

Breast cancer risk associated with ovulation-stimulating drugs

Louise A. Brinton^{1,7}, Bert Scoccia², Kamran S. Moghissi³, Carolyn L. Westhoff⁴, Michelle D. Althuis¹, Jerome E. Mabile⁵ and Emmet J. Lamb⁶

¹Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD, ²University of Illinois, Chicago, IL, ³Wayne State University, Detroit, MI, ⁴Columbia University, New York, NY, ⁵Information Management Services, Inc., Rockville, MD and ⁶Stanford University, Stanford, CA, USA

⁷To whom correspondence should be addressed at: 6120 Executive Boulevard, Room 7068, Bethesda, MD 20892-7234, USA.
E-mail: brinton@nih.gov

BACKGROUND: Despite the recognized role of hormones in the aetiology of breast cancer, there has been little evaluation of hormonal preparations used to treat infertility. **METHODS:** A retrospective cohort study of 12 193 women evaluated for infertility between 1965 and 1988 at five clinical sites identified 292 *in situ* and invasive breast cancers in follow-up through 1999. Standardized incidence ratios (SIRs) compared breast cancer risks with those of the general population. Analyses within the cohort estimated rate ratios (RRs) associated with medications after adjustment for other breast cancer predictors. **RESULTS:** Infertile patients had a significantly higher breast cancer risk than the general population [SIR = 1.29, 95% confidence interval (CI) 1.1–1.4]. Analyses within the cohort showed adjusted RRs of 1.02 for clomiphene citrate and 1.07 for gonadotrophins, and no substantial relationships to dosage or cycles of use. Slight and non-significant elevations in risk were seen for both drugs after ≥ 20 years of follow-up (RRs = 1.39 for clomiphene and 1.54 for gonadotrophins). However, the risk associated with clomiphene for invasive breast cancers was statistically significant (RR = 1.60, 95% CI 1.0–2.5). **CONCLUSIONS:** Although there was no overall increase in breast cancer risk associated with use of ovulation-stimulating drugs, long-term effects should continue to be monitored.

Key words: breast cancer/epidemiology/infertility/ovulation-stimulating medications/risk

Introduction

The epidemiology of breast cancer has been studied extensively, with many investigations supporting an important aetiological role for endogenous as well as exogenous hormones (Bernstein, 2002). Surprisingly few studies have addressed potential relationships with usage of infertility medications, despite their recognized effects on ovulation and endogenous hormone production. Although concern about potential adverse effects has been raised by a number of clinical reports (Bolton, 1977; Laing *et al.*, 1989; Arbour *et al.*, 1994; Brzezinski *et al.*, 1994; Jourdain *et al.*, 1996; Unkila-Kallio *et al.*, 1997), epidemiological studies have produced varying results. The majority of studies have found no relationships to risk (Ron *et al.*, 1987; Venn *et al.*, 1995; Braga *et al.*, 1996; Modan *et al.*, 1998; Ricci *et al.*, 1999; Doyle *et al.*, 2002; Klip *et al.*, 2002; Lerner-Geva *et al.*, 2003), but for the most part have been limited by small numbers of events or incomplete abilities to control for other correlates of risk, including a variety of well-recognized familial and reproductive risk factors.

A few studies, however, have supported the notion that fertility medications may affect breast cancer risk. Studies have

suggested that these drugs may both increase (Potashnik *et al.*, 1999; Burkman *et al.*, 2003) as well as decrease (Bernstein *et al.*, 1995; Rossing *et al.*, 1996) risk, although these conclusions have been based on relatively few events and results regarding different medications. The most recent investigation (Burkman *et al.*, 2003), a case-control study which involved large numbers of breast cancer patients and careful control for reproductive parameters, reported no association of risk with clomiphene citrate, but elevated risks among women with longer term use of menopausal gonadotrophins. This study, however, relied on patient reports of drug exposures, leading to questions regarding the validity of the implicated drugs. In addition, this study, as well as most others, was unable to fully account for indications for drug usage (i.e. causes of infertility), which may have independent effects on breast cancer risk (Cowan *et al.*, 1981; Brinton *et al.*, 1997; Gammon and Thompson, 1990, 1991; Moseson *et al.*, 1993; Garland *et al.*, 1998; Dor *et al.*, 2002).

The issue of whether infertility drugs are related to breast cancer risk is of public health concern, given the large numbers of women being medically evaluated for infertility and the high incidence of breast cancer. Given that

ovulation-stimulating drugs were first prescribed in the early 1960s, sufficient time has now elapsed to evaluate long-term effects. Clarification of effects is of importance given the substantial numbers of women seeking advice for infertility (Stephen and Chandra, 1998). Furthermore, IVF currently being used for many of these women (Wright *et al.*, 2003) involves high levels of exposure to ovulation-stimulating drugs.

In a retrospective cohort study, involving a large series of women evaluated for infertility beginning in the mid-1960s, we collected extensive information on drug histories, as documented in medical records, along with information on the indications for usage. Through additional information in the medical records as well as direct contact with the patients, we were able to evaluate effects of infertility medications independent of other breast cancer predictors.

Methods

The methods of this investigation have been described previously in relation to ovarian cancers (Brinton *et al.*, 2004). In brief, eligible study subjects comprised women who had sought advice for infertility between 1965 and 1988 at one of five large reproductive endocrinology practices in the following areas: Boston, MA, New York City, NY, Chicago, IL, Detroit, MI and the San Francisco Bay area, CA. These practices were chosen because they had retained all original records and had evaluated large numbers of infertile patients, many of whom received high doses of ovulation-stimulating drugs. The study was approved by the institutional review boards at the collaborating centres as well as at the National Cancer Institute.

Trained abstractors reviewed medical records of all patients evaluated for infertility at these practices to determine eligibility. Patients were eligible for inclusion in the study if they had a US address at the time of evaluation, and if they 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 for inclusion, but those who were evaluated for reversal of a tubal ligation were not. A total of 12 193 met eligibility criteria. Using standardized software, trained abstractors entered data directly into laptop computers. This included patient identifiers as well as information on the work-up for infertility, medications prescribed, menstrual and reproductive histories, and other factors that might affect health status. Abstracted information on infertility drugs included use of clomiphene citrate (hereafter referred to as clomiphene) and a variety of human gonadotrophins, namely Pergonal, Humegon or Metrodin. Details from the clinical work-up were used to define six potentially overlapping causes of infertility (endometriosis, anovulation, tubal disease/pelvic adhesions, male factor, cervical disorders and uterine disorders), with each cause coded on each patient as having no evidence, evidence or incomplete evaluation.

Location information for eligible study subjects was sought through a variety of sources, including clinic records, telephone directories, credit bureaus, postmasters and motor vehicle administration records. Additional information about vital status and development of cancers was obtained by administration of questionnaires to located, living subjects and through linkage of the cohort against selected cancer registries and the National Death Index (NDI). As detailed in Figure 1, a total of 9751 (80.0%) of the patients were traced successfully one or more years after first clinic registration. A total of 1319 (10.8%) of the patients indicated upon contact that

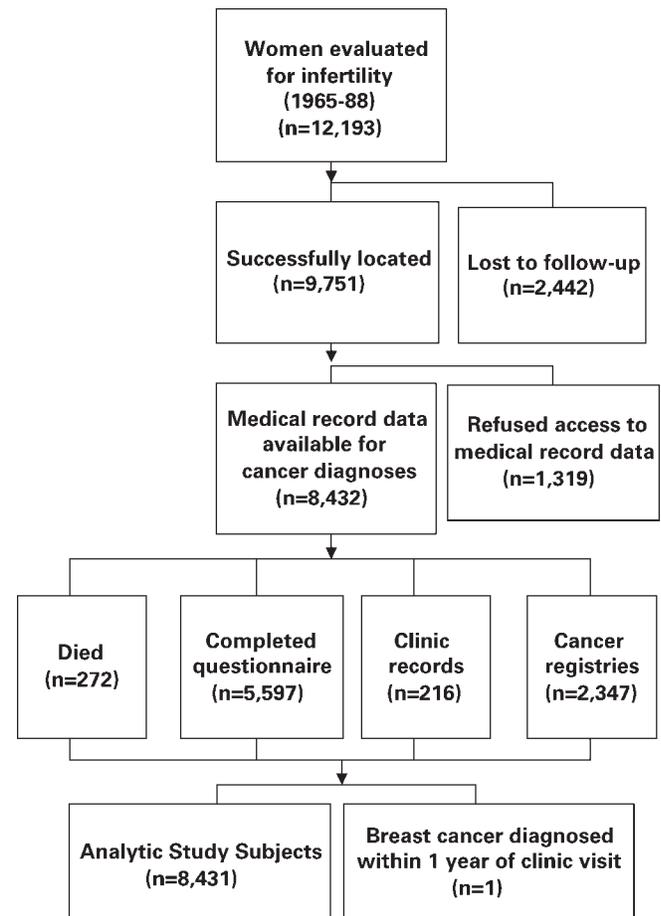


Figure 1. Field and analytical status of eligible study subjects, women evaluated for infertility, 1965–1988.

they did not want to participate in the study and would not allow access to data available in their medical records. Only descriptive information, i.e. calendar year at registration, age at registration and race, was retained for these patients.

A total of 272 of the patients were traced as deceased. For the patients traced as alive, information on the development of cancers was obtained from clinic records, completed questionnaires and cancer registries. Questionnaires initially were mailed to patients beginning in early 1998, with telephone follow-up attempted for non-respondents. A total of 5597 of the patients completed the questionnaire. The questionnaires ascertained information on demographic factors, updated health status, and lifestyle factors that could affect health, including menstrual, pregnancy and breastfeeding history; use of exogenous hormones; anthropometric factors; cigarette smoking; alcohol consumption; and breast and ovarian disease screening histories. An additional 216 patients had follow-up visits one or more years beyond their initial clinic visit. For 2347 patients for whom we were unable to obtain questionnaire data, we had accurate location information that enabled tracing through cancer registries in the states in which the majority of patients were last known to reside, i.e. California, Florida, Illinois, Massachusetts, Michigan, New Jersey, New York and Texas.

Attempts were made to verify medically any cancers reported in the questionnaires by obtaining discharge summaries, operative reports and pathology reports from the institutions where the diseases had been diagnosed and/or treated. Two self-reported cancers found to be benign based on medical record review were excluded.

Additional information on cancers was obtained from the cancer registries, from information on causes of death available from the NDI or copies of death certificates obtained from individual state vital statistics registries. Death certificates which noted cancer as a cause of death were searched for information on the duration of the disease to define an approximate diagnostic date.

Statistical methods

For the women with available medical records who were followed for subsequent cancer diagnoses, person-years were accrued beginning 1 year after clinic registration and continuing through the earliest date of cancer diagnosis, death or date last known alive and free of cancer (as indicated by last clinic visit, questionnaire completion or linkage against cancer registry data). Patients with cancer registry searches had variable study ending dates, depending on the completeness of registration in their states, which ranged from the end of 1997 to 1999. Otherwise, December 31, 1999 defined the end of the study period. Patients lost to follow-up after their initial clinic visit, those who denied access to their records and one woman who was diagnosed with breast cancer during the first year of follow-up were excluded from further analyses, leaving 8431 analytical study subjects and 155 652 person-years of follow-up. Within this cohort, a total of 292 women were found to have developed breast cancer; medical or cancer registry records confirmed 210 of these, death certificates defined 35 and the remaining 47 were self-reported via questionnaires.

We initially calculated standardized incidence ratios (SIRs) and 95% confidence intervals (CIs) comparing breast cancer within the cohort of infertile women with rates for US women. SIRs were computed as the number of observed cancer events among the infertility patients divided by the expected number of events based on age, race and calendar year-specific incidence disease rates for females from cancer registry rates available through the Surveillance Epidemiology and End Results (SEER) Program of the NCI. Standardized mortality ratios (SMRs) were calculated similarly, using US mortality rates to generate expected values. For this analysis, subjects who were located but did not respond to the questionnaire were assumed to be alive and their person-years accrued until the end of follow-up.

Additional analyses were conducted within the cohort of infertile women, allowing exposures to be evaluated after multivariable adjustment for other potential risk factors. Rate ratios (RRs) and their 95% CIs for developing breast cancer associated with administration of ovulation-stimulating drugs (ever use, total dosage, cycles prescribed, interval since first use) as compared with non-users were estimated by Poisson regression using standard likelihood ratio methods (Breslow and Day, 1987). For all analyses, the RRs were adjusted for calendar year (prior to 1980, 1980–1989 and 1990 or later) and age (<40, 40–49 and 50+) at follow-up. Other factors, such as study site, race and causes of infertility, were included in the regression models, as necessary, to evaluate their roles as potential confounding factors or to examine variations of the RRs. In addition, we used data obtained through clinic records or questionnaires to assess confounding and modifying influences of other breast cancer predictors, including gravidity, parity, age at first birth, family history of breast cancer, body mass and breast cancer screening histories.

Results

Table I shows the distribution of the entire cohort and the subjects excluded from analyses. The median years and ages at first evaluation were 1978 and 30 years, respectively.

Table I. Selected demographic factors of women evaluated for infertility

	Subjects in follow-up analysis (n = 8431)		Subjects excluded from analysis (n = 3762)	
	n	%	n	%
Calendar years of initial clinic evaluation				
< 1970	260	3.1	153	4.1
1970–1974	1898	22.5	848	22.5
1975–1979	2909	34.5	1377	36.6
1980–1984	2516	29.8	1048	27.8
1985–1988	848	10.1	336	9.0
Age at initial clinic evaluation				
<25 years	688	8.2	415	11.0
25–29 years	3315	39.3	1370	36.4
30–34 years	3072	36.4	1300	34.7
35–39 years	1124	13.3	557	14.8
40+ years	232	2.8	117	3.1
Unknown	0		3	
Race				
White	6660	79.0	2278	60.5
African-American	392	4.6	164	4.4
Other	471	5.6	191	5.1
Unknown	908	10.8	1129	30.0

Nearly 80% of the subjects were Caucasian. There were no significant differences according to calendar year or age at evaluation between the subjects included and excluded from analyses; however, a larger proportion of the excluded subjects had missing information on race. The median length of follow-up was 18.8 years, with >80% followed for ≥ 15 years. Forty-three percent of the women presented with primary infertility (i.e. no prior pregnancies).

The study subjects were found to have a significantly higher risk of developing breast cancer than the general population (SIR = 1.29, 95% CI 1.1–1.4) (Table II). A total of 3280 (39%) of the study subjects were prescribed clomiphene, while 867 (10%) received gonadotrophins. Breast cancer risks did not vary by histories of clomiphene exposure, with the SIRs being 1.28 (95% CI 1.1–1.5) for those unexposed versus 1.29 (1.1–1.6) for those exposed. Comparable SIRs for gonadotrophins were 1.28 (1.1–1.4) and 1.40 (0.9–2.0).

Cohort members were also compared with the general population with respect to their mortality experience. There were 40 deaths due to breast cancer, resulting in an SMR of 1.58 (95% CI 1.1–2.2). There was no evidence of higher mortality among subjects exposed to clomiphene (SMR, 95% CI = 1.26, 0.6–2.2 versus 1.78, 1.2–2.6 for those unexposed). The majority of deaths (38 or 95%) occurred among the patients not exposed to gonadotrophins.

To assess the effects of drug usage after accounting for other factors that might influence breast cancer risk among infertile women (including reproductive parameters), we focused subsequent analyses on internal comparisons that allowed the calculation of adjusted RRs. The majority of breast cancer risk factors identified in other populations prevailed among the infertility patients (Table III). Notably, higher risks of breast cancer were associated with later ages at first birth, nulliparity and a family history of breast cancer. Lower risks were observed among African-Americans as well as women with later ages at menarche. In addition,

Table II. Standardized incidence ratios comparing breast cancer risk among infertile patients with the general population^a, overall and stratified by infertility drug usage

	Person-years of observation	Observed no. of breast cancers	Expected no. of breast cancers	SIR	95% CI
All subjects	155 652	292	226.70	1.29	1.1–1.4
Ever exposed to clomiphene					
No	96 948	184	143.24	1.28	1.1–1.5
Yes	58 704	108	83.46	1.29	1.1–1.6
Ever exposed to gonadotrophins					
No	140 610	261	204.60	1.28	1.1–1.4
Yes	15 042	31	22.11	1.40	0.9–2.0

^aBased on data from the Surveillance, Epidemiology and End Results (SEER) Program.

we found that obesity was inversely related to risk, consistent with findings that obese women are at a low risk of developing early-onset breast cancers (Ursin *et al.*, 1995) (our average age of onset was 48 years).

We also examined risks according to specific, and often overlapping causes of infertility. There was little variation in risk across the different categories, and patients with endometriosis and anovulation were not at unusual risk (respective RRs of 0.78 and 1.10).

The only risk factor that exerted any confounding influence on the infertility medications was a family history of

breast cancer. After adjustment for this as well as year and age at follow-up, the RR associated with clomiphene use was 1.02 (95% CI 0.8–1.3) (Table IV), with no substantial difference in risk according to dosage or cycles (e.g. RR = 0.92 for ≥ 2251 mg of clomiphene). There was, however, a slight increase in risk with years since initial use, with the RR for clomiphene use being 1.39 (95% CI 0.9–2.1) after ≥ 20 years.

Gonadotrophin use was associated with an adjusted RR of 1.07 (95% CI 0.7–1.6). There were somewhat higher risks among subjects with the highest exposures, but the risks

Table III. Distribution of demographic and other determinants of breast cancer risk

	No cancer (n = 8139)	Breast cancer (n = 292)	Person-years of follow-up (155 652)	RR ^a	95% CI
Race					
White	6418	242	123 302	1.00	
African-American	384	8	6798	0.67	0.3–1.4
Other	450	21	8340	1.19	0.8–1.9
Missing	887	21	17 212	0.60	0.4–0.9
Age at first birth (years)					
< 25	803	18	15 580	1.00	
25–29	1297	44	26 843	1.43	0.8–2.5
≥ 30	1988	84	37 650	1.60	1.0–2.7
Nulliparous	1570	57	30 792	1.38	0.8–2.3
Missing	2481	89	44 787	1.64	1.0–2.7
Age at menarche (years)					
< 12	1619	61	31 512	1.00	
12	2224	82	42 333	1.01	0.7–1.4
13	2451	93	47 285	1.01	0.7–1.4
≥ 14	1650	51	31 173	0.84	0.6–1.2
Missing	195	5	3349	0.81	0.3–2.0
Mother or sister with breast cancer					
No	7554	258	143 540	1.00	
Yes	585	34	12 112	1.52	1.1–2.2
Body mass index at first clinic visit (quartiles, kg/m ²)					
≤ 20.0	1540	73	30 846	1.00	
20.1–21.6	1651	68	31 873	0.88	0.6–1.2
21.7–24.1	1638	50	30 762	0.70	0.5–1.0
≥ 24.2	1638	45	29 776	0.68	0.5–1.0
Missing	1672	56	32 395	0.67	0.5–1.0
Cause of infertility ^b					
Endometriosis	1864	57	35 240	0.78	0.6–1.1
Anovulation	2238	85	44 008	1.10	0.8–1.4
Tubal disease/pelvic adhesions	2897	102	54 031	0.95	0.7–1.2
Male factor	1875	78	36 897	1.22	0.9–1.6
Cervical disorder	556	20	10 664	0.97	0.6–1.6
Uterine disorder	928	28	17 035	0.76	0.5–1.1

^aRate ratios adjusted for calendar year and age at follow-up, study site and mother or sister with breast cancer. Inclusion of other variables in the table did not appreciably change risk estimates.

^bRisks are relative to women with no evidence of the condition, taking into account the adequacy of the evaluation. Conditions are not mutually exclusive, i.e. women could be classified as having more than one cause of infertility.

Table IV. Rate ratios of breast cancer according to usage of clomiphene and gonadotrophins

	Breast cancer (<i>n</i> = 292)	Person-years of follow-up (155 652)	RR ^a	95% CI
Clomiphene				
Never	184	96 948	1.00	
Ever	108	58 704	1.02	0.8–1.3
Dosage (mg)				
1–900	43	20 462	1.15	0.8–1.6
901–2250	33	18 402	0.99	0.7–1.4
≥2251	32	19 840	0.92	0.6–1.3
Cycles				
<6	72	38 069	1.03	0.8–1.4
6–11	27	14 191	1.08	0.7–1.6
≥12	9	6 444	0.88	0.4–1.7
Years since first use				
<10	18	26,448	0.72	0.4–1.2
10–19	52	22,853	0.98	0.7–1.3
≥20	29	5 105	1.39	0.9–2.1
Missing	9	3 391	1.46	0.7–2.9
Gonadotrophins				
Never	261	140 609	1.00	
Ever	31	15 043	1.07	0.7–1.6
Dosage (ampoules) ^b				
1–24	9	5 010	0.93	0.5–1.8
25–64	10	5 243	0.98	0.5–1.8
≥65	12	4 790	1.30	0.7–2.3
Cycles				
<6	24	12 590	0.98	0.6–1.5
≥6	7	2 453	1.50	0.7–3.2
Years since first use				
<10	8	7 888	0.87	0.4–1.8
10–19	15	5 244	1.06	0.6–1.8
≥20	8	1 186	1.54	0.8–3.2
Combination of clomiphene and gonadotrophins				
Neither	181	94 363	1.00	
Clomiphene only	80	46 245	0.97	0.7–1.3
Gonadotrophins only	3	2 585	0.59	0.2–1.8
Both	28	12 459	1.15	0.8–1.7

^aRate ratios adjusted for calendar year and age at follow-up, study site and mother or sister with breast cancer

^bEach ampoule of Pergonal or Humegon consisted of 75 IU of FSH and 75 IU of LH, while each ampoule of Metrodin consisted of 75 IU of FSH.

were modest, not statistically significant and based on relatively small numbers. As with clomiphene, we saw a somewhat higher risk among the subjects with ≥20 years since initial exposure (RR = 1.54, 95% CI 0.8–3.2).

Attempts to consider separate effects of clomiphene and gonadotrophins by adjusting one for the other or by assessing relationships only within those exposed to one agent failed to change previously derived conclusions. Furthermore, women who were exposed to both clomiphene and gonadotrophins did not exhibit an unusual cancer risk compared with non-users of either drug (RR = 1.15).

Cross-classifications of the different exposure measures (dosage, cycles, years since first use) of clomiphene were also pursued (limited numbers of women precluded similar analyses for gonadotrophins). Although these analyses were based on small numbers, it appeared that the major discriminator of risk was years since initial usage, with risk elevations associated with high dosages or multiple cycles only among subjects followed for at least 20 years. For example, among those with ≥20 years of follow-up, the RRs

for <6 and ≥6 cycles were 1.31 (95% CI 0.8–2.2) and 1.56 (0.9–2.8), respectively.

When analyses focused on the invasive cancers (*n* = 243) after excluding those cancers specifically identified as *in situ* (*n* = 49) (Table V), effects for clomiphene were somewhat stronger than for the total series of cancers. Ever usage of clomiphene was associated with an RR of 1.13 (95% CI 0.9–1.5). Although we observed no striking trends with dosage or number of cycles, elevations in risk persisted for subjects followed for ≥20 years (RR = 1.60, 95% CI 1.0–2.5). We further restricted analyses to the invasive cancers that had been medically validated (81% of the invasive cancers). These analyses showed results similar to the total series of invasive cases. For gonadotrophins, the risk of medically validated invasive cancers associated with high dosages (≥65 ampoules) was statistically significant (RR = 1.79, 95% CI 1.0–3.3).

Further analyses focused on whether the risks of breast cancers associated with clomiphene and gonadotrophin use varied by the presence of other risk factors (Table VI). For both clomiphene and gonadotrophins, the highest risks were observed among nulliparous women (respective RRs and 95% CIs of 1.18, 0.8–1.8 and 1.80, 1.0–3.2). There was no evidence that either clomiphene or gonadotrophins had substantially different effects in women with family histories of breast cancer. We also failed to observe striking effect modifications of drug usage according to causes of infertility.

Since the retrospective nature of the study resulted in our inability to include the complete cohort for analyses, we also conducted a number of analyses to define the impact of study losses. Since we were unable to obtain completed questionnaires from many of the study subjects, we had to rely on identification of cancer outcomes through cancer registry linkages. However, if the last known address was incorrect, we might have missed the true identification of cancer cases among these subjects and incorrectly assigned person-years until the end of the study. We conducted alternative analyses in which we limited the analysis to patients with questionnaires or a definite diagnosis of breast cancer confirmed by medical records, cancer registries or death registries. Although the number of person-years substantially decreased (109 034), the RRs associated with drug exposures changed little. For example, the resultant RR for clomiphene for the total series of breast cancers was 0.95 (95% CI 0.7–1.2).

Discussion

In this study, we found that infertile women had an ~30% higher risk of breast cancer compared with the general population. This undoubtedly reflects unique attributes of infertile women, including higher rates of nulliparity, a recognized breast cancer risk factor. Unresolved is whether infertility medications might also play a role, an issue that we attempted to address by internal analyses that allowed other breast cancer risk factors to be taken into account. These results were generally reassuring, although some slight increases after extended follow-up periods support the need for further evaluation of long-term effects of these drugs.

Table V. Rate ratios of breast cancers, restricted to invasive and medically validated invasive cases according to usage of clomiphene and gonadotrophins

	Invasive cancers		Medically validated invasive cancers	
	No. of cancers (<i>n</i> = 243)	RR ^a (95% CI)	No. of cancers (<i>n</i> = 198)	RR ^a (95% CI)
Clomiphene				
Never	148	1.00	121	1.00
Ever	95	1.13 (0.9–1.5)	77	1.11 (0.8–1.5)
Dosage (mg)				
1–900	35	1.18 (0.8–1.7)	30	1.23 (0.8–1.8)
901–2250	31	1.17 (0.8–1.8)	25	1.14 (0.7–1.8)
≥2251	29	1.00 (0.7–1.6)	22	0.96 (0.6–1.5)
Cycles				
<6	62	1.12 (0.8–1.5)	52	1.13 (0.8–1.6)
6–11	24	1.19 (0.8–1.8)	18	1.10 (0.7–1.8)
≥12	9	1.07 (0.5–2.1)	57	1.03 (0.5–2.2)
Years since first use				
<10	17	0.82 (0.5–1.4)	10	0.60 (0.3–1.2)
10–19	46	1.11 (0.8–1.6)	43	1.27 (0.9–1.8)
≥20	27	1.60 (1.0–2.5)	21	1.47 (0.9–2.4)
Missing	5	1.02 (0.4–2.5)	3	0.74 (0.2–2.3)
Gonadotrophins				
Never	217	1.00	174	1.00
Ever	26	1.10 (0.7–1.7)	24	1.25 (0.8–1.9)
Dosage (ampoules)				
1–24	7	0.89 (0.4–1.9)	6	0.93 (0.4–2.1)
25–64	8	0.97 (0.5–2.0)	7	1.04 (0.5–2.3)
≥65	11	1.47 (0.8–2.7)	11	1.79 (1.0–3.3)
Cycles				
<6	20	1.01 (0.6–1.6)	18	1.11 (0.7–1.8)
≥6	6	1.58 (0.7–3.6)	6	1.93 (0.9–4.4)
Years since first use				
<10	7	0.92 (0.4–2.0)	6	1.00 (0.4–2.3)
10–19	13	1.16 (0.7–2.0)	13	1.40 (0.8–2.5)
≥20	6	1.41 (0.6–3.2)	5	1.38 (0.6–3.4)

^aRate ratios adjusted for calendar year and age at follow-up, study site and mother or sister with breast cancer.

Table VI. Rate ratios of breast cancer by ever versus never use of clomiphene and gonadotrophins according to other risk factors

	Clomiphene (ever versus never)			Gonadotrophins (ever versus never)		
	Exposed cases (<i>n</i> = 108)	RR ^a	95% CI	Exposed cases (<i>n</i> = 31)	RR ^a	95% CI
Age at follow-up						
<40 years	13	0.90	0.4–1.8	0		
40–49 years	54	0.89	0.6–1.2	19	1.28	0.8–2.1
≥50 years	41	1.31	0.9–2.0	12	1.20	0.7–2.2
Reproductive status at follow-up						
Nulliparous	34	1.18	0.8–1.8	14	1.80	1.0–3.2
Parous	57	0.97	0.7–1.4	10	0.62	0.3–1.2
Unknown	17	0.99	0.6–1.8	7	1.36	0.6–3.0
Mother or sister with breast cancer						
No	94	1.00	0.8–1.3	27	1.09	0.7–1.6
Yes	14	1.17	0.6–2.4	4	0.99	0.3–2.9
Causes of infertility ^b						
Endometriosis	28	1.22	0.7–2.1	8	1.09	0.5–2.3
Anovulation	43	0.97	0.6–1.5	14	1.21	0.7–2.2
Tubal disease/pelvic adhesions	37	1.06	0.7–1.6	10	0.97	0.5–1.9
Male factor	31	1.12	0.7–1.8	10	1.47	0.7–2.9
Cervical disorder	6	0.55	0.2–1.5	3	0.87	0.2–3.1
Uterine disorder	11	1.03	0.5–2.2	4	1.23	0.4–3.6

^aBreast cancer risk for ovulation-stimulating drug use relative to never use, adjusted for calendar year and age at follow-up, and study site.

^bCauses of infertility are not mutually exclusive.

Although fertility drugs have received extensive attention with respect to ovarian cancers (Klip *et al.*, 2000), their impact on breast cancer risk remains less clear. There is, however, a clear rationale for studying their effects, especially given the recognized role of reproductive and hormonal factors in the aetiology of breast cancer. Of further concern are effects of infertility drugs in stimulating ovulation, given that ovulation is an established breast cancer risk factor (Henderson *et al.*, 1985; La Vecchia *et al.*, 1985; Parazzini *et al.*, 1993; Stoll, 1997). It has also been shown that breast mitotic activity reaches its peak during the luteal phase of the menstrual cycle (following ovulation) (Pike *et al.*, 1993) and that ovulation-stimulating agents raise estradiol and progesterone levels (Sovino *et al.*, 2002).

Previous epidemiological studies have had only limited ability to assess relationships of infertility medications to breast cancer risk. Of note are the numbers of breast cancer cases in most of the follow-up investigations, including 20 in the Beer-Sheba, Israel cohort (Potashnik *et al.*, 1999), 27 in the Seattle cohort (Rossing *et al.*, 1996), 55 in the British cohort (Doyle *et al.*, 2002) and 59 in the Tel Hashomer, Israel cohort (Modan *et al.*, 1998). Only one study, conducted in Australia (Venn *et al.*, 1999), had >100 observed breast cancer cases (143 in total), with only 87 of these exposed to ovulation-stimulating drugs. An additional limitation of most of the previous studies is that only a few have been able to assess effects of specific types of drugs, of importance given their possibly distinctive effects.

In one of the larger cohort studies, which focused on 3837 infertile women, a non-significantly decreased risk of invasive and *in situ* breast cancer associated with clomiphene usage was found (adjusted RR = 0.5, 95% CI 0.2–1.2) (Rossing *et al.*, 1996). This risk was based on only 12 exposed cases and there was no indication of any further risk reduction with extended duration of use. Since clomiphene is a selective estrogen receptor modulator (SERM), the finding was interpreted as possible support for a chemopreventive effect, similar to what has been observed for tamoxifen (Fisher *et al.*, 1998). Our study, however, provided no support for a protective effect of clomiphene on breast cancer risk. This may reflect the unique chemical properties of clomiphene or that it is administered for the treatment of infertility differently from most other SERMs, namely cyclically and at low dosages. Furthermore, most of the support for a chemopreventive mechanism of other SERMs has related to short-term rather than long-term effects.

Similar to our investigation, Burkman *et al.* (2003), in a case-control study involving 4575 breast cancer patients, found self-reported histories of clomiphene usage unrelated to risk (e.g. use for either ≥ 6 months or ≥ 6 cycles was associated with RRs of 1.0). However, they observed exposures to gonadotrophins for ≥ 6 months or at least six cycles associated with RRs ranging from 2.7 to 3.8. Although neither of the constituents of HMGs, i.e. FSH and LH, are thought to have direct effects on breast tissue, the therapy has been shown to result in increases in both estrogen and progesterone levels, prompting the suggestion that this might contribute to risk increases. It is unclear, however, whether

the relatively minimal increases in hormones that would be associated with six or more cycles of exposure would be sufficient to affect subsequent breast cancer risk substantially (Healy and Venn, 2003).

Although we found no relationship of breast cancer risk to ever use of clomiphene or gonadotrophins, we did observe some increase in risk for gonadotrophins prescribed at higher dosages and, for both drugs, when follow-up extended for ≥ 20 years. Although these long-term risks were based on small numbers (29 breast cancers for clomiphene use and eight for gonadotrophins) and were for the most part not statistically significant, the risks estimates (ranging between 1.4 and 1.6) are in line with risks observed for other hormonal exposures that have been found to have long latency effects on breast cancer risk, including diethylstilbestrol (Palmer *et al.*, 2002), a compound that is structurally similar to clomiphene (Sovino *et al.*, 2002). Thus, we believe that the elevations in risk that we observed after extended drug usage deserve monitoring in additional follow-up studies to assess their biological credibility.

The only other epidemiological investigation that had sufficient power to assess relationships according to detailed parameters of drug usage was an Australian follow-up study of IVF patients (Venn *et al.*, 1999). Although they found no overall association with ever use of various ovulation-stimulating drugs, an ~ 2 -fold increased risk of breast cancer was observed within 1 year of last treatment. This prompted the suggestion that ovulation-stimulating drugs might promote the rapid development of pre-existing tumours, similar to the short-term transient increase in breast cancer risk following a recent pregnancy (Lambe *et al.*, 1994). However, when we assessed detailed timing effects of last drug usage, we, like others (Klip *et al.*, 2002), found little evidence for a promotional effect on risk of either clomiphene or gonadotrophins.

We also had the opportunity to assess drug relationships according to the presence of other breast cancer risk factors, of interest given that some of these may be associated with unique hormonal influences. Although we observed no distinctive effects according to a family history of breast cancer, we did note somewhat higher risks associated with both clomiphene and gonadotrophin usage among women who never subsequently conceived. We initially thought that this might reflect an interaction with distinctive causes of infertility, but found no remarkable variation in drug effects within our categories of causes, including endometriosis and anovulation, both of which have been linked with possible elevations in breast cancer (Cowan *et al.*, 1981; Coulam *et al.*, 1983; Ron *et al.*, 1987; Moseson *et al.*, 1993; Rossing *et al.*, 1996; Brinton *et al.*, 1997; Venn *et al.*, 1999; Dor *et al.*, 2002). The somewhat higher risks associated with drug usage among nulliparous women could merely reflect a spurious association. Whether this subgroup finding has any biological credibility will require assessment in future investigations.

While our study had a number of strengths, there were some notable limitations. Given the retrospective nature of the study, we were unable to locate 20% of the study population, while another 11% did not provide us with permission

to access their medical records. Further, among those located as alive, 41% did not complete a questionnaire. Thus, a variety of selection biases could have affected our results. However, we were unable to detect any systematic biases in the analyses undertaken to assess relationships according to sources of subject inclusion or loss. In addition, a number of women had incomplete work-ups, leading to uncertainty regarding causes of infertility. However, among women with complete work-ups, adjustment for causes of infertility did not substantially change the risks associated with drug exposures. Furthermore, information on ovulation-stimulating drugs, although more complete than in most studies, was still less than optimal. Although information about later drug use was obtained via questionnaire, we could not account for drugs subsequently prescribed by other providers among women who did not complete the questionnaire. Finally, the pattern and dose of drug exposures for many women that we evaluated were quite different from those in current use. However, many of the women in our study received prolonged cycles and very high doses of clomiphene, and many subsequently underwent assisted reproductive technology procedures.

In summary, our results were largely reassuring, although we could not entirely rule out slight effects of ovulation-stimulating drugs on breast cancer risk after ≥ 20 years of follow-up. Although chance cannot be ruled out given that our observed risks were based on small numbers, long-term risks should continue to be monitored, particularly since our study subjects were only beginning to enter the breast cancer age range. If real, our observation of an $\sim 40\%$ increase in breast cancer risk associated with use of ovulation-stimulating drugs after ≥ 20 years of follow-up would translate into ~ 4 additional breast cancers per 1000 exposed women. Given that between 5.4 and 7.7 million women are projected to seek treatment for infertility annually by 2025 (Stephen and Chandra, 1998), additional long-term follow-up studies are needed to confirm and expand upon our findings.

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References

Arbour L, Narod S, Glendon G *et al.* (1994) In-vitro fertilisation and family history of breast cancer. *Lancet* 344,610–611.

Bernstein L (2002) Epidemiology of endocrine-related risk factors for breast cancer. *J Mammary Gland Biol Neoplasia* 7,3–15.

Bernstein L, Hanisch R, Sullivan-Halley J *et al.* (1995) Treatment with human chorionic gonadotropin and risk of breast cancer. *Cancer Epidemiol Biomarkers Prev* 4,437–440.

Bolton P (1977) Bilateral breast cancer associated with clomiphene. *Lancet* 2,1176.

Braga C, Negri E, La Vecchia C *et al.* (1996) Fertility treatment and risk of breast cancer. *Hum Reprod* 11,300–303.

Breslow NE and Day NE (1987) *Statistical Methods in Cancer Research, Volume II: The Design and Analysis of Cohort Studies*. International Agency for Research on Cancer, Lyon, France.

Brinton L, Gridley G, Persson I *et al.* (1997) Cancer risk after a hospital discharge diagnosis of endometriosis. *Am J Obstet Gynecol* 176,572–579.

Brinton LA, Lamb EJ, Moghissi KS, Scoccia B, Althuis MD, Mabie JE and Westhoff CL (2004) Ovarian cancer risk after use of ovulation-stimulating drugs. *Obstet Gynecol* 103, 1194–1203.

Brzezinski A, Peretz T, Mor-Yosef S *et al.* (1994) Ovarian stimulation and breast cancer: is there a link? *Gynecol Oncol* 52,292–295.

Burkman R, Tang M, Malone K *et al.* (2003) Infertility drugs and the risk of breast cancer: findings from the National Institute of Child Health and Human Development Women's Contraceptive and Reproductive Experiences Study. *Fertil Steril* 79,844–851.

Coulam CB, Annegers JF and Kranz JS (1983) Chronic anovulation syndrome and associated neoplasia. *Obstet Gynecol* 61,403–407.

Cowan LD, Gordis L, Tonascia JA *et al.* (1981) Breast cancer incidence in women with a history of progesterone deficiency. *Am J Epidemiol* 114, 209–217.

Dor J, Lerner-Geva L, Rabinovici J *et al.* (2002) Cancer incidence in a cohort of infertile women who underwent in vitro fertilization. *Fertil Steril* 77,324–327.

Doyle P, Maconochie N, Beral V *et al.* (2002) Cancer incidence following treatment for infertility at a clinic in the UK. *Hum Reprod* 17,2209–2213.

Fisher B, Costantino J, Wickerham D *et al.* (1998) Tamoxifen for prevention of breast cancer: report of the National Surgical Adjuvant Breast and Bowel Project P-1 Study. *J Natl Cancer Inst* 90,1371–1388.

Gammon MD and Thompson WD (1990) Infertility and breast cancer: a population-based case-control study. *Am J Epidemiol* 132,708–716.

Gammon MD and Thompson WD (1991) Polycystic ovaries and the risk of breast cancer. *Am J Epidemiol* 134,818–824.

Garland M, Hunter DJ, Colditz GA *et al.* (1998) Menstrual cycle characteristics and history of ovulatory infertility in relation to breast cancer risk in a large cohort of US women. *Am J Epidemiol* 147,636–643.

Healy D and Venn A (2003) Infertility medications and the risk of breast cancer. *Fertil Steril* 79,852–854.

Henderson BE, Ross RK, Judd HL *et al.* (1985) Do regular ovulatory cycles increase breast cancer risk? *Cancer* 56,1206–1208.

Jourdain O, Avril A, Mauriac L *et al.* (1996) Breast cancer and in vitro fertilization. About 32 cases. *Eur J Obstet Gynecol Reprod Biol* 67,47–52.

Klip H, Burger CW, Kenemans P *et al.* (2000) Cancer risk associated with subfertility and ovulation induction: a review. *Cancer Causes Control* 11, 319–344.

Klip H, Burger CW, van Leeuwen FE *et al.* (2002) Risk of hormone-related cancers after ovarian stimulation for in-vitro fertilisation in a cohort of 25 152 women. In *Long-term health effects of subfertility treatment*. PrintPartners Ipskamp BV, Enschede, The Netherlands, pp. 55–82.

La Vecchia C, Decarli A, di Pietro S *et al.* (1985) Menstrual cycle patterns and the risk of breast disease. *Eur J Cancer Clin Oncol* 21,417–422.

Laing RW, Glaser MG and Barrett GS (1989) A case of breast carcinoma in association with in vitro fertilization. *J R Soc Med* 82,503.

Lambe M, Hsieh C, Trichopoulos D *et al.* (1994) Transient increase in the risk of breast cancer after giving birth. *N Engl J Med* 331,5–9.

Lerner-Geva L, Geva E, Lessing J *et al.* (2003) The possible association between in vitro fertilization treatments and cancer development. *Int J Gynecol Cancer* 13,23–27.

Modan B, Ron E, Lerner-Geva L *et al.* (1998) Cancer incidence in a cohort of infertile women. *Am J Epidemiol* 147,1038–1042.

Moseson M, Koenig K, Shore RE *et al.* (1993) The influence of medical conditions associated with hormones on the risk of breast cancer. *Int J Epidemiol* 22,1000–1009.

Palmer J, Hatch E, Rosenberg C *et al.* (2002) Risk of breast cancer in women exposed to diethylstilbestrol in utero: preliminary results (United States). *Cancer Causes Control* 13,753–758.

Parazzini F, La Vecchia C, Negri EFS *et al.* (1993) Lifelong menstrual pattern and risk of breast cancer. *Oncology* 50,222–225.

Pike MC, Spicer DV, Dahmouch LPM *et al.* (1993) Estrogens, progestogens, normal breast cells proliferation, and breast cancer risk. *Epidemiol Rev* 15,17–35.

- Potashnik G, Lerner-Geva L, Genkin L et al. (1999) Fertility drugs and the risk of breast and ovarian cancers: results of a long-term follow-up study. *Fertil Steril* 71,853–859.
- Ricci E, Parazzini F, Negri E et al. (1999) Fertility drugs and the risk of breast cancer. *Hum Reprod* 14,1653–1655.
- Ron E, Lunenfeld B, Menczer J et al. (1987) Cancer incidence in a cohort of infertile women. *Am J Epidemiol* 125,780–790.
- Rossing MA, Daling JR, Weiss NS et al. (1996) Risk of breast cancer in a cohort of infertile women. *Gynecol Oncol* 60,3–7.
- Sovino H, Sir-Petermann T, Devoto L et al. (2002) Clomiphene citrate and ovulation induction. *Reprod Biomed Online* 4,303–310.
- Stephen E and Chandra A (1998) Updated projections of infertility in the United States: 1995-2025. *Fertil Steril* 70,30–34.
- Stoll BA (1997) Impaired ovulation and breast cancer risk. *Eur J Cancer* 33,1532–1535.
- Unkila-Kallio L, Leminen A, Tiitinen A et al. (1997) Malignant tumors of the ovary or the breast in association with infertility: a report of thirteen cases. *Acta Obstet Gynecol Scand* 76,177–181.
- Ursin G, Longnecker M, Haile R et al. (1995) A meta-analysis of body mass index and risk of premenopausal breast cancer. *Epidemiology* 6,137–141.
- Venn A, Watson L, Lumley J et al. (1995) Breast and ovarian cancer incidence after infertility and in vitro fertilisation. *Lancet* 346,995–1000.
- Venn A, Watson L, Bruinsma F et al. (1999) Risk of cancer after use of fertility drugs with in-vitro fertilisation. *Lancet* 354,1586–1590.
- Wright V, Schieve L, Reynolds M et al. (2003) Assisted reproductive technology surveillance—United States, 2000. *MMWR Surveill Summ* 52, 1–16.

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