ORIGINAl CONTRIBUTIONS

Effect of Anovulation Factors on Pre- and Postmenopausal Ovarian Cancer Risk: Revisiting the Incessant Ovulation Hypothesis

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Risk factors for ovarian cancer may differ between pre- and postmenopausal women. The authors used data from a multiethnic, population-based, case-control study, conducted between 1993 and 1999 in Hawaii and Los Angeles, California, to examine whether menopause modified the effect of ovulation on ovarian cancer risk. A structured questionnaire was administered to 558 histologically confirmed epithelial ovarian cancer cases and 607 population controls. Lifetime ovulatory (log) years were significantly associated with an increased risk of ovarian cancer (odds ratio = 1.78, 95% confidence interval: 1.24, 2.57), particularly among premenopausal women (odds ratio = 2.49) but not among postmenopausal women (odds ratio = 0.88) ($p_{interaction} = 0.006$). Factors that induced anovulation, including oral contraceptives, pregnancy, and breastfeeding, were associated with a reduced risk of ovarian cancer. Among anovulation factors, prolonged oral contraceptive pill use provided a greater protective effect against premenopausal ovarian cancer than against postmenopausal ovarian cancer (for ≥5.4 years of use vs. never use: odds ratio = 0.28, 95% confidence interval: 0.15, 0.52 vs. odds ratio = 0.58, 95% confidence interval: 0.31, 1.08, respectively), but the difference was not significant ($p_{interaction} = 0.20$). Association of breastfeeding and pregnancy with ovarian cancer risk was also similar between pre- and postmenopausal women (respective $p_{interaction} = 0.72$ and 0.43). The authors’ data support the hypothesis that lifetime ovulation is involved in the pathogenesis of pre- but not postmenopausal ovarian cancer, while the protective effects of anovulation factors persist from pre- to postmenopausal women.

Ovarian cancer is a common cancer among women in the United States and worldwide. Although much is known about risk factors for ovarian cancer, its underlying causative mechanisms remain elusive. Two long-held hypotheses, the incessant ovulation (1) and the gonadotropin (2) theories, propose that both repeated ovulation and gonadotropin hormones (follicle-stimulating hormone or luteinizing hormone) stimulate cell proliferation and malignant transformation of the ovarian epithelium. Although consistent findings from epidemiologic studies showing a protective effect of anovulatory events, such as oral contraceptive pill use, pregnancy, and lactation, against ovarian cancer support both of these hypotheses (3, 4), ovulation suppression alone is insufficient to explain the underlying effects. In part, the protective effects of anovulatory exposures exceed that expected from ovulatory suppression (4, 5), and the hypothesis fails to provide an explanation for the differential effects among anovulatory events. Alternatively, Risch (6) has hypothesized that progesterone stimulation or androgen reduction during pregnancy and oral contraceptive use may also account for the risk reduction in ovarian cancer.

While both pre- and postmenopausal ovarian cancer risk may be related to previous ovulation exposures, ovulation in conjunction with hormone stimulation may play a more important role in the carcinogenesis of premenopausal than postmenopausal ovaries (7). During ovulation, follicular and

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steroid hormones regulate the cell cycle dynamics of the ovarian epithelium. The sensitivity of ovarian cells to hormone stimulation can be quite variable during a woman’s lifetime, particularly at perimenopause. Loss of ovarian function during the menopause transition is accompanied by a dramatically decreased negative feedback of gonadal steroids and peptides on the hypothalamus and pituitary (8). Menopause accompanying follicular depletion leads to significant hormonal changes in women: Estrogen levels decline, while follicle-stimulating hormone and luteinizing hormone levels rise (9). Pike (10) has proposed that the effectiveness of suppression of ovulation against ovarian cancer may decline with age, especially near menopause when serum gonadotropins reach their highest concentrations. Exposure to high levels of estrogen and progesterone during pregnancy and oral contraceptive use appears to protect against ovarian cancer during a woman’s reproductive life, while postmenopausal hormone replacement therapy may actually increase ovarian cancer risk (11). Furthermore, some risk factors may influence ovulatory function and steroid hormonal levels differently in premenopausal and postmenopausal women (6). It is conceivable that menopause may modify the risk of ovarian cancer associated with ovulatory cycles.

In this study, we examine the hypothesis that ovulation is more strongly associated with premenopausal than postmenopausal ovarian cancer risk. Few studies to date have investigated if menopause modifies the beneficial effects of anovulatory factors, such as breastfeeding, on ovarian cancer risk (12).

MATERIALS AND METHODS

The details of this population-based, case-control study, conducted in Hawaii and Los Angeles, California, have been described elsewhere (13). Briefly, eligibility criteria for participation in this investigation included 1) residency in Hawaii or Los Angeles County for at least 1 year prior to the reference date of diagnosis for cases or interview for controls, 2) 18 or more years of age, 3) no prior history of ovarian cancer, and 4) at least one intact ovary for controls. Ovarian cases were identified through the rapid-reporting system of the Hawaii Tumor Registry and the Los Angeles Ovarian cases were identified through the rapid-reporting system of the Hawaii Tumor Registry and the Los Angeles

We calculated total years of menstruation as age at menarche subtracted from age at menopause for postmenopausal women and from age at interview or diagnosis for premenopausal women. Age at menopause was based on self-report. However, age at menopause was assigned for postmenopausal women with an unknown age at menopause as one of the following: 1) age at the very last menstrual period, 2) age at surgery or other medical treatment, 3) age started taking hormone replacement therapy, 4) age started having menopausal symptoms, or 5) age 55, if age was greater than 55 years. Among the 1,165 study participants, menopausal status was assigned to 26 percent, and age at menopause was assigned to 17 percent.

Lifetime ovulatory cycles were calculated by first subtracting the total years of any anovulatory periods due to pregnancies, lactation, use of oral contraceptives, and amenorrhea from the total menstrual years and then multiplying by the number of estimated cycles per year based upon a woman’s cycle length (365/cycle length). Finally, the

time ovulatory cycles were converted to total years of ovulatory cycles through dividing by 13 for all women (average cycles per year = 365/28). The estimated length of the anovulatory events was based on women’s self report. However, duration of lactation per birth was truncated at 6 months, as suppression of ovulation diminishes with prolonged breastfeeding (14). This algorithm was used so that years of ovulation would be proportional to the number of ovulatory cycles; for example, a woman with ovulatory cycles from age 14 to age 34 years and 13 cycles a year would have more “years of ovulation” than a woman with ovulatory cycles over the same age range but with only 11 cycles a year.

Unconditional logistic regression (15) was used to estimate the risk of ovarian cancer associated with the ovulation variables. All data were analyzed using SAS version 8.2 software (SAS Institute, Inc., Cary, North Carolina). Adjustment variables included age (continuous), race (indicator variable for Caucasian, Asian, other), study site (indicator variable for Hawaii, Los Angeles), education (continuous), tubal ligation (yes or no), and hormone replacement therapy (yes or no). We also considered other potential risk factors as adjustment variables, such as family history of breast and/or ovarian cancer, body mass index, and pack-years of smoking, but these did not materially alter the fit of the models. The duration of ovulation variables was parameterized in four ways: indicator variables representing quartiles or tertiles, a trend variable, years, and (log)years. The trend variable was assigned the median for the appropriate quartile or tertile and tested for significance with the likelihood ratio test. Odds ratios and 95 percent confidence intervals were computed for quartiles, years, and (log)years as the exponentiation of the regression parameter and its confidence interval. Years or (log)years of lifetime ovulatory cycles, oral contraceptive pill use, pregnancy, and breastfeeding were mutually adjusted for each other in the models. (Log)years provided a better fit to our data, but statistics are also provided for years, for comparison with the results of others. To statistically compare anovulatory event parameters in (log)years between menopausal subgroups, one model with all individuals was fit with an interaction term between menopausal status and the ovulatory event variable. The significance of this term was based on a Wald test. Covariate-adjusted means were computed by analysis of covariance. A separate model was also run for age-specific exposure periods (<20, 20–29, 30–39, and 40–49 years) among 956 women older than 40 years.

RESULTS

The distribution of subject demographics and risk factor information is shown in table 1. Cases and controls had similar ages (mean = 54.8 years), and the majority were of European or Asian ancestry. Controls were better educated than cases, had a greater number of full-term pregnancies, were more likely to have used oral contraceptives, and had a higher frequency of tubal ligation. These risk factors were used as adjustment variables in subsequent analyses.

Among the 558 ovarian cancer cases, 39 percent of the women were premenopausal, and 61 percent were postmenopausal. Forty-two percent of the 607 controls were premenopausal at interview, and 58 percent were postmenopausal. Covariate-adjusted mean years for ovulation cycles and anovulatory factors varied significantly between cases and controls, particularly among premenopausal women (table 2). Overall, years of oral contraceptive pill use (p < 0.0001), pregnancy (p < 0.0005), and breastfeeding (p < 0.001) were significantly lower among cases than among controls, while lifetime ovulation years were significantly greater among cases than among controls (p < 0.0001). Although similar differences between cases and controls for the ovulatory factors were found among postmenopausal women, none was statistically significant.

Years of lifetime ovulatory cycles were positively associated with overall ovarian cancer risk, and the duration of ovulation-suppressive factors was inversely associated with ovarian cancer risk (table 3). However, these patterns differed by menopausal status. Total ovulatory years were associated with an increased risk of premenopausal ovarian cancer but not of postmenopausal ovarian cancer (p interaction in (log)year model = 0.006). Also, the trend in the odds ratio with increasing total ovulatory years (by quartiles) was significant among premenopausal women (p trend < 0.0001) but not among postmenopausal women (p trend = 0.58). When the 146 cases and 155 controls who were assigned a menopausal status were removed from the analysis, the results were similar: Odds ratios for (log)years of ovulation, oral contraceptive use, pregnancy, and breastfeeding among premenopausal women were 2.18, 0.58, 0.61, and 0.51, respectively (data not shown). Ovulatory cycle years during the respondent’s twenties had the most beneficial effect on ovarian cancer risk (table 4).

Significant inverse dose-response relations were found for oral contraceptives and breastfeeding among premenopausal but not among postmenopausal women (table 3). Although the associations were somewhat stronger for premenopausal ovarian cancer, the differences between the menopausal groups were not significant (p interaction = 0.20 for oral contraceptive use, 0.72 for pregnancy, and 0.43 for breastfeeding). Oral contraceptive use and breastfeeding before age 20 years had the greatest influence on ovarian cancer risk (table 4).

DISCUSSION

Our results are consistent with those of previous studies that showed that lifetime ovulation was significantly and positively associated with the risk of ovarian cancer. We found that the risk reduction associated with anovulatory factors, such as oral contraceptive pill use, pregnancy, and lactation, was similar. Furthermore, we found that the positive relation of ovulation to the risk of ovarian cancer was modified by menopausal status, suggesting a strong influence of ovulation-related hormones on the pathogenesis of ovarian cancer.

Our data provide clear evidence for the hypothesis that ovulation is integral to the etiology of ovarian cancer. Experimental studies support the notion that successive ovulation and regenerative repairs enhance the possibility of genetic instability and subsequently lead to mutagenesis (7, 16, 17). A dysfunction in the mechanism for the recognition and
repair of DNA damage is likely to be the initial step in ovarian tumorigenesis. Epithelial ovarian cancers appear to arise from precursor lesions at the site of follicular rupture that are provoked by postovulatory wound repair. The ovarian epithelium is constantly exposed to several reactive oxidative and inflammatory substances during the process of ovulation (18–20). Concomitantly, oxidative DNA damage, p53 expression, and apoptosis occur at the ovulation site (21). In a ewe model, Murdoch (22) demonstrated that oxidative DNA adducts and p53 tumor suppressor gene expression are related in ovulated follicles and that down-regulation of p53 led to a failure to repair or remove DNA damage. Webb et al. (23) and Purdie et al. (24) both confirmed the association between ovulation and ovarian cancer risk.

In support of the incessant ovulation hypothesis, epidemiologic studies have consistently demonstrated that factors that suppress ovulation, including oral contraceptives, preg-

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cases (n = 558)</th>
<th>Controls (n = 607)</th>
<th>Odds ratio*</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Center</td>
<td></td>
<td></td>
<td></td>
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</tr>
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<td>200</td>
<td>35.8</td>
<td>283</td>
<td>46.6</td>
</tr>
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<td>Los Angeles</td>
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<td>64.2</td>
<td>324</td>
<td>53.4</td>
</tr>
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<td>130</td>
<td>23.3</td>
<td>149</td>
<td>24.5</td>
</tr>
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<td>45–54</td>
<td>160</td>
<td>28.7</td>
<td>171</td>
<td>28.2</td>
</tr>
<tr>
<td>55–64</td>
<td>117</td>
<td>21.0</td>
<td>98</td>
<td>16.1</td>
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<td>≥65</td>
<td>151</td>
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<td>189</td>
<td>31.1</td>
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<td>Ethnicity†</td>
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<td>266</td>
<td>43.8</td>
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<tr>
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<td>36.9</td>
<td>254</td>
<td>41.8</td>
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<td>Other</td>
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<td>14.3</td>
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<td>163</td>
<td>26.8</td>
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<tr>
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<td>34.8</td>
<td>214</td>
<td>35.2</td>
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<tr>
<td>15</td>
<td>113</td>
<td>20.2</td>
<td>150</td>
<td>24.7</td>
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<td>≥16</td>
<td>62</td>
<td>11.1</td>
<td>80</td>
<td>13.2</td>
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<tr>
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<td>17.6</td>
</tr>
<tr>
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<td>77</td>
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<td>14.8</td>
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<td>2</td>
<td>139</td>
<td>24.9</td>
<td>179</td>
<td>29.5</td>
</tr>
<tr>
<td>≥3</td>
<td>183</td>
<td>32.8</td>
<td>231</td>
<td>38.1</td>
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<td>Oral contraceptive pill use (years)</td>
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<tr>
<td>0</td>
<td>320</td>
<td>57.3</td>
<td>269</td>
<td>44.3</td>
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<tr>
<td>0.1–1.8</td>
<td>93</td>
<td>16.7</td>
<td>99</td>
<td>16.3</td>
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<td>1.9–5.3</td>
<td>82</td>
<td>14.7</td>
<td>110</td>
<td>18.1</td>
</tr>
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<td>≥5.4</td>
<td>63</td>
<td>11.3</td>
<td>129</td>
<td>21.2</td>
</tr>
<tr>
<td>History of tubal ligation</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>490</td>
<td>87.8</td>
<td>487</td>
<td>80.2</td>
</tr>
<tr>
<td>Yes</td>
<td>68</td>
<td>12.2</td>
<td>120</td>
<td>19.8</td>
</tr>
<tr>
<td>Hormone replacement therapy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>377</td>
<td>67.6</td>
<td>387</td>
<td>63.8</td>
</tr>
<tr>
<td>Yes</td>
<td>181</td>
<td>32.4</td>
<td>220</td>
<td>36.2</td>
</tr>
</tbody>
</table>

* After adjustment by unconditional multiple logistic regression for age, ethnicity, study center, education, oral contraceptive pill use, parity, and tubal ligation (where appropriate).
† Cases and controls were frequency matched on these variables.
‡ Referent category.
nancy, and lactation, protect against ovarian cancer. Under the ovulation model, the protective effects of anovulation factors per ovulation prevented would be similar. This was indeed the case in our analysis: A similar risk reduction in ovarian cancer was observed with 1 year of ovulatory suppression by oral contraceptives (odds ratio = 0.94), pregnancy (odds ratio = 0.88), and breastfeeding (odds ratio = 0.91). However, other studies showed that oral contraceptives and pregnancy decreased ovarian cancer risk to a greater extent than did lactation (24, 25), suggesting that the protective effects of oral contraceptives and pregnancy may not be attributed solely to anovulation. Risch (26) predicted that the risk reduction associated with 1 year of anovulation would not be greater than 5 percent under the assumption of at least 20 years of ovulation for most women, while the effect per year of anovulatory factors exceeded this level in our study and those of others.

Lifetime years of ovulation were more strongly associated with ovarian cancer among premenopausal women than among postmenopausal women in our study. Whittemore et al. (27) also reported that ovarian cancer risk associated with ovulation cycles was significantly greater for younger (<55 years) than for older (≥55 years) women in a pooled analysis of 12 US case-control studies. Pike (10) hypothesized that the incidence of ovarian cancer increases with ovarian age, corresponding to exposure to ovulation-inducing cell mitosis. It follows, therefore, that the risk reduction associated with anovulation declines with age as each year of anovulation is reduced by a fixed proportion. Consistent with the results of Purdie et al. (24), our results show that total years of ovulation at younger ages (20–29 years) had the greatest effect (odds ratio = 1.45 (per (log)year)) on ovarian cancer risk when compared with other age periods, as the anovulation events most likely occurred during this age period (data not shown). This finding supports the hypothesis that the susceptibility of the ovary to carcinogenic events associated with ovulation may be greatest in young adults. It is notable that Tokouka et al. (28) reported a minimum latency period for radiation-induced ovarian cancer among Hiroshima atomic bomb survivors of from 15 to 20 years. This would support an association of ovulatory exposure within the first decade or two of reproductive life with premenopausal ovarian cancer.

Patterns of pituitary-ovarian hormones change throughout a woman’s reproductive life corresponding to ovulation, pregnancy, breastfeeding, and stage of menopausal transition. Serum gonadotropin levels, predominately follicle-stimulating hormone, increase monotonically with age during the premenopausal period and rise significantly after perimenopause (9). Although estradiol levels do not change during the premenopausal period and rise significantly after perimenopause, the ovarian cells’ response to gonadotropin stimulation decreases progressively as a function of age, and higher gonadotropin levels may be required to induce ovulation for older premenopausal women. The frequency of anovulatory cycles also increases as menopause approaches, since follicular depletion accelerates dramatically in the last decade of menstrual life. This may explain the reduced effect of ovulation factors on ovarian cancer risk occurring later in a woman’s reproductive life.

We found that anovulatory factors provided a similar protective effect for overall ovarian cancer and that, while the effects were somewhat stronger for premenopausal cancer, the differences were not significant. The stronger association of the oral contraceptives with ovarian cancer among younger than among older women may have been influenced by the greater prevalence of oral contraceptive pill use in younger cohorts. We found that oral contraceptive use under age 20 years had the most beneficial effects on ovarian cancer. Similarly, Willett et al. (29) reported that the reduction in risk associated with oral contraceptives was strongest in younger (aged <35 years) women. In contrast, Whitemore et al. (27) reported that oral contraceptive pill use accounted for the greatest risk reduction for ovarian cancer among older (aged ≥55 years) women, but pregnancy

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**TABLE 2.** Mean* and standard error of total years for lifetime ovulation and anovulatory factors (oral contraceptives, pregnancy, and breastfeeding) by menopausal status, Hawaii and Los Angeles, California, 1993–1999

<table>
<thead>
<tr>
<th>Ovulatory factors (years)</th>
<th>Total (n = 1,165)</th>
<th>Premenopausal (n = 475)</th>
<th>Postmenopausal (n = 692)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases (n = 658)</td>
<td>Controls (n = 607)</td>
<td>Cases (n = 217)</td>
</tr>
<tr>
<td></td>
<td>Cases (n = 341)</td>
<td>Controls (n = 351)</td>
<td></td>
</tr>
<tr>
<td>Total ovulatory cycles</td>
<td>28.6 (0.32)</td>
<td>28.3 (0.30)</td>
<td>29.7 (0.59)</td>
</tr>
<tr>
<td></td>
<td>27.9 (0.45)</td>
<td>26.7 (0.46)</td>
<td></td>
</tr>
<tr>
<td>p value†</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.05</td>
</tr>
<tr>
<td>Oral contraceptives</td>
<td>1.83 (0.16)</td>
<td>2.88 (0.15)</td>
<td>1.47 (0.30)</td>
</tr>
<tr>
<td></td>
<td>2.07 (0.23)</td>
<td>2.56 (0.23)</td>
<td></td>
</tr>
<tr>
<td>p value</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.09</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>1.56 (0.05)</td>
<td>1.81 (0.05)</td>
<td>1.45 (0.10)</td>
</tr>
<tr>
<td></td>
<td>1.63 (0.07)</td>
<td>1.79 (0.07)</td>
<td></td>
</tr>
<tr>
<td>p value</td>
<td>0.0005</td>
<td>0.004</td>
<td>0.09</td>
</tr>
<tr>
<td>Breastfeeding</td>
<td>0.36 (0.03)</td>
<td>0.48 (0.03)</td>
<td>0.39 (0.05)</td>
</tr>
<tr>
<td></td>
<td>0.34 (0.04)</td>
<td>0.41 (0.04)</td>
<td></td>
</tr>
<tr>
<td>p value</td>
<td>0.001</td>
<td>0.001</td>
<td>0.15</td>
</tr>
</tbody>
</table>

*After adjustment by analysis of covariance for age, ethnicity, study site, education, and tubal ligation.
†p value for F test comparing means between cases and controls.
was more associated with risk reduction among younger women (aged <55 years). The results of Whittemore et al.
suggest that the cumulative risk reduction associated with
oral contraceptives may have a greater lag-time than that of
pregnancy. Although Ness et al. (30) did not find that oral
contraceptive formulation had an impact on the inverse asso-
ciation with ovarian cancer risk, it should be noted that the
data collected for the pooled analysis conducted by Whitte-
more et al. spanned a period when oral contraceptive pill
formulations contained greater amounts of estrogen and
progestin than in the 1990s when our study was conducted.

Ness et al. (30) reported that the ovarian cancer risk reduc-
tion associated with oral contraceptives may persist for 10–
30 years after cessation. Differences in the relation of oral
contraceptives and pregnancy to premenopausal ovarian
cancer may result from the greater potential for gonadotropin
suppression by oral contraceptives than by pregnancy,
particularly before a woman’s thirties. These differences

### TABLE 3. Odds ratios* and 95% confidence intervals for the association of ovulation variables with risk of ovarian cancer and menopausal status, Hawaii and Los Angeles, California, 1993–1999

<table>
<thead>
<tr>
<th>Ovulatory factors (years)</th>
<th>All women†</th>
<th>Menopausal status‡</th>
<th>Postmenopausal women</th>
<th>$p_{interaction}$§</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Odds ratio</td>
<td>95% confidence interval</td>
<td>Odds ratio</td>
<td>95% confidence interval</td>
</tr>
<tr>
<td>Ovulatory cycles</td>
<td></td>
<td></td>
<td>Premenopausal women</td>
<td></td>
</tr>
<tr>
<td>&lt;22.1</td>
<td>1¶</td>
<td>78</td>
<td>128</td>
<td>1¶</td>
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<tr>
<td>22.1–29.1</td>
<td>1.53</td>
<td>1.05, 2.23</td>
<td>58</td>
<td>82</td>
</tr>
<tr>
<td>29.2–34.1</td>
<td>1.91</td>
<td>1.28, 2.85</td>
<td>53</td>
<td>39</td>
</tr>
<tr>
<td>&gt;34.1</td>
<td>1.82</td>
<td>1.17, 2.85</td>
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<td>7</td>
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<tr>
<td>$p_{trend}$</td>
<td>0.003</td>
<td>&lt;0.0001</td>
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<tr>
<td>(Log)year#</td>
<td>1.78</td>
<td>1.24, 2.57</td>
<td>2.49</td>
<td>1.53, 4.05</td>
</tr>
<tr>
<td>Year#</td>
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<td>1.00, 1.04</td>
<td>1.07</td>
<td>1.04, 1.10</td>
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<td>Oral contraceptives</td>
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<td>89</td>
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<td>1¶</td>
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<td>0.1–1.8</td>
<td>0.74</td>
<td>0.50, 1.07</td>
<td>56</td>
<td>57</td>
</tr>
<tr>
<td>1.9–5.3</td>
<td>0.60</td>
<td>0.41, 0.88</td>
<td>38</td>
<td>67</td>
</tr>
<tr>
<td>≥5.4</td>
<td>0.45</td>
<td>0.30, 0.69</td>
<td>34</td>
<td>86</td>
</tr>
<tr>
<td>$p_{trend}$</td>
<td>0.0003</td>
<td></td>
<td>0.0003</td>
<td></td>
</tr>
<tr>
<td>(Log)year</td>
<td>0.74</td>
<td>0.63, 0.87</td>
<td>0.65</td>
<td>0.51, 0.82</td>
</tr>
<tr>
<td>Year</td>
<td>0.94</td>
<td>0.91, 0.98</td>
<td>0.94</td>
<td>0.89, 0.99</td>
</tr>
<tr>
<td>Pregnancy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;0.75</td>
<td>1¶</td>
<td>82</td>
<td>64</td>
<td>1¶</td>
</tr>
<tr>
<td>0.75–1.49</td>
<td>0.93</td>
<td>0.60, 1.45</td>
<td>47</td>
<td>43</td>
</tr>
<tr>
<td>1.50–2.25</td>
<td>0.75</td>
<td>0.51, 1.12</td>
<td>48</td>
<td>88</td>
</tr>
<tr>
<td>&gt;2.25</td>
<td>0.73</td>
<td>0.48, 1.10</td>
<td>40</td>
<td>61</td>
</tr>
<tr>
<td>$p_{trend}$</td>
<td>0.25</td>
<td></td>
<td>0.53</td>
<td></td>
</tr>
<tr>
<td>(Log)year</td>
<td>0.68</td>
<td>0.50, 0.92</td>
<td>0.61</td>
<td>0.36, 1.04</td>
</tr>
<tr>
<td>Year</td>
<td>0.88</td>
<td>0.78, 0.99</td>
<td>0.83</td>
<td>0.65, 1.05</td>
</tr>
<tr>
<td>Breastfeeding</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1¶</td>
<td>130</td>
<td>109</td>
<td>1¶</td>
</tr>
<tr>
<td>&lt;0.5</td>
<td>0.69</td>
<td>0.48, 1.01</td>
<td>33</td>
<td>42</td>
</tr>
<tr>
<td>0.5–1.0</td>
<td>0.62</td>
<td>0.42, 0.91</td>
<td>33</td>
<td>52</td>
</tr>
<tr>
<td>&gt;1.0</td>
<td>0.60</td>
<td>0.40, 0.89</td>
<td>21</td>
<td>53</td>
</tr>
<tr>
<td>$p_{trend}$</td>
<td>0.001</td>
<td></td>
<td>0.003</td>
<td></td>
</tr>
<tr>
<td>(Log)year</td>
<td>0.70</td>
<td>0.46, 1.05</td>
<td>0.51</td>
<td>0.22, 1.18</td>
</tr>
<tr>
<td>Year</td>
<td>0.91</td>
<td>0.72, 1.15</td>
<td>0.68</td>
<td>0.40, 1.17</td>
</tr>
</tbody>
</table>

* After adjustment for age, ethnicity, study site, education, tubal ligation, hormone replacement therapy, and ovulation variables.
† Overall models for quartiles, trend variables, and (log)years.
‡ Interaction models for quartiles, trend variables, and (log)years.
§ $p$ value was based on a Wald test.
¶ Referent category.
# Odds ratios and 95% confidence intervals are provided from a model using continuous years and another model using continuous (log)years. Using (log)years fit our data better, but we also present years for comparison with the others.
may not persist after menopause, since the reductions in risk associated with oral contraceptives (31) and pregnancy (27) appear to decline with age. However, we found that the ovarian cancer risk reduction associated with pregnancy also occurred at older age. This would support a pregnancy-induced apoptosis pathway in addition to inhibition of ovulation (32).

We found that breastfeeding reduced ovarian cancer risk as effectively as oral contraceptive use. The one other study investigating the effect of breastfeeding on ovarian cancer risk by menopausal status found that the protective effect was limited to premenopausal ovarian cancer risk (33). The protective association of breastfeeding with ovarian cancer may be attributed to the partial inhibition of ovulation resulting from elevated follicle-stimulating hormone and prolactin levels and lower luteinizing hormone levels among lactating women (34).

An important limitation of this study is that the lifetime ovulatory cycles may not be estimated accurately, as not all menstrual cycles will be ovulatory, especially at young ages.
or at perimenopause. Recall errors could occur if cases and
differences in their recollection of reproductive events,
such as ages at menarche and menopause, or the length of
breastfeeding or oral contraceptive use. Previous validation
studies have generally reported good reproducibility and
reliability for reproductive exposures, except for menstrual
cycle characteristics (35–37). Although nondifferential
recall bias may exist in estimating pre- and postmenopausal
ovarian cancer risk, the extent of attenuation may be greater
for post- than premenopausal women as postmenopausal
women may have trouble remembering events in the distant
past. To see if this was a problem, we stratified the analysis
by time between referent date and menopause among post-
menopausal women (≤15 years and >15 years). We found
that the associations for postmenopausal women did not
differ between the groups for lifetime ovulatory (log)years
(respective odds ratios of 0.91 and 0.90), (log)years of oral
contraceptive use (respective odds ratios of 0.83 and 0.75),
and (log)years of pregnancy (respective odds ratios of 0.70
and 0.85). Therefore, our results for these variables are
unlikely to be affected by recall. However, we found that
the protective effect for breastfeeding was much stronger for
women recalling long after menopause (for ≥15 years: odds
ratio for (log)years = 0.56) than for women recalling <15
years ago (odds ratio = 0.96). This could indicate that older
women are recalling breastfeeding events incorrectly or that
older postmenopausal women breastfed differently (longer
or with more intensity) from younger postmenopausal
women. Therefore, the breastfeeding comparison between
pre- and postmenopausal women must be viewed cautiously.
Another limitation is that the suboptimal response rates for
cases and controls may affect the generalizability and lead to
bias in the estimates. However, our results are generally
consistent with those of other investigators. Madigan et al.
(38) found that nonresponse had little effect on breast cancer
risk estimates, even though participating cases and controls
were more likely to be educated or to have used oral contra-
ceptives. However, it is unlikely that pregnancy or breast-
feeding histories would influence participation rates in
women aged 40 or more years. Therefore, we have no reason
to suspect that recall bias would be large in our study for
most reproductive factors.

In summary, our data suggest that lifetime ovulatory
cycles were predominantly associated with increased
premenopausal ovarian cancer risk. The reduction in ovarian
cancer risk afforded by anovulatory events, such as oral
contraceptives and pregnancy, was somewhat stronger in
premenopausal than postmenopausal women, although the
differences were not statistically significant, suggesting that
the beneficial effects of prolonged anovulatory exposures
persist from the pre- to the postmenopausal status. This anal-
ysis supports the hypothesis that ovulation is an etiologic
factor for ovarian cancer, particularly among premenopausal
women. Progestogen and other steroid hormones may
account for the prolonged effect of pregnancy and oral
contraceptives on postmenopausal ovarian cancer.

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