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Original Contribution

Reproductive and Hormonal Factors in Association With Ovarian Cancer in the Netherlands Cohort Study

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Parity, oral contraceptive use, and hysterectomy are known to protect against ovarian cancer, whereas the effect of other reproductive factors remains unclear. The authors investigated the association between several reproductive and hormonal factors and the risk of epithelial invasive ovarian cancer among postmenopausal women participating in the Netherlands Cohort Study on Diet and Cancer. Information on reproductive history and exogenous hormone use was obtained through a self-administered questionnaire at baseline in 1986. After 16.3 years of follow-up, 375 cases and 2,331 subcohort members were available for case-cohort analysis. Ovarian cancer risk was reduced for parous women, with increasing parity, and for hysterectomized women. Moreover, the authors found evidence that oral contraceptive use is protective against ovarian cancer, even when initiated at an older age. In addition, a reduced risk was observed for each year reduction in age at natural menopause and per year reduction in total menstrual life span. A small increased risk was observed with prolonged time to pregnancy, but no difference was found between ever-married nulliparous women and never-married nulliparous women. Moreover, no associations were observed for age at first birth, age at menarche, age at first and last use of oral contraceptives, and use of hormone replacement therapy.

hormones; infertility; ovarian neoplasms; prospective studies; reproductive history

Abbreviations: CI, confidence interval; HR, hazard ratio; HRT, hormone replacement therapy; OC, oral contraceptive.

Ovarian cancer is the fifth most common cancer among women in Europe (1). Unknown pathogenesis and late diagnosis contribute to poor survival. Different reproductive and hormonal factors have been studied to clarify their influence on ovarian carcinogenesis. Epidemiologic studies consistently show a protective effect of parity, oral contraceptive (OC) use, and hysterectomy (2–22). Less clear are the effects of age at first birth and timing of OC use. Moreover, results for other reproductive risk factors, such as age at menarche, age at menopause, and hormone replacement therapy (HRT), remain conflicting.

The increased risk for nulliparous women could, in part, reflect an association between ovarian cancer and subfertility. Generally, no strong overall associations have been found between subfertility and ovarian cancer (23–29). However, an increased risk has been observed in some studies for subfertile women who remained childless (20, 21, 23, 25, 26, 28).

Most studies that examined reproductive and hormonal factors used a case-control design, which may suffer from recall and selection bias. Moreover, interpretation of results has been hampered by inadequate control for potential confounding factors, small sample sizes, and differences in reference groups used. We conducted a large prospective study among postmenopausal women within the Netherlands Cohort Study on Diet and Cancer to examine the association of fertility and of reproductive and hormonal factors with the risk of epithelial ovarian cancer.

MATERIALS AND METHODS

The cohort

The prospective Netherlands Cohort Study on Diet and Cancer started in September 1986 with the enrollment of

participants aged 55–69 years (30). In total, 62,573 women were included, who were all presumed to be postmenopausal. For efficiency reasons, data processing and analysis were based on the case-cohort approach. Cases were derived from the entire cohort, and number of person-years at risk for the entire cohort was estimated from a subcohort of 2,589 women randomly sampled from the total cohort at baseline. The subcohort has been contacted by letter every 2 years regarding migration and vital status. In case of no response, the municipal population registries were contacted. No women were lost to follow-up. For more details on the Netherlands Cohort Study on Diet and Cancer, refer to the article by van den Brandt et al. (30). After exclusion of women with prevalent malignancy at baseline (other than nonmelanoma skin cancer) and women who, at baseline, reported they had undergone an oophorectomy, 2,406 female subcohort members remained available.

The study protocol of the Netherlands Cohort Study on Diet and Cancer was approved by the medical ethics committees of the University Hospital Maastricht in February 1985 and TNO Nutrition and Food Research in July 1986.

Identification of cases

Incident cancer cases were identified by computerized record linkage of the entire cohort to the Netherlands Cancer Registry and the Netherlands Pathology Registry (30, 31). The completeness of cancer follow-up was estimated to be more than 95% (32). During a follow-up period of 16.3 years, 394 microscopically confirmed cases of invasive epithelial ovarian cancer were identified. Women with incomplete covariate data (i.e., parity (parous/nulliparous, number of children) and OC use (never/ever)) were excluded, leaving 375 cases and 2,331 subcohort members for analysis.

Questionnaire data

The baseline questionnaire included self-reported information on year of first marriage, number of children, year of first birth, age at menarche, age at menopause, and how menopause was induced. Participants were asked whether they had ever used OCs (yes/no), at which age they started and stopped using OCs, and the total duration of use (in years). In addition, they were asked whether they had ever used HRT and in which year they started and stopped using it. In an open question, participants were asked which types of surgery they had undergone. This item enabled us to define whether women had undergone oophorectomy or hysterectomy, or a combination of both. For women reporting a hysterectomy, age at menopause could be misclassified because many of these women reported that their menopause started on the date of the surgery. Therefore, we restricted our analysis of age at menopause to women experiencing a natural menopause.

Reductions in years of menstrual life span were estimated using different indicators, according to the studies of Dossus et al. (33) and Pelucchi et al. (34). We estimated the influence on ovarian cancer for each year that menarche is delayed; for each year menopause is advanced in time (entering age at natural menopause into the model with a minus sign); per year of being pregnant (calculated as number of children $\times$ 0.75); per year of OC use; per year reduction in time between menarche and menopause; and per year reduction in total menstrual life span. The latter was estimated by calculating the time between age at menarche and age at natural menopause and subsequently subtracting years of pregnancy and years of OC use. Time to pregnancy was defined as the time between marriage and first birth. To analyze time to pregnancy, we excluded women who used OCs prior to the birth of their first child ($n = 5$).

Data analysis

Person-years at risk were calculated from the start of the study until ovarian cancer diagnosis, death, emigration, or end of follow-up (December 31, 2002). The association between various reproductive and hormonal factors and risk of ovarian cancer was evaluated in age-adjusted and multivariate case-cohort analyses using Cox proportional hazards models. Standard errors were estimated using the robust Huber-White sandwich estimator to account for additional variance introduced by sampling from the cohort. This method is equivalent to the variance-covariance estimator presented by Barlow (35).

Analyses were adjusted a priori for age, parity (number of children), and OC use (ever/never) because of their established influence on ovarian cancer development. We considered other potential confounders based on evidence from epidemiologic literature, including height (cm), body mass index (kg/m^2), family history of ovarian or breast cancer (yes/no), educational level (primary school, lower vocational school, high school/intermediate vocational school, higher vocational school/university), nonoccupational physical activity ($\leq$30 minutes/day, 31–60 minutes/day, 61–90 minutes/day, $>$90 minutes/day), smoking status (never, current, former), and all other reproductive and hormonal factors under study. Confounding was evaluated starting with a full multivariate model and using a backward elimination approach (36). If eliminating a covariate from the full Cox regression model changed the hazard ratio by 10% or more, the covariate was considered a confounder and was retained in the model. Otherwise, that covariate was dropped from the multivariate model. None of the potential confounders met this criterion. Therefore, all models were adjusted for only age, parity, and OC use. Moreover, ages at first and last use of OC and HRT were additionally adjusted for duration of use of OC and HRT, respectively.

We also examined whether results differed by age, parity, OC use, hysterectomy, family history of ovarian or breast cancer, body mass index, and smoking status. We used both stratified analyses and the likelihood ratio test to compare proportional hazards regression models with and without the interaction term (37). The proportional hazards assumption was tested using the scaled Schoenfeld residuals and with graphic tests (38). To calculate the P value for the trend test, we assigned participants the median value of each category and treated this variable as a continuous term in the model (36). Two-sided P values are reported throughout the paper and were considered statistically significant if <0.05. All

Table 1. Baseline Characteristics of Cases and Subcohort Members of the Netherlands Cohort Study on Diet and Cancer, 1986–2002

	Cases (n = 375)			Subcohort (n = 2,331)		
	Mean (SD)	No.	%	Mean (SD)	No.	%
Age, years	62.0 (4.3)			61.5 (4.3)		
Height, cm	165.8 (6.1)			165.2 (6.2)		
Weight, kg	69.7 (10.8)			68.5 (10.2)		
Body mass index, kg/m^2	25.2 (3.5)			25.1 (3.5)		
Family history of ovarian or breast cancer		30	8.0		199	8.5
Educational level						
Primary school		129	34.9		806	35.0
Lower vocational school		96	26.0		539	23.4
High school/intermediate vocational school		118	31.9		764	33.2
Higher vocational school/ university		27	7.3		191	8.3
Smoking status						
Never		241	64.3		1,361	58.4
Current		67	17.9		491	21.1
Former		67	17.9		479	20.6

Abbreviation: SD, standard deviation.

analyses were performed with the Stata statistical software package (release 9.1; Stata Corporation, College Station, Texas).

RESULTS

Baseline characteristics of cases and subcohort members are presented in Table 1. Compared with subcohort members, ovarian cancer cases were slightly taller and heavier, and they were more likely to be never smokers. Of the ovarian cancers, 182 were serous invasive (48.5%), 31 were endometrioid (8.3%), 35 were mucinous (9.3%), and 15 were clear-cell (4.0%). The mean age at diagnosis was 70.4 (standard deviation, 5.9) years.

Table 2 shows the associations between various reproductive factors and ovarian cancer risk. Compared with nulliparous women, parous women had a lower risk (hazard ratio (HR) = 0.71, 95% confidence interval (CI): 0.55, 0.93). Moreover, risk decreased by almost 10% for each additional livebirth, which showed a statistically significant trend ($P <$ 0.001). In addition, ovarian cancer risk was decreased for women with a history of hysterectomy (HR = 0.50, 95% CI: 0.34, 0.72). Age at first birth was not associated with ovarian cancer risk. Observations were essentially unchanged after further adjustment for number of full-term pregnancies.

Women who ever used OCs had an almost 30% reduced ovarian cancer risk compared with those who never used OCs (HR = 0.71, 95% CI: 0.52, 0.97; Table 3). This finding was most pronounced for women who used OCs for more than 5 years (HR = 0.47, 95% CI: 0.30, 0.76). We observed no statistically significant associations for age at first and last use of OC, ever use of HRT, and age at first and last use of HRT. For duration of HRT use, the proportional hazards assumption did not hold, and the number of cases in the predefined intervals subsequently became too small to validly interpret the results. The observed risk estimates remained essentially the same after adjustment for age at menopause and induced menopause.

We examined the association between ovarian cancer and different exposures known to reduce menstrual life span, mutually adjusted for each other (Table 4). For all of the exposures, except for delay in age at menarche, trends of decreasing risk with decreasing years of menstrual life span were found. We observed a lower ovarian cancer risk of 2% and 5% per year reduction in age at natural menopause and per year of OC use, respectively, and a 10% risk reduction per year of being pregnant. Furthermore, we observed a reduced risk for each year reduction in time between menarche and menopause (HR = 0.98, 95% CI: 0.95, 1.00). Moreover, we observed a 3% reduction in ovarian cancer risk for each year that total menstrual life span was reduced (HR = 0.97, 95% CI: 0.95, 0.99). This association did not change after exclusion of women whose menopause was induced.

Ovarian cancer risk increased by 4% per year delay in conception (HR = 1.04, 95% CI: 0.99, 1.09; Table 5). This observation remained essentially unchanged after adjustment for total number of children (results not shown). No difference in ovarian cancer risk was observed between ever-married nulliparous women and never-married nulliparous women (HR = 1.04, 95% CI: 0.65, 1.68; OC users were excluded).

We found no evidence of effect measure modification by any of the potential effect modifiers (results not shown). In addition, we observed no clear heterogeneity across the serous, endometrioid, and mucinous subtypes. As the number

Table 2. Reproductive Factors in Association With Ovarian Cancer Risk in the Netherlands Cohort Study on Diet and Cancer, 1986–2002

	No. of Cases	Person-Years in the Subcohort	Age Adjusted		Multivariate Adjusted[a]	
			HR	95% CI	HR	95% CI
Parity						
Nulliparous	88	5,961.0	1.00	Referent	1.00	Referent
Parous	287	28,624.5	0.68	0.53, 0.89	0.71	0.55, 0.93
No. of children						
0	88	5,961.0	1.00	Referent	1.00	Referent
1–2	130	10,539.9	0.85	0.63, 1.15	0.88	0.65, 1.19
3–4	108	11,653.7	0.64	0.47, 0.86	0.66	0.49, 0.90
>4	49	6,430.9	0.51	0.35, 0.74	0.53	0.36, 0.78
P for trend[b]				<0.001		<0.001
Overall trend per term pregnancy	375	34,585.5	0.90	0.85, 0.95	0.91	0.86, 0.96
Age at first birth, years						
<20	3	690.4	0.51	0.16, 1.61	0.51	0.15, 1.69
20–24	63	7,331.3	1.00	Referent	1.00	Referent
25–29	152	14,043.6	1.24	0.93, 1.67	1.25	0.91, 1.71
≥30	68	6,381.7	1.20	0.85, 1.69	1.21	0.83, 1.75
P for trend[b]				0.16		0.15
Overall trend per year increase	286	28,447.0	1.02	0.99, 1.05	1.02	0.99, 1.05
Hysterectomy						
No	342	28,825.2	1.00	Referent	1.00	Referent
Yes	33	5,760.2	0.49	0.34, 0.72	0.50[c]	0.34, 0.72

Abbreviations: CI, confidence interval; HR, hazard ratio.
[a] Adjusted for age and oral contraceptive use (ever/never).
[b] Calculated by using the median for each category and modeled as a continuous variable.
[c] Additionally adjusted for parity (number of children).

of cases became very small for this subanalysis, the confidence intervals became relatively wide.

DISCUSSION

In this prospective study, parity, OC use, and hysterectomy substantially reduced epithelial ovarian cancer risk. In addition, ovarian cancer risk was reduced with earlier age at menopause, per year of being pregnant, for shorter time intervals between menarche and menopause, and per year reduction in total menstrual life span. For pregnancies, the protective effect was strongest. Furthermore, we observed an increased ovarian cancer risk by increasing time to pregnancy.

We need to underscore some population characteristics that make this cohort unique but must be kept in mind when interpreting the results. In our cohort, birth rates were high, with a median of 3 and a range of 0–15. Only 24.1% of these women reported ever use of OCs. In addition, women started using OCs at a relatively late age: a mean of 40 years. All women were postmenopausal, and the mean age of participants at baseline was 62 years. Therefore, results may not be generalizable to premenopausal women.

The major strengths of our study include its prospective design, with detailed exposure and covariate assessment prior to diagnosis. Subjects were followed for up to 16.3 years, with a nearly complete follow-up.

A potential source of bias is possible underreporting of oophorectomies by hysterectomized women. Because excluding hysterectomized women did not alter the results, this factor is unlikely to have substantially affected our results. In addition, recall of reproductive factors and surgeries by women aged 55–69 years could lead to some misclassification. Pregnancies are expected to be recalled accurately regardless of age; however, other reproductive and hormonal factors are likely to be recalled less accurately. Exposure and covariate information was assessed independently of the outcome; therefore, misclassification is most likely undifferential. Another limitation of our study is that a proxy had to be used for reduced fertility. Although time to pregnancy is a validated measure of biologic fertility, we were not able to directly determine it (39–41). Therefore, we used time to childbirth after marriage as a surrogate measure for time to pregnancy. Because birth control measures were sparse during these women's reproductive life, especially in the years between their marriage and their first

Table 3. Exogenous Hormone Use in Association With Ovarian Cancer Risk in the Netherlands Cohort Study on Diet and Cancer, 1986–2002

	No. of Cases	Person-Years in the Subcohort	Age Adjusted		Multivariate Adjusted[a]	
			HR	95% CI	HR	95% CI
OC use						
Never	310	25,916.9	1.00	Referent	1.00	Referent
Ever	65	8,668.6	0.67	0.49, 0.91	0.71	0.52, 0.97
Duration of OC use, years						
Never	310	25,916.9	1.00	Referent	1.00	Referent
≤5	32	3,246.6	0.87	0.58, 1.31	0.92	0.61, 1.38
>5	22	4,443.2	0.44	0.28, 0.71	0.47	0.30, 0.76
Age at first OC use, years						
≤40	31	5,211.8	1.00	Referent	1.00	Referent
>40	32	3,170.7	1.36	0.78, 2.39	1.28[b]	0.68, 2.43
Age at last OC use, years						
≤45	18	2,910.8	0.91	0.51, 1.60	0.51[b]	0.24, 1.10
>45	42	5,282.9	1.00	Referent	1.00	Referent
HRT use						
Never	314	28,679.8	1.00	Referent	1.00	Referent
Ever	44	4,175.4	0.97	0.69, 1.36	0.97[c]	0.69, 1.37
Age at first HRT use, years						
≤50	22	2,114.4	1.00	Referent	1.00	Referent
>50	17	1,500.2	1.10	0.56, 2.18	0.96[c]	0.47, 1.97
Age at last HRT use, years						
≤50	12	1,275.0	1.00	Referent	1.00	Referent
>50	28	2,241.0	1.28	0.61, 2.66	1.39[c]	0.63, 3.03

Abbreviations: CI, confidence interval; HR, hazard ratio; HRT, hormone replacement therapy; OC, oral contraceptive.

[a] Adjusted for age and parity (number of children).

[b] Additionally adjusted for duration of OC use.

[c] Additionally adjusted for duration of HRT use.

child, this measure should adequately estimate time to pregnancy. For the same reason, nulliparity among married women might be seen as a valuable proxy for reduced fertility. Nevertheless, results should be interpreted with caution. Finally, the sample size limited our ability to analyze data by histologic subtype.

In line with our results, other studies consistently observed a decreased ovarian cancer risk for parous women, with increasing parity (2, 3, 5, 6, 11, 15, 16, 18, 19, 21, 22), for OC users, and with increasing duration of OC use (7, 12–15, 21, 22, 42–44). OCs were introduced in the early 1960s, when women in our study population were aged 33–47 years. Mean age at first OC use was 40 years. Our results thus imply that OC use is preventive against ovarian cancer, even when initiated at an older age. The incessant ovulation hypothesis, proposed by Fathalla (45), postulates that ovarian cancer develops through repeated trauma to the covering epithelium of the ovary during ovulation.

Recent findings implicate the fallopian tube fimbria as a possible site of origin of ovarian carcinomas (46–48). Piek et al. (49) revisited the incessant ovulation hypothesis and suggested that incessant ovulation increases ovarian cancer risk by increasing the risk of inclusion of exfoliated tubal epithelial cells into the ovarian stroma and by increasing mitotic activity within tubal epithelium. According to this hypothesis, parity and OC use reduce ovarian cancer risk by reducing the lifetime ovulation numbers. Also concordant with this hypothesis, we observed a clear trend per year reduction in total menstrual life span. Although we could estimate menstrual life span only crudely, our observation of a 3% decrease in risk for each year reduction in total menstrual life span is consistent with the 2.5%–6% increase in ovarian cancer risk associated with each ovulation year observed in other studies (19, 34, 50).

When all factors relating to menstrual life span were analyzed simultaneously, we found that pregnancies had

Table 4. Reductions in Years of Menstrual Life Span in Association With Ovarian Cancer Risk in the Netherlands Cohort Study on Diet and Cancer, 1986–2002

	No. of Cases	Person-Years in the Subcohort	HR	95% CI
Risk for each year that menarche is delayed	313	26,501.9	1.02[a]	0.95, 1.09
Risk for each year that menopause is advanced in time[b]	313	26,501.9	0.98[a]	0.95, 1.01
Risk per year of OC use	313	26,501.9	0.95[a]	0.91, 0.99
Risk per year of being pregnant[c]	313	26,501.9	0.90[a]	0.83, 0.98
Risk per year reduction in time between menarche and menopause[d]	349	31,796.0	0.98[e]	0.95, 1.00
Risk per year reduction in total menstrual life span[f]	313	26,501.9	0.97[e]	0.95, 0.99

Abbreviations: CI, confidence interval; HR, hazard ratio; OC, oral contraceptive.

[a] Mutually adjusted for the other risk factors in the table (except for year reduction in time between menarche and menopause and year reduction in total menstrual life span) and for age.

[b] Age at natural menopause was entered in the model with a minus sign.

[c] Calculated as follows: (number of children × 0.75).

[d] Calculated as the time interval between menarche and menopause and entered into the model with a minus sign.

[e] Age adjusted.

[f] Calculated as follows: (age at natural menopause − age at menarche − duration of OC use − total years of being pregnant) and entered into the model with a minus sign.

the strongest protective effect on ovarian cancer. This observation, along with the observed protective effect of OCs initiated at an older age, suggests that development of ovarian cancer is more complex than could be explained by anovulatory action alone. However, the result regarding reductions in total menstrual life span is limited by the lack of information on breastfeeding, menstrual patterns, and incomplete pregnancies. Besides, accurate estimation of menstrual life span is difficult because menstrual cycles vary within and between women, and not all menstrual cycles are ovulatory. Therefore, our association for total menstrual life span might be underestimated. The World Cancer Research Fund concluded in 2007, based on 1 cohort and 10 case-control studies, that there is only limited evidence suggesting that lactation protects against ovarian cancer (51). However, 1 cohort and 1 case-control study, published after the World Cancer Research Fund report, found inverse associations between total duration of breastfeeding and ovarian cancer (52, 53). Therefore, our observation of a stronger protective effect of pregnancies compared with other factors could reflect a protective role of breastfeeding.

Table 5. Reduced Fertility in Association With Ovarian Cancer Risk in the Netherlands Cohort Study on Diet and Cancer, 1986–2002

	No. of Cases	Person-Years in the Subcohort	HR[a]	95% CI
Time to pregnancy[b]	282	28,017.8	1.04	0.99, 1.09
Nulliparity among ever-married women[c]	39	5,961.0	1.04	0.65, 1.68

Abbreviations: CI, confidence interval; HR, hazard ratio.

[a] Adjusted for age.

[b] Calculated as time between marriage and first birth (in years).

[c] Ever-married women versus never-married women.

Excessive stimulation of ovarian tissue by hormones such as pituitary gonadotropins, estrogens, and androgens is also suggested to increase ovarian cancer risk (54, 55). Pregnancies and OCs suppress pituitary gonadotropin secretion. Moreover, OCs reduce endogenous androgen and estrogen levels. Another mechanism by which parity and OCs might reduce ovarian cancer risk is by increasing circulating progesterone levels. Moreover, it has been proposed that pregnancies clear malignantly transformed cells from the ovaries (2). This alternate hypothesis was recently extended to the cell clearance hypothesis supported by Rostgaard et al. (56), which is based on the idea that a fraction of the genetically modified (premalignant) cells are cleared after each pregnancy.

In line with other studies (4, 8–10, 13, 17, 21), we found that risk of ovarian cancer was decreased for women with a history of hysterectomy. According to the androgen hypothesis, hysterectomy might reduce ovarian cancer by reducing testosterone levels (57). It also eliminates or reduces uterine growth factors involved in ovarian cancer pathogenesis (8). Moreover, hysterectomy alters ovarian blood flow and consequently impairs ovarian function (58–60). Furthermore, hysterectomy may reduce ovarian cancer development by blocking access of ovarian carcinogens that enter the peritoneal cavity via the vagina (4). Recently, a novel hypothesis regarding the origin of ovarian cancer was proposed by Massuger et al. (61), in which serous ovarian cancer is hypothesized to originate in the uterus. This hypothesis, if correct, easily explains the protective effect of hysterectomies.

In contrast with other studies (2, 18, 21, 22, 62), we did not observe a reduced ovarian cancer risk with increasing age at first birth. A higher age at first birth in other studies could indicate a longer duration of OC use; in our population, as stated before, women started using OCs at an older age.

We observed a decreased ovarian cancer risk per year that menopause was advanced in time, which is consistent with most studies (9, 34, 63–66) but contrasts with others (18, 21, 67). A younger age at menopause indicates less exposure to ovulatory cycles and might therefore decrease ovarian cancer risk according to the incessant ovulation hypothesis.

Our finding of a lack of association between age at menarche and ovarian cancer risk is in line with most studies (18, 34, 63, 67, 68), but not all (15, 21). We also did not observe a clear association of age at first and last use of OCs with ovarian cancer risk, which again is consistent with most studies (12, 14, 42, 44), but not all (43, 69).

In this study, ever use of HRT was not associated with ovarian cancer risk. This result agrees with results of

a collaborative analysis of 12 case-control studies by Whittemore et al. (21), but it contradicts results from most studies, including 4 meta-analyses, that found an increased ovarian cancer risk for ever users of HRT (15, 21, 70–74). Consistent with the estrogen hypothesis, associations in these studies were mostly stronger for unopposed estrogen therapy users (54, 55). Because progestins were added to HRT in 1985 (75), we assume that almost all HRT prescribed before the start of the Netherlands Cohort Study on Diet and Cancer consisted of oral estrogen therapy. Most likely, the group of women who used HRT in our population was too small to detect associations.

If increased ovarian cancer risk for nulliparous women stems from difficulties in conceiving, ovarian cancer risk should be higher with prolonged time to pregnancy and for nulliparous women who married compared with nulliparous women who never married. Indeed, prolonged time to pregnancy elevated ovarian cancer risk in our study, which is consistent with previous studies (20, 21, 25, 26, 28). However, we did not observe a difference between ever-married nulliparous women and never-married nulliparous women. This lack of association could be due to the relatively small number of cases for this particular analysis. Therefore, we cannot form any reliable conclusion regarding the effect of subfertility on ovarian cancer risk.

The analysis according to histologic subtype was limited by the small number of cases in the different strata and the relatively high number of cases with not-otherwise-specified adenocarcinoma (*International Classification of Diseases for Oncology* code 8140/3). Therefore, we were unable to observe clear differences between these subtypes.

In conclusion, we observed a reduced ovarian cancer risk with increasing parity, increasing duration of OC use, hysterectomy, younger age at natural menopause, and per year reduction of total menstrual life span. We provided evidence that OC use is protective, even when initiated at an older age. Moreover, we found an increased ovarian cancer risk with prolonged time to pregnancy. Additional research is needed to further elucidate the different biologic pathways of ovarian carcinogenesis.

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