Research Article

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Long-term Relationship of Ovulation-Stimulating Drugs to Breast Cancer Risk

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Abstract

Background: Although fertility drugs stimulate ovulation and raise estradiol levels, their effect on breast cancer risk remains unresolved.

Methods: An extended follow-up was conducted among a cohort of 12,193 women evaluated for infertility between 1965 and 1988 at five U.S. sites. Follow-up through 2010 was achieved for 9,892 women (81.1% of the eligible population) via passive as well as active (questionnaires) means. Cox regression determined HRs and 95% confidence intervals (CI) for fertility treatments adjusted for breast cancer risk factors and causes of infertility.

Results: During 30.0 median years of follow-up (285,332 person-years), 749 breast cancers were observed. Ever use of clomiphene citrate among 38.1% of patients was not associated with risk (HR = 1.05; 95% CI, 0.90–1.22 vs. never use). However, somewhat higher risks were seen for patients who received multiple cycles, with the risk for invasive cancers confirmed by medical records being significantly elevated (HR = 1.69; 95% CI, 1.17–2.46). This risk remained relatively unchanged after adjustment for causes of infertility and multiple breast cancer predictors. Gonadotropins, used by 9.6% of patients, mainly in conjunction with clomiphene, showed inconsistent associations with risk, although a significant relationship of use with invasive cancers was seen among women who remained nulligravid (HR = 1.98; 95% CI, 1.04–3.60).

Conclusions: Although the increased breast cancer risk among nulligravid women associated with gonadotropins most likely reflects an effect of underlying causes of infertility, reasons for the elevated risk associated with multiple clomiphene cycles are less clear.

Impact: Given our focus on a relatively young population, additional evaluation of long-term fertility drug effects on breast cancer is warranted. *Cancer Epidemiol Biomarkers Prev; 23(4); 584–93. ©2014 AACR.*

Introduction

Although there has been extensive debate about the effects of fertility drugs on ovarian cancer risk, less attention has focused on relationships with breast cancer. The concern surrounding ovarian cancer has centered around the incessant ovulation hypothesis (1), given the effectiveness of fertility drugs at stimulating ovulation. Although also relevant for breast cancer (2), further concerns are raised by the fact that these drugs increase estradiol levels (3), another mechanism by which risk could be enhanced (4).

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Despite the biologic plausibility, results of epidemiologic studies of fertility drugs and breast cancer present a mixed picture, with some showing increases in risk (5–8), others showing decreases (9,10) and still others showing no substantial associations (11–18). Some of the conflicting results may be because of limited power, or to imprecise information on drug usage, particularly in case–control studies where exposures are self-reported. Some investigations have combined all drugs, despite seemingly different biologic effects. For example, it has been suggested that clomiphene citrate, a selective estrogen receptor modulator chemically similar to tamoxifen, may lead to risk reductions (9). Many studies have also been limited by an inability to control for breast cancer risk factors that are highly correlated with drug exposures, such as reproductive status, causes of infertility, and personal and family disease histories.

We assembled a large cohort of infertile women with detailed information on causes of infertility, fertility drugs, and potential breast cancer risk factors. In a previous follow-up involving a median of 18.8 years of follow-up (11), we found no substantial associations of either clomiphene or gonadotropins on risk, but were hampered in assessing detailed relationships by the

relatively young age of the women and limited numbers of breast cancers ($n = 292$). We have updated the follow-up to further clarify these relationships.

Materials and Methods

Study subject eligibility

Study subjects were women who had sought infertility advice between 1965 and 1988 at 5 reproductive endocrinology practices in Boston, MA; Chicago, IL; Detroit, MI; Palo Alto, CA; and New York City, NY. These practices were chosen because they retained all records and had evaluated large numbers of infertility patients, many of whom received high doses of ovulation-stimulating drugs. This study was approved by institutional review boards at the National Cancer Institute and the participating institutions.

Patients were eligible for study if they had a U.S. address at first evaluation and were seen more than once or had been referred by another physician who provided relevant medical information. Patients with either primary or secondary infertility were eligible, but those evaluated for reversal of a tubal ligation were not. A total of 12,193 patients met eligibility criteria.

Trained personnel abstracted data about the infertility workup (all procedures and tests), medications prescribed, menstrual and reproductive histories, and other factors that might affect health (e.g., weight). Information on the clinical workup was used to define causes of infertility, as previously described (19).

Follow-up of patients

An initial attempt at follow-up was pursued during 1998 to 2001 (11). Because of the relatively young age of the patients at that time, a second follow-up attempt was initiated in 2010. Follow-up procedures included searches for deaths and updated addresses through several publically available and proprietary databases (Social Security Administration Death Master File, SSA DMF; MaxCOA, a change of address service; LexisNexis, a legal database service; U.S. Postal Service National Change of Address); and the Center for Disease Control National Death Index. Attempts were made to mail a short questionnaire to located subjects who did not expressly indicate that they wanted no further follow-up. This questionnaire focused on the development of cancers and cancer risk factors that might have changed over time (e.g., reproductive and menopause status).

In addition to information on cancers identified through death records and completed questionnaires, we completed linkages against cancer registries in the 14 states in which the majority of patients resided (Arizona, California, Connecticut, Florida, Illinois, Indiana, Massachusetts, Michigan, New Hampshire, New Jersey, New York, Ohio, Pennsylvania, and Texas). For the 12.4% of patients who resided outside of these states, outcome information was dependent on completed questionnaires, with attempts to validate any self-reports of cancers by requesting records from the patients' treating physicians. Another SSA DMF search was completed at the end of the study in 2010 to identify new deaths.

The flow chart for inclusion and exclusion of study subjects is shown in Figure 1. After excluding the 1,319 patients who requested no additional follow-up, 8 who were enrolled twice, 6 found to be <18 years of age, 1 who requested removal from the study, and 1 with a missing date of birth, we were able to obtain information related to death, development of cancer, or date last known alive and free of cancer for 10,018 patients—or all but 840 subjects (7.7%) of the remaining 10,858 study subjects. Information on last known vital status and the development of incident cancers through 2010 was available from completed questionnaires or cancer registry linkages for 9,404 patients, from earlier follow-up efforts for 469 patients, and from information available one or more years after first infertility evaluation in their original clinic records for 145 patients. A total of 749 breast cancers were identified: 607 had documented information in cancer registry records, 61 were verified through medical records, 28 were identified from death certificates or NDI Plus, and 53 comprised self-reports from completed questionnaires.

Analytic approaches

Person-years were accrued beginning 1 year after the date of first infertility evaluation at study clinics and continued through the earliest date of any cancer occurrence, death, date last known alive, and free of cancer, or, if vital status depended on cancer registry linkage, a variable ending date, depending on when each registry had complete information (range of 2008–2010). We excluded from analysis 15 patients with missing information on a cancer diagnosis date and 111 with less than 1 year of follow-up (11 of whom had a diagnosis of breast cancer), leaving 9,892 analytic study subjects and 285,332 person-years of follow-up.

Information on clomiphene and gonadotropins that was abstracted from medical records included age at first use, treatment cycles, and total cumulative dosage. Race, gravidity, and/or parity at study entry, causes of infertility and body mass at study entry were also defined through clinic records. Other potential confounding factors were obtained through questionnaire data, supplemented, as appropriate, by information in clinic records. The 1998 to 2001 questionnaire obtained extensive information on menstrual and reproductive history, use of exogenous hormones, anthropometric factors, cigarette smoking, alcohol consumption, and screening for breast and ovarian diseases. The 2010 questionnaire obtained updated information on reproductive history, body size, gynecologic operations, use of menopausal hormones, and mammographic screening history. Questionnaires were obtained from 6,756 patients (68.3% of the analysis subjects), 5,511 completed the 1998 to 2001 questionnaire, and 4,824 the 2010 questionnaire (3,579 completed both). From these questionnaires, 543 women with a first cancer of breast cancer were identified.

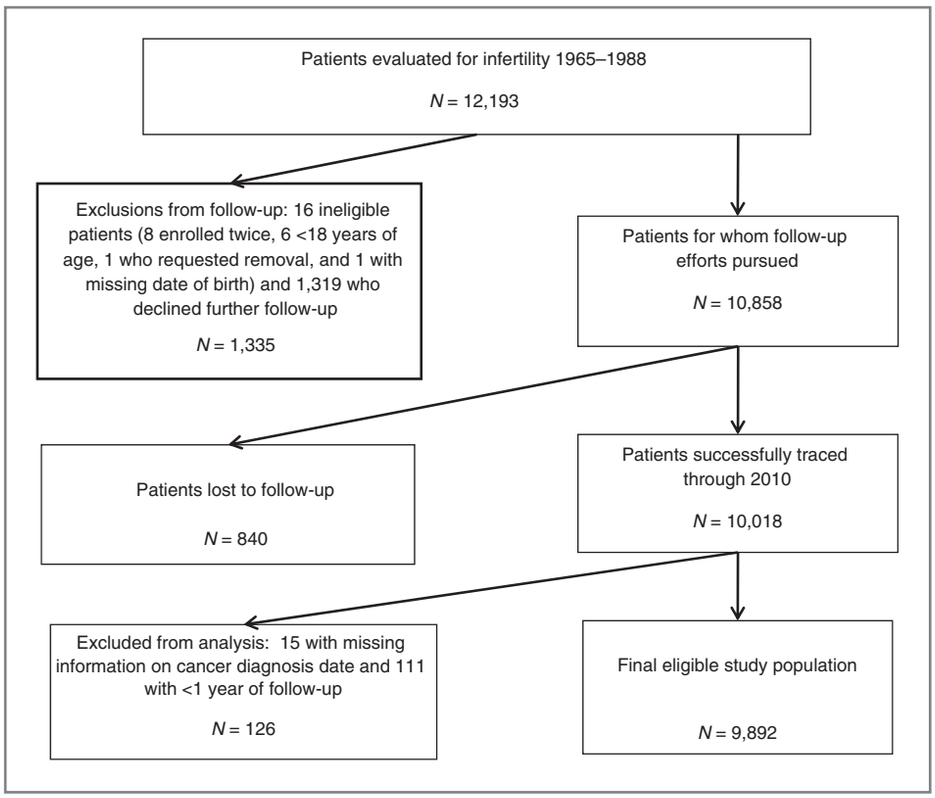

Figure 1. Flow chart of inclusion and exclusion of study participants.

Statistical analyses

HRs and 95% confidence intervals (CI) for breast cancer associated with fertility treatments, with adjustment for study site, calendar year of first infertility evaluation, and gravidity at first clinic visit, were obtained using Cox proportional hazards regression with age as the time metric. We considered the impact on risk of a variety of additional potential confounding factors, including race, age at first birth, age at menarche, family history of breast cancer, BMI at first clinic visit, and various causes of infertility; however, these had minimal effects on drug relationships and we thus chose to present risks based on a parsimonious model. Missing information was assigned a separate level for each exposure and included in the models. Tests for linear trends across cycle and dose categories were calculated using an ordinal variable. We also tested the assumption of proportional hazards for fertility treatments using the Wald test of interaction with the time-scale (continuous).

Results

After exclusion of the 16 ineligible subjects, a comparison of demographic information for the 9,892 patients who were traced for cancer outcomes versus the 2,285 for whom follow-up information was not available showed larger proportions of exclusions from analysis for subjects from the New York and Boston practices (primarily because of the incompleteness of social security numbers for these patients, which hindered location efforts) and for those with missing information on race (Table 1). Less substantial differences in exclusion rates were seen according to calendar year and age at first clinic visit.

Among the analytic cohort of 9,892 women, the mean age at first evaluation for infertility was 30.1 years. During a median of 30.0 years of follow-up, 749 breast cancers were identified among study participants, with a mean age at diagnosis of 52.7 years.

The identified risk factors for breast cancer generally mirrored those found in other populations of young women, including somewhat higher risks associated with being nulligravid at either the first clinic visit or at follow-up, late ages at first birth and having a mother or sister with breast cancer, and somewhat lower risks with late ages at menarche and being heavy (Table 2). Other factors, including use of exogenous hormones (either oral contraceptives or menopausal hormones), cigarette smoking, alcohol consumption, and mammographic screening history, were not substantially or significantly related to risk (data not shown). In this cohort of infertile women, endometriosis and anovulation were the infertility causes most strongly related to risk, although neither was a significant predictor.

A total of 38.1% of the patients had been exposed to clomiphene and 9.6% to gonadotropins. After adjustment for study site and calendar year and gravidity at first clinic visit, ever use of clomiphene was unrelated to risk as compared with nonuse of either clomiphene or gonadotropins (HR = 1.05; 95% CI, 0.90–1.22; Table 3). There was some evidence of increasing risk with increasing cycles of

Table 1. Comparison of demographic factors of subjects included and excluded from analyses

	Subjects in follow-up analysis ($n = 9,892$)		Subjects excluded from analysis ($n = 2,285$)[a]		
	n	%	*n*	%	χ^2 *P* value
Study site					<0.001
New York and Boston	3,211	32.5	879	38.5	
Chicago	2,882	29.1	602	26.4	
Detroit	2,168	21.9	454	19.9	
Palo Alto	1,631	16.5	350	15.3	
Calendar year at first clinic visit					0.03
<1975	2,546	25.7	607	26.6	
1975–1979	3,433	34.7	848	37.1	
1980–1984	2,941	29.7	618	27.1	
1985–1988	972	9.8	212	9.3	
Age at first clinic visit					
<25 years	879	8.9	219	9.6	0.001
25–29 years	3,875	39.2	803	35.2	
30–34 years	3,542	35.8	831	36.3	
35–39 years	1,323	13.4	355	15.5	
40+ years	273	2.8	77	3.4	
Race					
White	7,514	76.0	1413	61.8	<0.0001
African-American	451	4.6	105	4.6	
Other	535	5.4	126	5.5	
Unknown	1,392	14.1	641	28.1	

[a]Includes 1,319 patients who did not wish to be followed, 840 who were lost to follow-up, 111 with less than 1 year of follow-up, and 15 with missing information on a date of cancer diagnosis. Does not include 16 patients who were considered ineligible for study: 8 enrolled twice and 6 less than 18 years at first visit, 1 who requested removal from the study, and 1 with a missing birthdate.

clomiphene, with the risk rising to 1.37 (0.97–1.92) for those who received ≥12 cycles. This did not seem to reflect longer follow-up times among those with multiple cycles, given that we found no relationship of follow-up interval to risk when examined as a time-varying covariate. Slightly elevated, although nonsignificant, risks were also seen for women who received the highest cumulative dosages (≥2,251 mg; HR = 1.20; 95% CI, 0.97–1.48). When we assessed risks among women who had both a high cumulative dose ≥2251 mg and ≥6 cycles, the risk was statistically significantly elevated (HR = 1.27; 95% CI, 1.02–1.59, 96 exposed cases). We also saw some risk elevation among women whose first use of clomiphene occurred at 35 years or older (HR = 1.31; 95% CI, 1.00–1.73).

Ever use of gonadotropins was not significantly associated with breast cancer risk (HR = 1.14; 95% CI, 0.89–1.44). Furthermore, there were no trends according to dosage, number of cycles, or age at first use. Analyses considering whether women only received clomiphene or gonadotropins as opposed to both drugs sequentially revealed that the majority of women (81.5%) prescribed gonadotropins had also received clomiphene initially. Risks for use of either drug alone were similar to those identified when there was not consideration of joint exposure. Furthermore, those using both drugs sequentially were not at further elevated risk.

To separately assess risks for the invasive versus *in situ* cancers, we focused on the 696 of the 749 breast cancer (92.9%) for whom it was possible to obtain medical validation through cancer registry or medical records. When analyses were restricted to these validated cancers (Table 4), an increased risk emerged for women exposed to 12 or more clomiphene cycles (HR = 1.45; 95% CI, 1.02–2.05; $P_{trend} = 0.20$), with a significantly elevated risk (HR = 1.69; 95% CI, 1.17–2.46; $P_{trend} = 0.16$) seen for the validated invasive cancers. An increased risk associated with clomiphene use was not seen for the validated *in situ* cancers, although small numbers of users were involved. A nonstatistically significant risk was observed for the validated invasive cancers related to use of gonadotropins (HR = 1.28; 95% CI, 0.98–1.67), although without convincing trends according to dosage, number of cycles, or age at first use.

Hormone receptor status was available for 39% of the validated breast cancers. To the extent that we could evaluate meaningful differences, there did not seem to be discrepant results about the relationship of fertility drug use according to hormone receptor status; however,

Table 2. Relationship of selected demographic and other factors to breast cancer risk

Selected demographic and other factors	Breast cancers ($n = 749$)	Person-years	HR[a]	95% CI
Race				
White	590	216,237	1.00	Referent
African-American	29	12,392	0.97	0.66–1.42
Other	39	14,864	0.91	0.66–1.27
Unknown	91	41,839	0.77	0.62–0.96
Reproductive status at first clinic visit				
Gravid	417	163,834	1.00	Referent
Nulligravid	332	121,498	1.11	0.96–1.28
Reproductive status at follow up		285,332		
Gravid	515	203,492	1.00	Referent
Nulligravid	120	41,300	1.13	0.92–1.38
Unknown	114	40,540	1.13	0.92–1.38
Number of births at follow up				
≥3	72	30,983	1.00	Referent
2	150	51,899	1.21	0.92–1.61
1	118	44,285	1.04	0.78–1.40
0	177	61,282	1.16	0.88–1.53
Unknown number of births	67	32,868	0.82	0.59–1.14
Missing information on parity	165	64,015	1.07	0.81–1.42
Age at first birth (years)				
<25	56	31,012	1.00	Referent
25–29	113	49,038	1.25	0.91–1.73
≥30	226	72,241	1.50	1.12–2.02
Nulliparous	177	61,282	1.45	1.07–1.96
Unknown	177	71,759	1.27	0.94–1.72
Age at menarche				
<12	155	56,605	1.00	Referent
12	224	77,421	1.06	0.86–1.30
13	212	84,922	0.91	0.74–1.12
≥14	139	58,404	0.85	0.68–1.07
Unknown	19	7,980	0.88	0.54–1.42
Mother or sister with breast cancer				
No	348	129,572	1.00	Referent
Yes	76	18,087	1.54	1.20–1.97
Unknown	325	137,673	0.86	0.74–1.00
Body mass index at first clinic visit (quartiles, kg/m^2)				
<18.5	44	15,616	1.01	0.74–1.38
18.5–22.9	371	127,608	1.00	Referent
23.0–24.9	72	31,414	0.81	0.63–1.04
25–29.9	64	28,962	0.80	0.61–1.04
≥30.0	23	12,683	0.69	0.45–1.06
Unknown	175	69,049	0.84	0.68–1.02
Cause of infertility[b]				
Endometriosis	189	63,979	1.12	0.93–1.35
Anovulation	225	81,152	1.13	0.96–1.32
Tubal disease/pelvic adhesions	269	99,781	1.03	0.88–1.21
Cervical disorder	52	17,725	1.06	0.79–1.42
Uterine disorder	86	30,092	0.96	0.76–1.21
Male factor	177	65,421	1.02	0.85–1.22

[a]HRs adjusted for study site and calendar year of first infertility evaluation. Inclusion of other variables in the table did not appreciably change risk estimates.

[b]Risks are relative to women with no evidence of the condition, taking into account the adequacy of the evaluation. Conditions are not mutually exclusive, that is, women could be classified as having more than one cause of infertility.

Table 3. Relationship of clomiphene and gonadotropin use to breast cancer risk

	Breast cancers ($n = 749$)	Person-years	HR[a]	95% CI
Never use of clomiphene or gonadotropins	450	173,457	1.00	referent
Clomiphene use				
Ever use	284	107,036	1.05	0.90–1.22
Dosage (mg)				
1–900	99	36,338	1.06	0.85–1.32
901–2,250	77	34,323	0.88	0.69–1.12
≥2,251	108	36,375	1.20	0.97–1.48
P_{trend}			0.39	
Cycles				
<6	175	69,954	0.97	0.81–1.15
6–11	73	25,670	1.15	0.89–1.48
≥12	36	11,412	1.37	0.97–1.92
P_{trend}			0.21	
Age at first use				
<30	116	49,620	1.07	0.87–1.32
30–34	90	36,504	0.92	0.73–1.16
≥35	62	13,927	1.31	1.00–1.73
Unknown	16	6,985	0.87	0.53–1.44
Gonadotropin use				
Ever use	82	26,639	1.14	0.89–1.44
Dosage (ampules)[b]				
1–25	30	8,927	1.26	0.87–1.83
26–64	25	8,907	1.03	0.69–1.54
≥65	27	8,805	1.12	0.76–1.66
P_{trend}			0.40	
Cycles				
<6	67	22,001	1.12	0.87–1.46
≥6	15	4,638	1.19	0.71–2.00
P_{trend}			0.33	
Age at first use				
<30	24	7,993	1.33	0.88–2.02
30–34	25	11,150	0.85	0.56–1.27
≥35	31	7,123	1.32	0.91–1.92
Unknown	2	373	1.83	0.45–7.36
Combination of clomiphene and gonadotropins				
Clomiphene only	217	85,236	1.02	0.87–1.21
Gonadotropins only	15	4,839	1.12	0.67–1.88
Both	67	21,800	1.14	0.88–1.48

[a]HRs adjusted for study site, calendar year of first infertility evaluation, gravidity at first clinic visit.
[b]One ampule = 75 IU of gonadotropins.

the number of hormone receptor negative tumors was limited (data not shown).

We assessed whether the associations of fertility drugs with breast cancer risk were modified by risk predictors and causes of infertility (Table 5). Although no significant effect modifications were observed, slightly higher risks associated with gonadotropin use was seen among women nulligravid at either first clinic visit or follow-up. The risk among nulligravid women at follow-up was associated with a statistically significant risk for validated invasive breast cancers (HR = 1.98; 95% CI, 1.04–3.60; data not shown). Risks for clomiphene use were similar for nulligravid and gravid women.

Discussion

This study offered an opportunity, within a large cohort of patients with well-documented causes of infertility, to evaluate relationships between fertility drug usage and breast cancer risk, with many of these patients having received extensive exposures. Similar to our previous analysis, the results were generally reassuring, although we did detect some increases among

Table 4. Relationship of clomiphene and gonadotropin use to validated breast cancer cases

	All validated cancers ($n = 696$)			Validated invasive cancers ($n = 536$)			Validated *in situ* cancers ($n = 160$)		
Selected breast cancer risk factors	**No. exposed cases**	**HR[a]**	**95% CI**	**No. exposed cases**	**HR[a]**	**95% CI**	**No. exposed cases**	**HR[a]**	**95% CI**
Never use of clomiphene or gonadotropins	417	1.00	Referent	323	1.00	Referent	94	1.00	Referent
Clomiphene use									
Ever use	264	1.04	0.89–1.22	202	1.04	0.87–1.24	62	1.05	0.76–1.45
Dosage (mg)									
1–900	91	1.04	0.83–1.31	69	1.02	0.79–1.33	22	1.10	0.69–1.75
901–2,250	70	0.85	0.66–1.10	52	0.83	0.61–1.11	18	0.94	0.56–1.56
≥2,251	103	1.22	0.98–1.52	81	1.26	0.99–1.62	22	1.10	0.69–1.76
P_{trend}		0.40			0.41			0.80	
Cycles									
<6	160	0.94	0.78–1.13	119	0.91	0.73–1.12	41	1.05	0.72–1.52
6–11	69	1.16	0.90–1.50	52	1.15	0.86–1.55	17	1.20	0.71–2.02
≥12	35	1.45	1.02–2.05	31	1.69	1.17–2.46	4	0.68	0.25–1.87
P_{trend}		0.20			0.16			0.90	
Gonadotropin use									
Ever use	80	1.17	0.92–1.50	67	1.28	0.98–1.67	13	0.82	0.46–1.48
Dosage (ampules)									
1–25	29	1.30	0.89–1.90	25	1.44	0.96–2.18	4	0.79	0.29–2.17
26–64	24	1.05	0.69–1.59	20	1.13	0.72–1.79	4	0.76	0.28–2.08
≥65	27	1.18	0.80–1.75	22	1.27	0.82–1.96	5	0.91	0.37–2.26
P_{trend}		0.30			0.18			0.78	
Cycles									
<6	65	1.16	0.89–1.51	55	1.27	0.95–1.70	10	0.77	0.40–1.49
≥6	15	1.26	0.75–2.12	12	1.32	0.74–2.36	3	1.07	0.34–3.39
P_{trend}		0.27			0.19			0.93	
Combination of clomiphene and gonadotropins									
Clomiphene only	199	1.00	0.85–1.19	146	0.96	0.79–1.17	53	1.14	0.81–1.60
Gonadotropins only	15	1.18	0.70–1.98	11	1.14	0.62–2.08	4	1.33	0.48–3.64
Both	65	1.17	0.90–1.53	56	1.31	0.99–1.75	9	0.71	0.35–1.41

[a]HRs adjusted for study site, calendar year of first infertility evaluation, gravidity at first clinic visit.

Table 5. Relationship of clomiphene and gonadotropin use to breast cancer risk according to selected breast cancer risk factors and causes of infertility

	Clomiphene (ever vs. never use of clomiphene or gonadotropins)			Gonadotropins (ever vs. never use of clomiphene or gonadotropins)		
	No. exposed cases	HR[a]	95% CI	No. exposed cases	HR[a]	95% CI
Age at follow-up						
<50 y	100	0.86	0.67–1.11	25	0.90	0.58–1.38
50–59 y	134	1.09	0.87–1.37	44	1.38	0.99–1.93
≥60 y	50	0.94	0.66–1.33	13	0.82	0.45–1.47
Reproductive status at first clinic visit						
Gravid	170	1.11	0.91–1.35	46	1.10	0.80–1.51
Nulligravid	114	0.97	0.77–1.22	36	1.22	0.85–1.75
Reproductive status at follow-up						
Gravid	200	1.02	0.85–1.23	54	1.04	0.78–1.39
Nulligravid	48	1.20	0.82–1.75	19	1.58	0.93–2.68
Unknown	36	1.04	0.70–1.57	9	1.12	0.55–2.27
Mother or sister with breast cancer						
No	146	1.05	0.85–1.31	45	1.17	0.84–1.64
Yes	36	1.41	0.88–2.27	11	1.29	0.64–2.58
Unknown	102	0.92	0.72–1.17	26	0.95	0.63–1.44
Causes of infertility						
Endometriosis	92	1.23	0.92–1.65	24	1.15	0.72–1.83
Anovulation	120	1.03	0.78–1.35	33	1.02	0.68–1.53
Tubal disease/pelvic adhesions	103	1.12	0.87–1.45	29	1.16	0.77–1.74
Cervical disorder	21	0.77	0.41–1.42	9	0.80	0.36–1.80
Uterine disease	31	1.02	0.64–1.62	10	1.25	0.62–2.51
Male factor	74	1.25	0.92–1.69	20	1.29	0.79–2.11

[a]HRs adjusted for study site, calendar year of first infertility evaluation, gravidity at first clinic visit.

women who had been prescribed the highest dosages of clomiphene.

Previous studies have provided conflicting results about the effects of fertility drugs on breast cancer risk. Some of the discrepancy in findings may relate to small numbers (many studies had less than 100 breast cancer cases; refs. 10, 13, 15, and 20–25) and/or short follow-up durations. The most informative studies have been those that focused on infertile women, allowing for adjustment for potential confounding factors. This includes studies in western Australia (384 cases, 16.3 years; ref. 8), the Netherlands (116 cases, 5.6 years; ref. 18), Denmark (331 cases, 8.8 years; ref. 6), and Israel (153 cases, >30 years; ref. 7). Of these studies, one observed no association between fertility drugs and breast cancer risk (18), whereas the others (6–8) showed some evidence of elevated risks, although restricted in several of the studies to either women exposed at young ages (8) or to those who received progesterone supplementation (6).

With 749 breast cancer cases and 30.0 median years of follow-up, our study had considerably more power than previous studies to evaluate relationships. Overall, clomiphene use, our most extensively used drug, was not associated with risk; however, we observed nonsignificant risk increases with number of cycles prescribed. Risk associated with 12 or more cycles was significantly elevated for validated invasive cancers. In contrast, we observed no increased risk for *in situ* breast cancers, providing little support for the notion (26) of closer surveillance of treated women—as has been suggested for the association of fertility drugs with borderline ovarian cancers (27–29).

We considered whether the increased risks among women with multiple clomiphene cycles might reflect either the indications for usage or a propensity for these subjects to remain nulligravid, but saw no interaction according to causes of infertility or gravidity, nor were the risks confounded by reproductive parameters. It is, however, possible that women with the heaviest drug exposures were also those with resistant infertility, a notion receiving some support given that women with later ages at first exposure were at highest risks. Chance can also not be entirely dismissed given the multiple comparisons undertaken. Nonetheless, some attention may be warranted about the possibility that the increased risk reflects clomiphene's ability to substantially increase endogenous estrogen levels and for these elevations to persist (3). Clomiphene also effectively stimulates ovulation, but whether such a mechanism would be independent of increased estradiol levels is unclear. Although the period of treatment of most women

was relatively short, even with multiple cycles, the increased risk is consistent with another hormonal exposure of short duration, namely diethylstilbestrol given during pregnancy, which has been related to significant increases in breast cancer risk many years after initial exposure (30).

We had less opportunity to evaluate the effects on breast cancer risk of gonadotropins, which are increasingly being used for *in vitro* fertilization (IVF). Although we noted no overall increased breast cancer risk associated with this exposure, we did detect some increases associated with usage among women who remained nulligravid—a relationship seen in one other study (6). A similar subgroup association has been noted for ovarian (27,31,32) cancers, with suggestions that such patients have either resistant types of infertility linked with higher cancer risks, genetic predispositions to both infertility and cancer, or higher drug exposures. Given that the majority of our women who received gonadotropins also received clomiphene, it is likely that the increased risk among nulligravid women reflects an effect on risk of their infertility rather than that of drug usage.

Previous studies have suggested that fertility drugs may have preferential effects among certain subgroups of users, including women with a family history of breast cancer (17) or those who are exposed at either younger (8) or older ages (24). Our study found no drug interactions according to a family history, but did demonstrate slightly higher clomiphene risks for those with later ages at first infertility evaluations. Although a number of tumor characteristics are recognized as important in defining etiologic subsets of breast cancer (33), with limited data we did not detect substantial differences in fertility drug associations for hormone receptor positive versus negative tumors. However, given that other exogenous hormones, including menopausal hormones, have been shown to preferentially affect estrogen receptor positive tumors (34), this issue deserves future attention.

Although our study had a number of strengths, it also had some limitations. Most notably, the precision of some of our derived risks, particularly within subgroups, was limited, requiring cautious interpretation. Furthermore, we had constraints on contacting some women who did not wish continued study participation, which could have affected the generalizability of our results (however, our loss to follow-up rate of 7.7% was quite low given the observation time). Although we had substantially longer follow-up than in our previous analyses, our subjects were still relatively young (average age of patients with breast cancer of 52.7 years). We were also dependent on assessing fertility drug exposures only as recorded in records from our study hospitals and some patients may have sought fertility advice elsewhere. However, patient reports of subsequent treatment were rare (most likely reflecting the relative paucity of specialists from which treatment could be obtained during the period of our study) and adjustment for such reports did not substantially affect the risk estimates based on recorded exposure information. Finally, we did not have information on potential confounders for all women, although we found little evidence of confounding on the basis of available risk factors.

In summary, in this large study of women treated for infertility, we found generally reassuring results about the long-term effects on breast cancer risk of ovulation-stimulating drugs. Gonadotropins were unrelated to risk, except in nulligravid women, most likely reflecting indications for usage. For clomiphene, we found evidence of statistically increased risks among the relatively small group whose drug exposures far exceeded current practices. Continued monitoring of the long-term effects of these drugs seems warranted, especially given that our study participants were still relatively young (and had not yet reached their peak incidence for breast cancer) and that ovulation-stimulating drugs, including gonadotropins—potent ovulation stimulators (40)—are increasingly being used in infertility treatment protocols.

Disclosure of Potential Conflicts of Interest

Carolyn L. Westhoff is a consultant/advisory board member of Merck, Bayer, Agile, and Elsevier. No potential conflicts of interest were disclosed by the other authors.

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