

1 Association Between Menopausal Estrogen-Only Therapy and Ovarian Carcinoma Risk

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87

88 **Precis**

89

90 Menopausal estrogen-only therapy use is significantly associated with increased risk of serous
91 and endometrioid ovarian carcinomas, especially among current, long-term users.

92 **Abstract**

93 Objective: To describe the association between postmenopausal estrogen-only therapy use and
94 risk of ovarian carcinoma, specifically with regard to disease histotype and duration and timing
95 of use.

96
97 Methods: We conducted a pooled analysis of 906 women with ovarian carcinoma and 1,220
98 controls; all 2,126 women included reported having had a hysterectomy. Ten population-based
99 case-control studies participating in the Ovarian Cancer Association Consortium (OCAC), an
100 international consortium whose goal is to combine data from many studies with similar methods
101 so reliable assessments of risk factors can be determined, were included. Self-reported
102 questionnaire data from each study were harmonized and conditional logistic regression was
103 used to examine estrogen therapy's histotype-specific and duration and recency of use
104 associations.

105
106 Results: 43.5% of the controls reported previous use of estrogen therapy. Compared to them,
107 current-or-recent estrogen therapy use was associated with an increased risk for the serous
108 (51.4%, OR=1.63, 95% CI 1.27-2.09) and endometrioid (48.6%, OR=2.00, 95% CI 1.17-3.41).
109 In addition, statistically significant trends in risk according to duration of use were seen among
110 current-or-recent postmenopausal estrogen therapy users for both ovarian carcinoma histotypes
111 ($p_{\text{trend}} < 0.001$ for serous and endometrioid). Compared to controls, current-or-recent users for ten
112 years or more had increased risks of serous ovarian carcinoma (36.8%, OR=1.73, 95% CI 1.26-
113 2.38) and endometrioid ovarian carcinoma (34.9%, OR=4.03, 95% CI 1.91-8.49).

114

115 Conclusions: We found evidence of an increased risk of serous and endometrioid ovarian
116 carcinoma associated with postmenopausal estrogen therapy use, particularly of long duration.
117 These findings emphasize that risk may be associated with extended estrogen therapy use.
118

119 **Introduction**

120 Menopausal hormone therapy (HT) containing estrogens is used to relieve climacteric
121 symptoms and prevent osteoporosis among postmenopausal women. Prior to the results of the
122 Women’s Health Initiative (WHI) in 2002,¹ approximately 13 million women in the United
123 States used HT, and while this number declined after the WHI, there are still approximately 5
124 million HT users.²

125 A comprehensive meta-analysis by Pearce et al, which included 14 population-based
126 studies of women ages 18 to 79, showed that use of estrogen-only therapy (ET) was associated
127 with increased risk of ovarian carcinoma (relative risk per 5 years of use=1.22).³ Recent studies
128 since then have shown similar results^{2,4-6}, but important aspects remain unclear including
129 whether differences exist by disease histotype or by duration and timing of use. The recent
130 pooled analysis by the Collaborative Group on Epidemiological Studies on Ovarian Cancer
131 (Collaborative Group)² did report histotype-specific findings for serous and endometrioid
132 cancers, but not for mucinous and clear cell cancers. They also found little trend in association
133 with duration of use, contrary to the results of several studies.^{3,4,6-9} Notably, the Collaborative
134 Group’s analysis included the majority of studies in Pearce et al’s meta-analysis in which a
135 duration association was found. Clarifying these features could have important implications
136 clinically and for risk stratification purposes.

137 Estrogen-only therapy is one of the most commonly used HT types, hence a more
138 complete characterization of the ET-ovarian carcinoma association is warranted. We have
139 undertaken a pooled analysis of data from the Ovarian Cancer Association Consortium (OCAC)
140 to assess ET’s histotype-specific, duration and recency of use associations with risk of ovarian
141 carcinoma.

142 **Materials and Methods**

143 The OCAC is an international multidisciplinary consortium founded in 2005
144 (<http://apps.ccge.medschl.cam.ac.uk/consortia/ocac/index.html>). Since many groups worldwide
145 are conducting studies to identify risk factors and genetic variation associated with ovarian
146 carcinoma risk, the goal of the OCAC is to provide a forum in which data from many individual
147 studies with similar methods can be combined so reliable assessments of the risks associated
148 with these factors can be determined. Data were sent by each study investigator to the
149 consortium data coordinating center at Duke University, which cleaned and harmonized these
150 data.

151 For the pooled analysis presented here, 10 population-based case-control studies that
152 were individually conducted and contributed data to the OCAC were included, with seven
153 conducted in the United States and three in Europe. Details regarding each study have been
154 published previously,¹⁰⁻²⁰ but their main characteristics as well as any overlap with the
155 Collaborative Group's pooled analysis are presented in Table 1. Cases were women with initial
156 diagnoses of primary ovarian carcinoma (women with primary fallopian tube and peritoneal
157 tumors were excluded). Eligible tumor types included serous, mucinous, endometrioid, and clear
158 cell ovarian carcinomas as well as other epithelial tumor types that were not classified as one of
159 these four main ovarian carcinoma histotypes including mixed cell and Brenner tumors;
160 borderline-malignant tumors were excluded. Controls were women with ovaries (a single ovary
161 was acceptable), who had not been diagnosed with ovarian carcinoma at the time of interview.
162 Reference dates for the women in the studies were usually the dates of diagnosis for the cases
163 and the dates of interview for the controls. The data used in this analysis considered events

164 occurring only prior to the reference dates. All studies included in this analysis had approval
165 from ethics committees and written informed consent was obtained from all study participants.

166 There was a total of 8,095 ovarian carcinoma patients and 13,434 controls across the ten
167 OCAC studies. However, only women who reported having had a simple hysterectomy (without
168 bilateral oophorectomy) were included in our analysis since estrogen-only therapy use is very
169 infrequent among women with intact uteri as it is a confirmed risk factor for endometrial
170 cancer,^{21,22} leaving us with 1,432 cases and 1,995 controls. Additional exclusions included
171 women who were less than 50 years of age at reference date (n=387), had a prior primary cancer
172 diagnosis (excluding non-melanoma skin cancer) (n=399), or were missing or had unknown HT
173 information (n=141). We also excluded women who had used HT in an estrogen-progestin
174 combined form (n=246) for simplicity of presentation and since its use is likely to skew the
175 primary effect of estrogen-only therapy. Only women classified as non-Hispanic white, Hispanic
176 white, or black were considered, hence our final subject set consisted of 2,126 women who had
177 undergone hysterectomy, with 906 ovarian carcinoma cases and 1,220 controls (Figure 1).

178 Information regarding HT use in all forms as well as potential confounding variables
179 selected a priori, including age, race-ethnicity, education, oral contraceptive (OC) use, parity,
180 endometriosis, tubal ligation, age at menarche, and body mass index (typically one year before
181 the reference date), was reported by means of self-completed questionnaires or in-person or
182 phone interviews; we did not have information on previous salpingectomy or *BRCA* status at the
183 time of this analysis. The questions used to ascertain HT use and, more specifically, estrogen-
184 only therapy use are presented in Appendix 1, available online at <http://links.lww.com/xxx>.

185 Age at menopause among women who have had a simple hysterectomy cannot be
186 determined since the women are no longer menstruating but may still have functioning ovaries.

187 Hence, in our primary analysis here, we have only considered estrogen-only therapy use after age
188 50 given that 50 is the approximate average age at menopause for women in these populations.²³
189 The majority of estrogen-only therapy use before age 50 is thus likely to be use when the women
190 were still having regular ovulatory cycles. Given that menopause plays a central role in ovarian
191 carcinoma etiology, it is possible that the added estrogen exposure during the period when
192 endogenous levels of estrogen are naturally high (i.e., before menopause) is less important than
193 exposure at older ages, the majority of which will be in the postmenopausal period.²⁴ Hence, for
194 the analysis presented here, we have defined estrogen-only therapy use as use after age 50, with
195 women who only used estrogen-only therapy before age 50 included in the baseline ‘never’ users
196 group. We also conducted sensitivity analyses to see if the results were affected if true ‘never’
197 users were used as the baseline comparison group and if estrogen-only therapy use was
198 considered regardless of age at use.

199 A common approach to dealing with the problem of an unknown age at menopause for
200 women who had a hysterectomy is to use their age at simple hysterectomy as their age at
201 menopause. Hence, we conducted a sensitivity analysis to assess the association between
202 estrogen-only therapy use and ovarian carcinoma risk using such an approach. We also
203 conducted sensitivity analyses using ages 48 and 52 instead of 50 as the age at menopause.

204 Estrogen-only therapy use was categorized in terms of its recency and its duration of use
205 (in years). Current use was defined as having last used estrogen-only therapy within the past
206 year, recent use as within the last one to four years, and past use as five or more years before the
207 reference date. Because current and recent estrogen-only therapy users showed similar effects,
208 they were combined in the analyses presented here. Duration of estrogen-only therapy use was
209 summed over all episodes of use and the total categorized into the following groups: ‘never’

210 (including <1 year), 1 to <5 years, 5 to <10 years, and 10 or more years of use. Women who used
211 estrogen-only therapy for less than one year were included in the baseline ‘never’ users group as
212 the recall of such short-term use may be greater in cases than controls. All data were cleaned and
213 checked for internal consistency and clarifications were requested from the study investigators
214 when needed.

215 Study, age, race-ethnicity, education, and OC use were included in all statistical models.
216 We conditioned on study, age in five-year groups (50-54, 55-59, 60-64, 65-69, 70-74, 75+; finer
217 stratification after age 75 was not warranted due to small numbers), race-ethnicity (non-Hispanic
218 white, Hispanic white, and black), and education (less than high school, high school, some
219 college, and college graduate or higher) and we adjusted for OC use in categories as ordinal
220 variables (‘never’ (including <1 year), 1 to <2 years, 2 to <5 years, 5 to <10 years, and 10 or
221 more years for OC use). Tubal ligation, endometriosis, parity, body mass index, and age at
222 menarche were also considered, but their inclusion did not change the beta coefficients for the
223 association between ET use and ovarian carcinoma (including overall, serous, or endometrioid)
224 by more than 10% so the results given below are only adjusted for OC use. Overall, cases were
225 missing 1.7% and 1.1% and controls 1.4% and 0.7% for OC use and education, respectively;
226 missing categories were created for these women so their data could be used in the analysis.

227 Conditional logistic regression was used to calculate odds ratios (ORs) and their 95%
228 confidence intervals (CIs) for the association between estrogen-only therapy use and risk of
229 ovarian carcinoma. This was done for all ovarian carcinoma cases combined and for its four
230 main histotypes. Similar analytic approaches were applied when assessing the effects of recency
231 and duration of use. All p-values reported are two-sided. All analyses were performed using SAS
232 9.4.

233 **Results**

234 Data from 906 women with ovarian carcinoma (567 serous, 113 endometrioid, 49
235 mucinous, 42 clear cell, 135 epithelial but not specified as one of the four main histotypes) and
236 1,220 controls, all of whom had a simple hysterectomy, were included in our analysis. Of these
237 women, 460 cases (50.8%) and 531 controls (43.5%) reported ever having used estrogen-only
238 therapy after age 50. Compared with the women in the control group, women who had used
239 estrogen-only therapy after age 50 had a 30% increased risk of ovarian carcinoma as shown in
240 Table 2 (50.8%, OR=1.30, 95% CI 1.06-1.59). Most of this risk elevation was observed among
241 long-term users of estrogen-only therapy for 10 years or more (both current or recent and past
242 users).

243 In addition, the estrogen-only therapy-ovarian carcinoma association appeared to show
244 distinct histotype-specific associations as presented in Table 3 (serous and endometrioid) and
245 Appendix 2, available online at <http://links.lww.com/xxx> (mucinous and clear cell). Compared
246 with the women in the control group, current or recent estrogen-only therapy use was statistically
247 significantly associated with an increased risk of both serous (51.4%, OR=1.63, 95% CI 1.27-
248 2.09) and endometrioid (48.6%, OR=2.00, 95% CI 1.17-3.41) histotypes, but not mucinous
249 (31.3%, OR=0.93, 95% CI 0.43-2.00) and clear cell (39.0%, OR=0.87, 95% CI 0.40-1.88)
250 histotypes, although the confidence limits for the mucinous and clear cell effect estimates were
251 wide due to small numbers of cases. When we looked at high-grade (moderately differentiated,
252 poorly differentiated, undifferentiated) and low-grade (well differentiated) serous ovarian
253 carcinomas separately, we found increased risks for both and hence the results for all serous
254 cases combined are given.

255 Trends in association with duration of estrogen-only therapy use were observed for the
256 serous ($p_{\text{trend}} < 0.001$) and endometrioid ($p_{\text{trend}} < 0.001$) histotypes among current or recent
257 estrogen-only therapy users. Across all histotypes and duration and timing categories, estrogen-
258 only therapy appeared to have the strongest association with risk of endometrioid ovarian
259 carcinoma; compared with the women in the control group, current or recent, long-term users of
260 estrogen-only therapy for 10 years or more had over a four-fold increased risk (34.9%, OR=4.03,
261 95% CI 1.91-8.49). Current or recent, long-term users also had nearly a two-fold increased risk
262 of serous ovarian carcinoma (36.8%, OR=1.73, 95% CI 1.26-2.38) when compared with women
263 in the control group. In addition, there appeared to be elevated risks of 1.49, 2.07, and 1.82 for
264 overall, serous, and endometrioid ovarian carcinoma, respectively, when we compared past,
265 long-term ET users to our baseline ‘never’ user group (Tables 2 and 3).

266 Because we assumed that all women in our analysis had an age at menopause of 50, we
267 conducted a sensitivity analysis in which each woman’s age at simple hysterectomy was used as
268 her age at menopause, with the duration and timing of use variables re-categorized as such. The
269 results by duration, timing of ET use, and histotype slightly attenuated with ORs of 1.46, 1.64,
270 and 3.72 among current-or-recent ET users of 10 years or more for ovarian carcinoma overall
271 and the serous and endometrioid histotypes, respectively (Appendix 3, available online at
272 <http://links.lww.com/xxx>). Sensitivity analyses that used a true ‘never’ user baseline group and
273 redefined ET use regardless of age at menopause or with ages 48 and 52 as the age at menopause
274 did not affect the overall findings (data not shown).

275

276 **Discussion**

277 Most population-based case-control studies and cohort studies have shown that estrogen-
278 only therapy use is associated with an increased risk of ovarian carcinoma and considering our
279 findings together with those recently published by the Collaborative Group,² it seems clear that
280 estrogen-only therapy is associated with risk for the serous and endometrioid histotypes of the
281 disease. We found greater increased risk for those who used estrogen-only therapy for 10 years
282 or more, including those who last used it more than 5 years in the past, whereas the Collaborative
283 Group² did not. This was surprising given that the individual studies that contributed the most
284 statistical information to their analysis (the Million Women Study (MWS)⁷ and the Danish Sex
285 Hormone Register Study (DaHoRS)⁴) reported duration associations with estrogen-only therapy
286 use in their primary publications. The meta-analysis from Pearce et al³ showed evidence of an
287 estrogen-only therapy duration-ovarian carcinoma risk association as well.

288 From a biological standpoint, an elevated risk of endometrioid ovarian carcinoma with
289 estrogen-only therapy use is not surprising given that the cells of origin are histologically similar
290 to endometrial tissue,²⁵ and estrogen-only therapy use is a confirmed risk factor for endometrial
291 cancer.²¹ Danforth et al²⁶ had suggested that estrogen-only therapy may act through similar
292 biologic mechanisms in the development of endometrioid tumors as it does in endometrial
293 cancer. Given the increased risk we see for endometrioid ovarian carcinoma and the well-
294 established association between endometriosis and the endometrioid and clear cell histotypes,²⁷
295 we assessed the estrogen-only therapy risk association according to previous history of
296 endometriosis or not, but did not see any heterogeneity in risk (data not shown).

297 Although the exact mechanism by which estrogen-only therapy might affect serous and
298 endometrioid ovarian carcinoma risk remains unknown, estrogens have long been implicated as
299 etiologic factors.²⁸ Ovarian carcinogenesis may be a result of the direct effects of unopposed

300 estrogen and an estrogen-rich environment, which would potentially be enhanced by estrogen-
301 only therapy use. The use of estrogen-only therapy may also directly stimulate the growth of
302 premalignant or early malignant cells with long-term use increasing the risk of transformation or
303 proliferation.²⁹ In addition, the fallopian tube fimbriae , a proposed cell of origin for high-grade
304 serous carcinoma, have been shown to proliferate at times when estrogenic influences are greater
305 during the menstrual cycle,^{30,31} and this increased activity results in greater cell proliferation
306 which may enhance the risk of mutations and malignant transformation. Estradiol has also been
307 shown to increase ovarian carcinoma cell proliferation in vitro³² and influence the growth of
308 ovarian tumors in a transplanted mouse model.³³ Therefore, while several hypotheses have been
309 put forth to explain ovarian carcinoma etiology, unopposed estrogen appear to play an important
310 role.

311 Limitations of our analysis include the self-reported nature of our data. Because case-
312 control studies inquire about previous exposures when subjects are already aware of their disease
313 status, recall bias is possible as cases may be more likely to search for explanations for their
314 disease and assign greater significance to past events than controls. However, studies have
315 shown high agreement between self-reported estrogen use and prescription data.³⁴ In addition,
316 case patients have not been shown to preferentially report HT use more than controls.³⁵ We
317 considered estrogen-only therapy use only after age 50 to be relevant in an attempt to mainly
318 consider only use after ovarian function had ceased. Sensitivity analysis showed little effect
319 when changing this to age 48 or 52, the latter which will only include use that is almost all in the
320 postmenopausal period.

321 A potential concern with case-control studies such as those included in our analysis is
322 that some ineligible women (those who had a bilateral oophorectomy) could have been recruited

323 as controls even though they would not be at risk of developing ovarian carcinoma. However,
324 oophorectomy results in a loss of estrogen production, which may make such women more likely
325 to use estrogen-only therapy, thus potentially biasing our findings towards the null. If this type
326 of bias is present, any association between estrogen-only therapy use and risk of ovarian
327 carcinoma would be underestimated.

328 Our analysis offers evidence of an increased risk of ovarian carcinoma with ET use after
329 the age of 50. This is especially true for risk of serous and endometrioid tumors for long
330 durations of use, shedding light on the distinct histotype-specific etiologies. Although ET use has
331 declined since the WHI, a significant number of women continue to use it today. Physicians and
332 patients should be aware of the risk of ovarian carcinoma associated with its long-term use.

333

334 **References**

- 335 1. Rossouw JE, Anderson GL, Prentice RL, et al. Risks and benefits of estrogen plus
336 progestin in healthy postmenopausal women: principal results From the Women's Health
337 Initiative randomized controlled trial. *JAMA*. 2002;288(3):321-333.
- 338 2. Collaborative Group On Epidemiological Studies Of Ovarian C, Beral V, Gaitskell K, et
339 al. Menopausal hormone use and ovarian cancer risk: individual participant meta-analysis
340 of 52 epidemiological studies. *Lancet*. 2015;385(9980):1835-1842.
- 341 3. Pearce CL, Chung K, Pike MC, Wu AH. Increased ovarian cancer risk associated with
342 menopausal estrogen therapy is reduced by adding a progestin. *Cancer*. 2009;115(3):531-
343 539.
- 344 4. Morch LS, Lokkegaard E, Andreassen AH, Kruger-Kjaer S, Lidegaard O. Hormone
345 therapy and ovarian cancer. *JAMA*. 2009;302(3):298-305.
- 346 5. Tsilidis KK, Allen NE, Key TJ, et al. Menopausal hormone therapy and risk of ovarian
347 cancer in the European prospective investigation into cancer and nutrition. *Cancer*
348 *Causes Control*. 2011;22(8):1075-1084.
- 349 6. Hildebrand JS, Gapstur SM, Feigelson HS, Teras LR, Thun MJ, Patel AV.
350 Postmenopausal hormone use and incident ovarian cancer: Associations differ by
351 regimen. *Int J Cancer*. 2010;127(12):2928-2935.
- 352 7. Beral V, Million Women Study C, Bull D, Green J, Reeves G. Ovarian cancer and
353 hormone replacement therapy in the Million Women Study. *Lancet*.
354 2007;369(9574):1703-1710.
- 355 8. Riman T, Dickman PW, Nilsson S, et al. Hormone replacement therapy and the risk of
356 invasive epithelial ovarian cancer in Swedish women. *J Natl Cancer Inst*.
357 2002;94(7):497-504.
- 358 9. Moorman PG, Schildkraut JM, Calingaert B, Halabi S, Berchuck A. Menopausal
359 hormones and risk of ovarian cancer. *Am J Obstet Gynecol*. 2005;193(1):76-82.
- 360 10. Royar J, Becher H, Chang-Claude J. Low-dose oral contraceptives: protective effect on
361 ovarian cancer risk. *Int J Cancer*. 2001;95(6):370-374.
- 362 11. Balogun N, Gentry-Maharaj A, Wozniak EL, et al. Recruitment of newly diagnosed
363 ovarian cancer patients proved challenging in a multicentre biobanking study. *J Clin*
364 *Epidemiol*. 2011;64(5):525-530.
- 365 12. Lurie G, Terry KL, Wilkens LR, et al. Pooled analysis of the association of PTGS2
366 rs5275 polymorphism and NSAID use with invasive ovarian carcinoma risk. *Cancer*
367 *Causes Control*. 2010;21(10):1731-1741.
- 368 13. Ness RB, Dodge RC, Edwards RP, Baker JA, Moysich KB. Contraception methods,
369 beyond oral contraceptives and tubal ligation, and risk of ovarian cancer. *Ann Epidemiol*.
370 2011;21(3):188-196.
- 371 14. Moorman PG, Calingaert B, Palmieri RT, et al. Hormonal risk factors for ovarian cancer
372 in premenopausal and postmenopausal women. *Am J Epidemiol*. 2008;167(9):1059-1069.
- 373 15. Wu AH, Pearce CL, Tseng CC, Templeman C, Pike MC. Markers of inflammation and
374 risk of ovarian cancer in Los Angeles County. *Int J Cancer*. 2009;124(6):1409-1415.
- 375 16. Terry KL, De Vivo I, Titus-Ernstoff L, Shih MC, Cramer DW. Androgen receptor
376 cytosine, adenine, guanine repeats, and haplotypes in relation to ovarian cancer risk.
377 *Cancer Res*. 2005;65(13):5974-5981.

- 378 17. Pike MC, Pearce CL, Peters R, Cozen W, Wan P, Wu AH. Hormonal factors and the risk
379 of invasive ovarian cancer: a population-based case-control study. *Fertil Steril*.
380 2004;82(1):186-195.
- 381 18. Bodelon C, Cushing-Haugen KL, Wicklund KG, Doherty JA, Rossing MA. Sun exposure
382 and risk of epithelial ovarian cancer. *Cancer Causes Control*. 2012;23(12):1985-1994.
- 383 19. Risch HA, Bale AE, Beck PA, Zheng W. PGR +331 A/G and increased risk of epithelial
384 ovarian cancer. *Cancer Epidemiol Biomarkers Prev*. 2006;15(9):1738-1741.
- 385 20. Glud E, Kjaer SK, Thomsen BL, et al. Hormone therapy and the impact of estrogen
386 intake on the risk of ovarian cancer. *Arch Intern Med*. 2004;164(20):2253-2259.
- 387 21. Beral V, Bull D, Reeves G, Million Women Study C. Endometrial cancer and hormone-
388 replacement therapy in the Million Women Study. *Lancet*. 2005;365(9470):1543-1551.
- 389 22. Grady D, Gebretsadik T, Kerlikowske K, Ernster V, Petitti D. Hormone replacement
390 therapy and endometrial cancer risk: a meta-analysis. *Obstet Gynecol*. 1995;85(2):304-
391 313.
- 392 23. Morabia A, Costanza MC. International variability in ages at menarche, first livebirth,
393 and menopause. World Health Organization Collaborative Study of Neoplasia and
394 Steroid Contraceptives. *Am J Epidemiol*. 1998;148(12):1195-1205.
- 395 24. Pike MC. Age-related factors in cancers of the breast, ovary, and endometrium. *J*
396 *Chronic Dis*. 1987;40 Suppl 2:59S-69S.
- 397 25. Kumar V CR, Robbins SL. *Basic Pathology*. 6 ed. Philadelphia: W.B. Saunders; 1997.
- 398 26. Danforth KN, Tworoger SS, Hecht JL, Rosner BA, Colditz GA, Hankinson SE. A
399 prospective study of postmenopausal hormone use and ovarian cancer risk. *Br J Cancer*.
400 2007;96(1):151-156.
- 401 27. Pearce CL, Templeman C, Rossing MA, et al. Association between endometriosis and
402 risk of histological subtypes of ovarian cancer: a pooled analysis of case-control studies.
403 *Lancet Oncol*. 2012;13(4):385-394.
- 404 28. Ho SM. Estrogen, progesterone and epithelial ovarian cancer. *Reprod Biol Endocrinol*.
405 2003;1:73.
- 406 29. Zhou H, Luo MP, Schonthal AH, et al. Effect of reproductive hormones on ovarian
407 epithelial tumors: I. Effect on cell cycle activity. *Cancer Biol Ther*. 2002;1(3):300-306.
- 408 30. Donnez J, Casanas-Roux F, Caprasse J, Ferin J, Thomas K. Cyclic changes in ciliation,
409 cell height, and mitotic activity in human tubal epithelium during reproductive life. *Fertil*
410 *Steril*. 1985;43(4):554-559.
- 411 31. George SH, Milea A, Shaw PA. Proliferation in the normal FTE is a hallmark of the
412 follicular phase, not BRCA mutation status. *Clin Cancer Res*. 2012;18(22):6199-6207.
- 413 32. Nash JD, Ozols RF, Smyth JF, Hamilton TC. Estrogen and anti-estrogen effects on the
414 growth of human epithelial ovarian cancer in vitro. *Obstet Gynecol*. 1989;73(6):1009-
415 1016.
- 416 33. Laviolette LA, Hodgkinson KM, Minhas N, Perez-Iratxeta C, Vanderhyden BC. 17beta-
417 estradiol upregulates GREB1 and accelerates ovarian tumor progression in vivo. *Int J*
418 *Cancer*. 2014.
- 419 34. Sandini L, Pentti K, Tuppurainen M, Kroger H, Honkanen R. Agreement of self-reported
420 estrogen use with prescription data: an analysis of women from the Kuopio Osteoporosis
421 Risk Factor and Prevention Study. *Menopause*. 2008;15(2):282-289.
- 422 35. Paganini-Hill A, Clark LJ. Comparison of patient recall of hormone therapy with
423 physician records. *Menopause*. 2007;14(2):230-234.

424 **Table 1.** Description of studies included in analysis

Study Name	Time Period	Location	Case Ascertainment	Control Ascertainment	Controls (mean/IQR for age)	Cases (mean/IQR for age)	Serous	Mucinous	Endometrioid	Clear cell
Connecticut Ovary Study (CON) ¹⁹	2002-2009	USA; CT	Cancer registry or hospital records	Random digit dialing, Health Care Financing Administration records	49 (60.9/13)	54 (62.2/12)	28	4	12	4
Disease of the Ovary Study and their Evaluation (DOV) ¹⁸	2002-2009	USA; WA	Cancer registry	Random digit dialing	224 (66.8/12)	159 (62.7/8)	108	3	15	3
German Ovarian Cancer Study (GER) ^{10,*}	1992-1998	Germany	Admissions to all hospitals serving the study regions	Population registries	89 (60.9/11)	34 (61.5/12)	17	2	2	1
Hawaii Ovarian Cancer Study (HAW) ¹²	1994-2007	USA; HI	Cancer registry	Department of Health Annual Survey, Health Care Financing Administration records	40 (67.3/16)	32 (66.1/17)	18	1	5	2
Hormones and Ovarian Cancer Prediction (HOP) ^{13,*}	2003-2008	USA; western PA, northeast OH, western NY	Cancer registries, pathology databases, physicians' offices	Random digit dialing	201 (65.5/16)	100 (66.6/15.6)	57	5	17	4
Malignant Ovarian Cancer Study (MAL) ²⁰	1994-1999	Denmark	Cancer registry, gynecological departments	Random digit dialing	84 (62.6/13)	47 (61.7/11)	25	4	8	5
North Carolina Ovarian Cancer Study (NCO) ¹⁴	1999-2008	USA; NC	Cancer registry	Random digit dialing	126 (62.7/11)	153 (62.9/10)	94	5	16	11
New England Case-Control Study of Ovarian Cancer (NEC) ¹⁶	1999-2008	USA; NH and eastern MA	Cancer registries, hospital tumor boards	Random digit dialing, town books, drivers' license lists	67 (63.2/11)	50 (63.4/12)	38	1	6	1
United Kingdom Ovarian Cancer Population Study (UKO) ¹¹	2006-2007	United Kingdom	Gynecological Oncology NHS centers	Women in the general population participating in the United Kingdom Collaborative Trial of Ovarian Cancer Screening (UKCTOCS)	116 (64.0/9)	56 (67.0/12)	30	7	12	4
University of Southern California. Study of Lifestyle and Women's Health (USC) ^{15,17,*}	1993-2005	USA; Los Angeles, CA	Cancer registry	Neighborhood controls	224 (62.9/12.5)	217 (64.7/12)	152	17	20	7
Total:					1220	906 [†]	567 [†]	49 [†]	113 [†]	42 [†]

425 Note: All studies used in-person interviews except GER, which used self-completed questionnaires. MAL used either in-person or phone interviews.

426 * Some of the study's data were included in the Collaborative Group's² analysis.

427 † Sum of numbers do not equal total number of cases because some cases were not classified as one of the four main histotypes considered.

Table 2. Association between estrogen-only therapy use over age 50 and risk of ovarian carcinoma overall

Categories of ET Use	Number of controls	Number of cases	Median duration (years)	OR*	95% CI	p-value
Never used	689	446	--	1.00	--	--
Ever	531	460	9.20	1.30	1.06 – 1.59	0.013
1 to <5 years	149	92	2.70	1.00	0.72 – 1.39	0.99
5 to <10 years	155	135	7.45	1.27	0.93 – 1.72	0.13
10+ years	227	233	15.12	1.54	1.18 – 2.01	0.002
					p-trend:	0.001
Current-or-recent users†	432	392	10.00	1.35	1.09 – 1.67	0.006
1 to <5 years	103	67	3.00	1.00	0.68 – 1.48	0.99
5 to <10 years	120	112	7.20	1.35	0.96 – 1.90	0.087
10+ years	209	213	15.20	1.53	1.17 – 2.02	0.002
					p-trend:	<0.001
Past users	99	68	6.20	1.07	0.74 – 1.56	0.72
1 to <5 years	46	25	2.20	1.01	0.59 – 1.74	0.97
5 to <10 years	35	23	8.20	1.03	0.57 – 1.86	0.93
10+ years	18	20	13.28	1.49	0.71 – 3.13	0.29
					p-trend:	0.95

Note: OR=odds ratio, CI = confidence interval.

* Adjusted for oral contraceptive use (never (including <1), 1 to <2, 2 to <5, 5 to <10, 10+ years) and conditioned on age (50-54, 55-59, 60-64, 65-69, 70-74, 75+), education (less than high school, high school, some college, college graduate or higher), race-ethnicity (non-Hispanic white, Hispanic white, black), and study.

† Current-or-recent users included those who used estrogen-only therapy within the last five years prior to their reference age.

Table 3. Association between estrogen-only therapy use after age 50 and risk of serous and endometrioid ovarian carcinoma

Categories of ET Use	Number of controls	SEROUS (N=567)				ENDOMETRIOID (N=113)			
		Number of cases	OR*	95% CI	p-value	Number of cases	OR*	95% CI	p-value
Never used	689	252	1.00	--	--	54	1.00	--	--
Ever	531	315	1.57	1.23 – 2.00	<0.001	59	1.82	1.10 – 3.03	0.021
1 to <5 years	149	62	1.26	0.86 – 1.83	0.24	10	0.98	0.45 – 2.15	0.97
5 to <10 years	155	92	1.58	1.11 – 2.25	0.012	17	1.64	0.78 – 3.47	0.19
10+ years	227	161	1.79	1.31 – 2.43	<0.001	32	3.58	1.74 – 7.36	<0.001
				p-trend:	<0.001			p-trend:	<0.001
Current-or-recent users [†]	432	267	1.63	1.27 – 2.09	<0.001	51	2.00	1.17 – 3.41	0.011
1 to <5 years	103	46	1.37	0.88 – 2.14	0.16	7	0.88	0.35 – 2.19	0.78
5 to <10 years	120	74	1.69	1.14 – 2.52	0.010	15	1.72	0.76 – 3.87	0.19
10+ years	209	147	1.73	1.26 – 2.38	<0.001	29	4.03	1.91 – 8.49	<0.001
				p-trend:	<0.001			p-trend:	<0.001
Past users	99	48	1.28	0.83 – 1.96	0.27	8	1.20	0.48 – 3.01	0.69
1 to <5 years	46	16	1.05	0.55 – 2.01	0.89	3	1.35	0.37 – 4.94	0.66
5 to <10 years	35	18	1.26	0.65 – 2.43	0.50	2	1.46	0.25 – 8.66	0.68
10+ years	18	14	2.07	0.89 – 4.79	0.091	3	1.82	0.40 – 8.19	0.44
				p-trend:	0.46			p-trend:	0.35

Note: OR=odds ratio, CI=confidence interval

* Adjusted for oral contraceptive use (never (including <1), 1 to <2, 2 to <5, 5 to <10, 10+ years) and conditioned on age (50-54, 55-59, 60-64, 65-69, 70-74, 75+), education (less than high school, high school, some college, college graduate or higher), race-ethnicity (non-Hispanic white, Hispanic white, black), and study.

[†] Current or recent users included those who used estrogen-only therapy within the last five years prior to their reference age.

Figure 1. Flowchart of analysis exclusions

