis of the NECC study, we observed that reproductive factors, such as OC use, were more strongly associated with non-mucinous tumors, while current smoking was associated with an increased risk of mucinous but not other histologic subtypes of tumors (D. Cramer, personal communication).

Strengths of the current study include the large sample size, which allowed the assessment of risk factor associations among the less common subgroups such as the low and high-grade endometrioid and clear cell tumors. However, by dividing the cases into subgroups, this also could limit the power of these particular analyses. Furthermore, assessment of risk factor associations across several case subgroups required multiple statistical tests; therefore, some significant findings could have been due to chance. Selection bias is possible since not all of the invited participants took part in the study; however, it is unlikely that the response rate would have had a strong influence on the reported results since study participation is not likely to be related to reproductive exposures. In the analyses of self-reported endometriosis, there is potential for misclassification since it has previously been shown that self-reported endometriosis does not always correlate with evidence of a clinical diagnosis (Missmer *et al.*, 2010). If there was a tendency to over-report a diagnosis of endometriosis in our study, then this could potentially inflate the association that we observed between endometriosis and ovarian cancer. Lastly, since cases were enrolled after their diagnosis, the most aggressive cases could be missed leading to potential survival bias. We would expect this to influence the risk estimates from the most aggressive subgroups (type II/high-grade serous tumors). However, on a reassuring note, we did not observe differences in the distribution of enrolled and unenrolled cases by the major histologic subtypes; percentages of serous borderline, invasive serous, mucinous, endometrioid and clear cell types were 10, 45, 10 and 29%, respectively, for the enrolled cases and 12, 43, 12 and 28, respectively, for the unenrolled cases (unpublished data). Furthermore, we observed a similar histologic distribution of the NECC study cases to those in SEER data (Howlader *et al.*, 2012) suggesting that our findings should not be greatly influenced by survival bias.

In summary, we have identified differences in reproductive risk factor associations between tumors categorized as type I/II and among pathway-based histologic classifications of EOC. Briefly, the risk for type II tumors increased with older age and a higher number of ovulatory cycles. In contrast, a history of endometriosis increased the risk for a type I tumor while strong protective factors for type I tumors included parity and having a previous tubal ligation or hysterectomy. In analyses of histologic pathways, a higher number of ovulatory cycles was associated with an increased risk of high-grade serous/other tumors, and a striking increase in risk was observed for high-grade endometrioid and clear cell tumors. The protective factors mentioned above for type I tumors (parity, tubal ligation and hysterectomy) showed a strong inverse association with risk of endometrioid/clear cell tumors. Together these findings highlight differences in etiologic pathways that can be integrated with these models of ovarian carcinogenesis. These data may assist in efforts to develop improved strategies for prevention while building on existing models that account for ovarian tumor heterogeneity.

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Authors' roles

K.L.T. conceived the study and provided guidance for the epidemiologic analyses. M.A.M. carried out the analyses with the assistance of M.D.P. and A.F.V., D.W.C. and L.J.T. coordinated the NECC study, led the data collection efforts and provided scientific guidance. M.A.M. drafted the manuscript with assistance from K.L.T. and all of the authors critically evaluated the results and edited the manuscript.

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Conflict of interest

None declared.

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