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EPIDEMIOLOGY

Infertility, ovulation induction treatments and the incidence of breast cancer—a historical prospective cohort of Israeli women

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Abstract
Context Ovulation induction drugs may be associated with increased breast cancer risk. Results so far have been inconclusive.
Objective To evaluate the association between infertility, exposure to ovulation induction drugs and the incidence of breast cancer.
Design Historical prospective cohort and nested case–control study.
Setting Institutional practice
Patients About 5,788 women attending five infertility centers in Israel between 1964 and 1984.
Intention Abstracting of medical records and telephone interviews.
Main outcome measure Breast cancer incidence was determined through linkage with the National Cancer Registry database. Standardized incidence ratios (SIRs) and 95% confidence intervals were computed by comparing the observed to the expected cancer rates in the general population. In addition, a nested case–control study within the cohort was performed with interviews of breast cancer cases and two matched controls.
Results The study cohort included 120,895 women years of follow-up. Compared to 115.2 expected breast cancer cases, 131 cases were observed (SIR = 1.1; 95% CI 0.9–1.4). Risk for breast cancer was significantly higher for women treated with clomiphene citrate (SIR = 1.4; 95% CI 1.0–1.8). Similar results were noted when comparisons were carried out between treated and untreated women, and when multivariate models were applied.In the nested case–control study, higher cycle index (OR = 2.2; 95% CI 1.0–4.8) and treatment with clomiphene citrate (OR=2.7; 95% CI 1.3–5.7) were associated with higher risk for breast cancer.
Conclusion Infertility and usage of infertility drugs in general are not associated with increased risk for breast cancer. However, for infertile women treated with clomiphene citrate, breast cancer risk is elevated.

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Keywords Breast cancer · Clomiphene citrate · Infertility · Ovulation induction · Prospective

Introduction

More than 15% of couples will be facing infertility problems during their reproductive years, and seek treatment [1]. Many adverse effects have been related to infertility treatments, some of which are short termed, i.e., multiple pregnancies [2, 3], ectopic pregnancies [4], and hyper-stimulation syndrome [5–7], while others are long-termed, genital tract cancer, for instance [8, 9].

Like in many other developed countries, breast cancer is the leading cancer in women in Israel, with an age-adjusted incidence rate of 95/100,000 cases per year [10]. A number of risk factors that are related to endocrine function have been proposed in the etiology of breast cancer, including early age at menarche, late menopause, late age at first birth and nulliparity [11, 12].

Ovulation induction might be associated with breast cancer risk. High estrogen levels during the follicular phase of ovulation induction cycles combined with high progesterone levels produced during the simultaneous ovulation of multiple follicles may expose infertile women to an environment that potentially favors the development of breast cancer [3]. On the other hand, one commonly used ovulation inducing agent, clomiphene citrate (CC), is structurally similar to tamoxifen (a specific estrogen receptor modulator, SERM) and, like tamoxifen, has been reported to exert anti-proliferative effects on human breast cancer cells [13].

However, to date there are few epidemiological studies investigating the associations between infertility, type of infertility, treatments with ovulation induction drugs, and breast cancer risk. Of them, four are case–control studies [14–17] and 15 are prospective studies [18–32]. These studies are very heterogeneous; some of them referred to cohorts of women undergoing in vitro fertilizations (IVF) [26–30] while others referred to other types of infertility treatments [14–23], and only few studies referred to the type and dosage of fertility treatments [17, 22–24, 29, 30, 32]. Results, so far, have been inconclusive, and are summarized in Table 1.

Although only around 15% of the population experience infertility, infertility treatments in Israel are covered by the National Health Insurance in a very liberal manner until the age of 45. If there is a positive association between infertility treatment and breast cancer occurrence, it may carry serious implications for the clinical practice of infertility treatment and for the current public health policy.

The aim of the present study is to prospectively assess the association between infertility, exposure to ovulation induction drugs and the incidence of breast cancer in a large cohort of Israeli women.

Materials and methods

This is a historical prospective cohort.

Study population

The study population included women who attended infertility clinics at five medical centers in Israel between 1964 and 1984.

Each participating infertility center had a registry of clinic visits, which included personal details of the patients. According to the registries there were 3,532 medical records in total, of which we have located 3,519 (99.6%). Additional 2,496 records were available from a previous study conduced by Ron et al. [20], and were included in our current database, thus reaching a total of 6,015 medical records. Subsequently, the cohort database was linked to the National Population Registry to verify participants' identity and vital status by using a unique ID number, first name, last name, year of birth, country of birth and year of immigration (when applied). If a woman was not identified in the Registry, an attempt was made at individual linkage in cases of incomplete ID number or name change following change of marital status. Sixty-four women (1.06%) were not identified and therefore were excluded from the study cohort. Some women ($n = 163$) had double records due to visiting more than one medical center; they were included only once, in the initial medical center they had approached (according to date of first visit). Thus, the final study population consisted of 5,788 women.

Data abstraction

Medical records were abstracted using a constructed questionnaire which included information on general and demographic variables, past and current morbidity, gynecological and obstetrical history, infertility definition and work up, infertility diagnosis and treatments. Women with normal ovulatory cycle (including women with mechanical infertility, male factor infertility and unexplained infertility) were defined as having non-hormonal infertility while non-ovulating women were defined as having hormonal infertility. The data was then computerized to establish the study cohort database.

Table 1 Summary of studies focusing on infertility, infertility treatments and breast cancer risk

Author/reference	Publication year, place	Population	Main findings	Comments
I. Case–control studies				
Gammon et al. [14]	1990, USA (8 areas in 6 states)	4,730 breast cancer cases (20–54 years) 4,688 population-based controls	OR [95% CI] for infertility in *gravid*: 1.01 [0.89; 1.15]; for infertility in *nulligravid*: 0.82 (0.59; 1.14)	Exposure defined as infertility. (Self reported & physician reported)
Braga et al. [15]	1996, Italy (6 provinces)	2,569 breast cancer cases (23–74 years) 2,588 hospital-based controls	OR [95% CI] for fertility treatments Premenopausal: 0.9 [0.5; 1.5] Postmenopausal: 1.3 [0.8; 1.9] OR by infertility types Mechanical: 0.6 [0.3; 1.3] Hormonal: 1.0 [0.5; 2.0] Other/unspec.: 1.3 [0.9; 2.0]	Exposure defined as infertility and use of fertility drugs (Self reported)
Ricci et al. [16]	1999, Italy (Greater Milan)	3,415 breast cancer cases (23–74 years) 2,916 hospital-based controls	OR [95% CI] for fertility treatments: 0.8 [0.5; 1.5] In nulliparous: 0.6 [0.2; 2.3] In parous: 1.2 [0.5; 2.6]	Exposure defined as fertility treatment (self-reported)
Burkman et al. [17]	2003, USA (5 states)	4,575 breast cancer cases (35–64 years) 4,682 population-based controls	OR [95% CI] (users vs. never users) hMG[a] users (> 6 mns): 2.1 [1.0; 4.4] hMG[a] users (> 6 cycles): 2.7 [1.0; 6.9]	Exposure defined as use of fertility drugs (self reported) Similar results when only infertile women (who were or were not treated by fertility drugs) are included
II. Prospective design studies				
Cowan et al. [18]	1981, USA (Johns Hopkins clinic, MD)	Prospective cohort of infertile women (1945–1965) 1,083 white women, divided into two subgroups "Hormonal" and "non-hormonal" infertility	RR [95% CI] of "hormonal" vs. "non-hormonal" Premenopausal: 5.4 [1.1; 49.0] Postmenopausal: 0.3 [0.01; 2.9]	Exposure defined as infertility ("hormonal" or "non-hormonal"); "hormonal" infertility = progesterone deficiency ("unopposed estrogen")
Coulam et al. [19]	1983, USA (Mayo clinic, MN)	Retrospective cohort of women diagnosed with anovulation (1935–1980) 1,270 women; 12 breast cancer cases; 14,500 person years	SIR* [95% CI] of "anovulation" vs. general population: 1.5 [0.8; 2.6] In premenopausal: 1.3 [0.3; 3.2] In perimenopausal: 0.9 [0.2; 2.5] In postmenopausal: 3.6 [1.2; 8.3]	Exposure defined as diagnosis of anovulation syndrome
Ron et al. [20]	1987, Israel (Ramat Gan)	Retrospective cohort of infertile women (1964–1974) 2,575 women; 10 breast cancer cases; 31,622 person years	SIR [95%] of infertility type vs. general population Hormonal: 1.4 [ns] (high E, low P): 1.8 [ns] Mechanical: 0.3 [ns] Male: 1.7 [ns] Unspecified: 0.8 [ns] All: 1.1 [ns]	Exposure defined as infertility Hormonal infertility subsequently divided into: high estrogen (E), low progesterone (P) = unopposed estrogen; normal E, normal P; low E, low P

Table 1 continued

Author/reference	Publication year, place	Population	Main findings	Comments
Brinton et al. [21]	1989, USA (Mayo clinic. MN)	Retrospective cohort of infertile women (1935–1980) 2,335 women; 49 breast cancer cases; 45,408 person years	SIR [95% CI] of infertile women vs. general population: 0.9 [0.7; 1.2]	Expansion of the study by Coulam et al. Exposure defined as infertility Analyses by causes of infertility showed no excess of breast cancer in any category
Modan et al. [22]	1998, Israel (Ramat Gan)	Retrospective cohort of infertile women (1964–1974) 2,496 infertile women; 59 breast cancer cases; 54,413 person years	SIR [95% CI] in infertile women vs. general population: 1.3 [1.0–1.6] By fertility drugs CC only: 1.2 [0.7; 1.6] CC + hMG: 1.6 [0.7; 3.4] Untreated: 1.4 [1.0; 2.0]	Expansion of the study by Ron et al. Exposure defined as infertility and use of fertility drugs
Rossing et al. [23]	1996, USA (Seattle, WA)	Case-cohort study derived from a prospective cohort of infertile women (1974–1985) 3,837 infertile women; 27 breast cancer cases	SIR [95% CI] in infertile women vs. general population: 0.9 [0.6–1.4] RR (95% CI): for ever vs. never users of CC: 0.5 (0.2; 1.2) For ever vs. never users of hCG: 0.5 (0.2; 1.8)	Exposure defined as infertility and use of fertility drugs Number of treatment cycles does not change results
Potashnik et al. [24]	1999, Israel (Beer Sheva)	Retrospective cohort of infertile women (1960–1984) 1,197 women; 20 breast cancer cases; 21,407 person years	SIR [95% CI] in infertile women vs. general population: 1.4 [0.8; 2.1] Exposed: 1.7 [0.9; 2.7] Unexposed: 0.8 [0.2; 2.0] By number of CC cycles 1–2: 2.6 [1.2; 5.0] 3–5: 1.3 [0.3; 3.4] 6+: 0.9 [0.2; 2.7] By cumulative CC dose < or = 1,000 mg: 2.5 [1.2; 4.6] 1,001–2,000 mg: no observed cases > 3,000 mg: 1.4 [0.3; 4.2]	Exposure defined as use of fertility drugs.
Doyle et al. [25]	2002, UK (London)	Prospective cohort of infertile women (1975–1989) 5,556 infertile women; 55 breast cancer cases Median follow-up: 15.5 years	SIR [95% CI] in infertile women vs. general population: 1.2 [0.9; 1.5] Exposed: 1.2 [0.8; 1.6] Unexposed: 1.2 [0.6; 2.0] RR [95% CI] exposed vs. unexposed: 1.0 [0.5; 1.9]	Exposure defined as use of fertility drugs

Table 1 continued

Author/reference	Publication year, place	Population	Main findings	Comments
Gauthier et al. [31]	2004 France	Prospective cohort of 6602 women treated for infertility (1990–2000); 183 breast cancer cases	RR in infertile women vs. rest of cohort 0.95 [0.82–1.11] RR in treated infertile women with family history of breast cancer 1.37 [0.99–1.87]	Self-reports of infertility and infertility treatments
Brinton et al [32]	2004 USA (5 clinics)	Retrospective cohort of 12,193 women evaluated for infertility (1965–1988); 292 breast cancer cases	SIR in infertile women [95% CI]: 1.29 [1.1–1.4] Treatment with CC ≥ 20 years RR=1.6 [1.0–2.5]	Exposure based on abstraction of medical records
III. IVF cohorts				
Brzezinski et al. [26]	1994, Israel (Jerusalem)	Case series in a cohort of IVF patients 950 women; 16 breast cancer cases	2.2-fold increase in breast cancer occurrence in the cohort as compared to general population rates for women in the same age range	4 out of 16 breast cancer cases diagnosed 1–6 mns following IVF
Venn et al. [27]	1995, Australia (Victoria)	Prospective cohort of IVF patients (1978–1992) 10,358 women, of them; 5,564 exposed; 4,794 unexposed	SIR [95% CI] (vs. general population) Exposed: 0.9 [0.6; 1.5] Unexposed: 1.0 [0.6; 1.6] RR (exposed vs. unexposed) [95% CI]: 1.1 [0.6; 2.2]	Exposure defined as use of fertility drugs
Venn et al. [28]	1999, Australia (Victoria)	Prospective cohort of IVF patients (before 1994) 29,700 women, of them 20,656 exposed; 9,044 unexposed	SIR [95% CI] (vs. general population) Exposed: 0.9 [0.7; 1.1] Unexposed: 1.0 [0.7; 1.2] SIR for women diagnosed within 12 mns of IVF: 2.0 [1.2; 3.1]	Expansion the study by Venn et al. [27] Exposure defined as use of fertility drugs
Dor et al. [29]	2002, Israel (Tel Aviv)	Retrospective cohort of IVF patients (1981–1992) 5,026 women; 11 breast cancer cases	SIR [95% CI] (vs. general population): 0.7 [0.5; 1.7] Type of infertility, number of IVF cycles, and treatment outcome did not significantly affect cancer risk	Exposure defined as infertility and use of fertility drugs
Lerner-Geva et al. [30]	2003, Israel (Tel Aviv)	Retrospective cohort of IVF patients (1984–1992) 1,082 women; 5 breast cancer cases	SIR[95% CI] (vs. general population): 1.0 [0.3; 2.4] Excluding cases diagnosed less than 12 mns following IVF: SIR = 0.8 [0.2; 2.1]	Exposure defined as infertility and use of fertility drugs

[a]hMG—human menopausal gonadotropin (pergonal)

*SIR of observed to expected cases according to national rates, unless indicated otherwise

Table abbreviations: SIR, standardized incidence ratio; RR, relative risk; CC, clomiphene citrate; hCG, human chorio-gonadotropin; hMG, human menopausal gonadotropin; mns, months

Breast cancer ascertainment

The study cohort computerized file was linked to the National Cancer Registry to identify cancer cases through December 1996. The Israeli Cancer Registry was established in 1960 and maintains data on all malignancies in Israel, including borderline and some benign tumors (primarily in the central nervous system). The Registry receives notifications of all incident malignancies from hospital discharge reports as well as oncology and pathology departments throughout the country. Depending on the cancer site, completeness of the data was found to be 90–95% [33]. The records were linked by computer matching of patients' identification numbers, names and demographic variables with the Cancer Registry data file. For all patient matches, the Cancer Registry provided diagnosis details coded according to the International Classification of Diseases, Ninth Revision, along with date and place of diagnosis. Diagnoses were verified by reviewing the original histopathologic report for each identified case.

All analyses were performed excluding breast cancer cases that were diagnosed within 12 months of the initiation of fertility treatment, to allow for a minimal latency period between exposure and cancer development.

Data analysis

Descriptive data on the study cohort, such as age, continent of birth, type of infertility, type of infertility treatment etc., are presented as absolute figures, rates or percentages.

In order to assess the association between infertility and breast cancer risk, we compared the observed breast cancer cases in the study cohort of infertile women to the expected rates in the general population. In order to assess the association between infertility treatments and breast cancer risk we compared the breast cancer incidence rates in treated women to those observed in untreated infertile women.

Comparison to the general population

The observed number of breast cancer cases in the cohort was obtained through linkage with the National Cancer Registry. Expected numbers of breast cancer in women were computed based on age, continent of birth and year-specific national breast cancer incidence rates. Standardized incidence ratios (SIR) were computed as the ratio of observed to expected breast cancer cases with estimated 95% confidence intervals (95% CI) [34]. The SIR calculations took into account person years, which were calculated from the date of first treatment until the end of follow-up (December 31st, 1996, the last update of the National Cancer Registry at the time), or until the date of incident breast cancer diagnosis, whatever came first.

Univariate SIRs were computed for participants by categories of age, continent of birth, medical center, age at menarche, infertility years, parity, type of infertility (Non-hormonal vs. hormonal), status of infertility treatment (treated/untreated) and type of infertility treatment.

Multivariate Poisson regression models were used to evaluate the possible effects of age group, continent of origin, parity, type of infertility and type of infertility treatment on the risk for breast cancer in the cohort of infertile women as compared to the general population. These variables were chosen based on the univariate analysis results as well as their role as established risk factors for breast cancer. The Poisson models incorporate the expected number of cancer cases in the tested group in place of person years and may, therefore, be viewed as the multivariate generalization of the SIR method.

Comparison between treated and untreated infertile women

The relative risk (RR) for breast cancer incidence was calculated as the ratio of breast cancer incidence in the treated subgroup to the breast cancer incidence in the untreated group with respected 95% confidence intervals.

The univariate and multivariate statistical analyses that were used for the comparisons to the general population were also applied for this comparison.

In addition, the role of risk factors (such as family history of breast/ovarian cancer) that were not available from the medical records was evaluated using a nested case–control study design. Cases and controls were chosen from the study cohort. The eligible cases were infertile women who developed breast cancer and were alive at the time of study. For each eligible case, two infertile controls matched for age (± 5 years), continent of birth and medical center were abstracted from the cohort. Cases and controls were interviewed by telephone using a pre-constructed questionnaire. The variables included in the questionnaire enabled the assessment of life long exposure to hormonal cycles, which was defined as cycle index. Cycle index was calculated as follows:

$$\text{Cycles index} = \frac{\text{Total menstrual years} * 365}{\text{Length of cycle (days)}}.$$

Total menstrual years included: (1) menarche to menopause interval (If premenopausal: [age at interview]−[age at menarche] and if postmenopausal: [age at menopause]−[age at menarche]; (2) total years pregnant ($0.75 \times$ no. of full-term pregnancies, $0.25 \times$ no. of abortions spontaneous and induced); (3) total years nursing as follows:

$$\text{Total menstrual years} = \leftarrow [\text{menarche to menopause interval}] - [\text{years pregnant} + \text{years nursing}].$$

In addition, the questionnaire included information regarding family history of cancer, use of oral contraceptives and diagnosis of infertility in four categories: mechanical, male, unexplained and an-ovulation that were subsequently grouped into two categories of non-hormonal (mechanical, male and unexplained) and hormonal (anovulatory) infertility. Ovulation induction treatments were classified as CC, hMG or none.

Stepwise conditional logistic regression models were performed to assess the contribution of each possible risk factor adjusted for others to breast cancer development, in the nested case–control study. The level of 0.10 was set for entering and removing variables from the model.

Two models were used in multivariate analysis; both included: BMI, family history, use of oral contraceptives and cycle index, while the first one included also the combination variable of infertility diagnosis and treatment, and the other alternatively included the variable ovulation induction treatment.

The combination variable consisted of four categories: hormonal infertility treated with ovulation induction, hormonal infertility untreated, non-hormonal infertility treated and non-hormonal infertility untreated which served as the reference group.

Due to the dependence between the diagnosis of infertility and ovulation induction treatment, the solution for including both in the same model was to create a combination variable.

All statistical tests were two-sided. Analyses were done using SAS statistical package, version 6.11 [35].

Results

The study cohort included 5,788 women who contributed 120,895 person years of follow-up with a mean follow-up period of 20.9 ± 6.6 years.

Mean age at first visit to infertility clinic was 28.6 ± 5.6 years, and mean age at end of follow-up was 49.9 ± 8.2 years. Almost half of the study participants were Israeli-born (n = 2,735, 47.3%), a third (n = 1,927, 33.3%) were born in Asia or Africa and the rest (n = 1,126, 19.4%) were born in Europe, America or elsewhere.

Table 2 Reproductive and obstetrical characteristics of the study participants (n=5,788)

	n (%)
Age at menarche	
9–11 years	377 (6.5)
12–15 years	3,711 (64.1)
16–20 years	247 (4.3)
Unknown	1,453 (25.1)
Menstrual cycles	
Regular menstruation	3,044 (52.6)
Irregular menstruation	2,744 (47.4)
Ovulatory cycles	
Yes	1,991 (34.4)
No	1,140 (19.7)
Unknown	2,657 (45.9)
Number of infertility years	
1–2	1,758 (30.4)
3–5	1,630 (28.2)
6+	1,216 (21.0)
Unknown	1,184 (20.4)
Parity	
Nulliparous	3,264 (56.4)
Parous	2,377 (41.1)
Unknown	147 (2.5)
Type of infertility	
Hormonal	2,822 (48.8)
Non-hormonal[a]	2,966 (51.2)
Treatment with ovulation induction	
Yes	3,076 (53.1)
No	2,712 (46.9)
Type of treatment (for treated women only, n=3,076)	
CC only	1,943 (63.1)
hMG only	325 (10.6)
CC followed by hMG	808 (26.3)

[a]Mechanical, male factor and unexplained

Mean age at menarche was 13.1 ± 1.4 years (range: 9–20). Categorization of the study population by regularity of the menstrual cycle, ovulatory status of the cycles, parity, type of infertility and type of infertility treatments is presented in Table 2. Almost half of the participants were diagnosed with hormonal infertility and were treated with ovulation induction drugs. Of the 3,076 women with known treatment cycles, the vast majority (around 90%) were treated with clomiphene citrate (CC) or CC followed by hMG. Approximately 10% were treated with hMG only (Table 2).

Comparison with the general population

Following linkage with the National Cancer Registry, 131 breast cancer cases were diagnosed. The mean age at diagnosis was 47.2 ± 8.4. Compared to the 131 cases

of breast cases observed, 115.18 cases were expected (SIR = 1.14; 95% CI 0.95–1.40). In univariate analyses of demographic variables (data not shown), infertile women aged 30–34 at the time of their first visit to the infertility clinic demonstrated higher than expected rate of breast cancer as compared to the general population cancer rates (SIR = 1.41; 95% CI 1.03–1.90). Women born in Asia–Africa also had higher than expected breast cancer rates with SIR = 1.33 (95% CI 0.98–1.76). Additionally, women with early age at menarche, women with 3–5 years of infertility, women who were diagnosed with non-hormonal infertility and women who were treated with CC had significantly higher risk for breast cancer as compared to the general population (Table 3).

Multivariate analysis including the general population cancer rates and adjusted for age, origin, type of infertility, diagnosis of infertility and treatment, revealed that the risk for developing breast cancer remains significantly elevated in women who were treated with CC (as compared to women who were not treated with ovulation induction) (Hazard ratio 1.49; 95% CI 1.15–1.93) (data not shown).

Comparison between treated and untreated infertile women

In total there was no significant excess of breast cancer incidence in the treated subgroup (n = 3,076, with 73 incident breast cancer cases) as compared to the untreated subgroup (n = 2,712, with 58 incident breast cancer cases): RR 1.11 (95% CI 0.79–1.57). Also, no significant excess of breast cancer incidence was noted for categories of age at first visit to the infertility clinic, continent of birth, treating medical center, age at menarche, number of infertility years, parity, type of infertility and type of treatment (data not shown).

Multivariate analysis revealed that the risk for women who were treated with CC (as compared to women who were not treated with ovulation induction) was significantly elevated (Hazard ratio 1.45; 95% CI 1.10–1.89) (data not shown).

Table 3 Observed and expected breast cancer cases by selected characteristics (univariate analysis)

Total	*n* 5,788	Observed (*n*) 131	Expected (*n*) 115.18	SIR 1.14	95% CI 0.95; 1.40
Age at first visit					
< 25	1,814	25	23.90	1.05	0.70–1.50
25–29	1,839	29	34.15	0.85	0.57–1.22
30–34	1,267	44	31.16	1.41	1.03–1.90
35+	868	33	25.90	1.27	0.90–1.80
Age at menarche					
9–11	377	13	7.06	1.84	0.98; 3.15
12–15	3,711	81	71.92	1.13	0.89; 1.40
16–20	247	4	4.82	0.83	0.22; 2.13
Unknown	1,453	33	31.38	1.05	0.72; 1.48
Number of infertility years					
1–2	1,758	38	31.87	1.19	0.84; 1.64
3–5	1,630	45	32.73	1.37	1.00; 1.84
6+	1,216	35	31.79	1.10	0.77; 1.53
Unknown	1,184	13	18.62	0.70	0.37; 1.19
Parity					
Nulliparous	3,264	68	62.01	1.10	0.85; 1.39
Parous	2,377	62	49.35	1.26	0.96; 1.61
Unknown	147	1	3.82	0.26	0.00; 1.46
Type of infertility					
Hormonal	2,822	61	57.41	1.06	0.81; 1.36
Non-hormonal[a]	2,966	70	57.75	1.21	0.95; 1.53
Treatment status					
Hormonal treatment	3,076	73	59.73	1.22	0.96; 1.54
No hormonal treatment	2,712	58	55.45	1.02	0.79; 1.35
Type of ovulation induction treatment					
Untreated	2,712	58	55.45	1.05	0.79; 1.35
CC only	1,943	53	37.98	1.40	1.05; 1.83
hMG only	325	5	7.59	0.66	0.21; 1.54
CC followed by hMG	808	15	14.16	1.06	0.59; 1.75

[a]Mechanical, male factor and unexplained

Nested case–control study

Of the 131 breast cancer cases diagnosed in the cohort, 61 (47%) were interviewed; another 46 (35%) were deceased at the time of the current nested case–control study and additional 24 (18%) were not traced or refused to participate. A total of 120 matched controls were interviewed as well. In univariate analyses, women who were past smokers, had BMI ≥ 30, had cycle index > 400, were diagnosed with non-hormonal infertility and were treated with ovulation induction drugs, especially CC, were at a borderline significantly higher risk for breast cancer (Table 4).

In both multivariate models, used for the assessment of breast cancer risk, post-smoking and cycle index were associated with higher risk. Additionally, the combination of non-hormonal infertility and ovulation induction treatment, as well as treatment with CC, was statistically significantly associated with higher risk, in the first and second models, respectively (Table 5).

Discussion

The current study aimed to assess the risk for breast cancer in relation to infertility and infertility treatments. Its main results regarding the association between infertility and breast cancer suggest no excess of the disease in infertile women compared to the general population. However, in women treated with clomiphene citrate, breast cancer risk was increased. These results reached statistical significance even when well-known risk factors for breast cancer (such as family history and cycle index) were controlled for.

Although no association between infertility and breast cancer in general was previously reported by many other studies [20, 21, 23–25, 27–31], the current findings are in accordance with one previous publication [32].

Women with non-hormonal infertility presented higher risk for breast cancer although not significantly. This finding is also supported by other studies, showing that higher number of ovulatory cycles (as seen in non-hormonal infertility) is associated with increased risk for breast cancer [36–38]. Although the role of hormone-related factors in the etiology of breast cancer is well established, the precise mechanisms underlying this association are not fully understood [12, 39]. The subgroup of women diagnosed with non-hormonal infertility may be at a significantly higher risk for breast cancer, due to their long-term exposure to ovulation cycles, which is presumably favoring the occurrence of breast cancer [18, 19]. On the other hand, woman with hormonal infertility may be at a higher risk for breast cancer due to their exposure to unopposed estrogen environment. However, in many studies including the current one, the group of hormonal infertility is comprised of a mix of women with unopposed estrogen

Table 4 Nested case–control (univariate)

	Cases		Control		OR	(95% CI)	*P*
	n=61		*n*=120				
	n	%	*n*	%			
BMI							
15–29	53	(86.9)	107	(89.2)	1.0		
30 +	8	(13.1)	13	(10.8)	1.9	(0.9–3.7)	0.07
Family history							
Yes	53	(86.9)	108	(90.0)	1.0		
No	8	(13.1)	12	(10.0)	1.3	(0.5–3.3)	0.53
Cycle index							
≤ 400	33	(54.1)	79	(65.8)	1.0		
> 400	28	(45.9)	41	(34.2)	1.7	(0.9–2.9)	0.07
Oral contraceptive							
Yes	47	(77.0)	103	(85.8)	1.0		
No	14	(23.0)	17	(14.2)	1.9	(0.8–4.3)	0.13
Diagnosis and treatment							
Hormonal + treatment	18	(29.5)	45	(37.5)	0.9	(0.40–2.0)	0.75
Hormonal − treatment	8	(13.1)	21	(17.5)	0.9	(0.30–2.4)	0.78
Non-hormonal + treatment	20	(32.8)	20	(16.7)	2.4	(0.96–6.2)	0.06
Non-hormonal − treatment	15	(24.6)	34	(28.3)	1.0		
Ovulation induction treatment							
No	23	(37.7)	55	(45.8)	1.0		
CC	27	(44.3)	32	(26.7)	2.1	(0.99–4.3)	0.05
hMG	3	(4.9)	11	(9.2)	0.6	(0.10–2.2)	0.40
CC + hMG	8	(13.1)	22	(18.3)	0.8	(0.30–2.2)	0.68

Table 5 Nested case–control (multivariate analysis), stepwise logistic regression

	Model 1		*P*	Model 2		*P*
	OR	(95% CI)		OR	(95% CI)	
Cycle index						
> 400	1.9	(0.9–4.1)	0.10	2.2	(0.99–4.8)	0.05
Diagnosis and treatment						
Non-hormonal + treatment	2.5	(1.1–5.6)	0.03	–	–	–
Ovulation induction treatment						
CC	–	–	–	2.7	(1.3–5.7)	0.01

(that might indeed be at a higher risk for the disease), women with hypogonadic–hypgonadotropic (low estrogen, low progesterone) syndrome that are not at an increased risk for the disease, and women with normal hormone levels who do not ovulate, thus diluting the effect of breast cancer excess risk generally attributed to the unopposed estrogen syndrome [40]. In the results of the nested case–control study women with higher number of cycle index representing higher number of ovulatory cycles were at increased risk for breast cancer [41]. Furthermore, women with non-hormonal infertility (normal ovulation) who were treated with ovulation induction drugs were at increased risk for breast cancer as well.

The observed association between CC use and breast cancer risk in the current study is not supported by two recent studies [17, 31] which disclosed no association between the two variables, and is partially contradicted by the findings of the study by Rossing et al. [23] which implied a non-significant protective effect of CC. On the other hand, Brinton et al. [32] observed a 60% increased risk for breast cancer in infertile women treated with CC for more than 20 years (RR = 1.6; 95% CI 1.0–2.5). In another Israeli study [24] based on the Soroka Medical Center cohort (that was also included in the cohort presented here), CC users were at a significantly higher risk for breast cancer, though only when treated with lower dosage (≤ 1,000 mg) and with fewer treatment cycles (1–2 cycles as opposed to 3–5 or 6+ CC treatment cycles) [24].

CC is one of the selective estrogen receptor modulators (SERM). It is an orally active, non-steroidal agent that shows structural similarity to endogenous estrogen and therefore is able to bind to estrogen receptors (ERs). The drug exerts only a very weak ER-mediated estrogenic effect (transcriptional activity) but at the same time it blocks the ERs to endogenous estradiol and occupies them for longer periods of time [42–44]. CC acts through modification of the hypothalamic activity by binding to the hypothalamic ERs and reducing the concentration of the receptors through inhibition of the process of receptor replenishment [43, 44]. Consequently, the receptor capacity is reduced, true endogenous estradiol signal is falsely lowered, negative feedback is diminished and gonadotropin releasing hormone (GnRH) secretion is activated, which, in turn, increases the production of gonadotropins and elevates their blood levels [45].

Higher circulating levels of gonadotropins (the expected outcome of CC administration) are not directly associated with breast cancer risk although, there is some evidence that ovarian hyperstimulation by leutinizing hormone (LH) may lead to mammary gland hyperplasia and cancer predisposition in transgenic mice [46]. However, CC may influence breast cancer risk directly. Although CC binds to the ER for long periods of time and blocks the ERs to endogenous estradiol (thus, acts as an anti-estrogen), it is also capable of evoking some transcriptional activity (thus, estrogenic). Some phytoestrogens—plant substances that structurally resemble estrogen and are able to bind to the ER—were also reported to evoke both estrogenic and anti-estrogenic effects [47, 48]. Subsequently, the net effect of these phytoestrogens in relation to breast cancer risk was questioned [49, 50]. A recent study has suggested that phytoestrogens, genistein mostly, have biphasic trait, and that their net effect is difficult to predict and strongly depends on the exposure dose, the exposure timing, the tissue metabolism of phytoestrogens and the endogenous hormonal environment [51]. CC may also share some of the biphasic characteristics shown for phytoestrogens and act as a weak estrogen (thus promoting and stimulating proliferation of breast cancer cells) under certain conditions.

Furthermore, although, CC may have a direct anti-estrogenic effects on the breast (not as potent as tamoxifen), this effect may be overridden by the elevated estradiol levels induced by CC in women of reproductive age [52, 53].

Elevated risk for breast cancer (as observed in the current study) was noticed for diethylstilbestrol (DES), a compound that is structurally similar to CC [32, 54]. These two drugs (CC and DES) were used, unlike tamoxifen, in younger women, and therefore, most of their effects on the genital tract and ovaries have been studied only in the context of fertility therapy [55].

Although CC was explored as a possible treatment against human breast cancer cells [56], additional studies are needed to establish its long term effects in different target organs [55].

The strengths of this study lie in the size of the cohort (n = 5,788) that enables the detection of breast cancer cases through follow-up period of more than 120,000 person years, and the fact that potential confounding factors were adjusted for in the additional nested case–control study. The inclusion of the nested case–control component in the study enabled us to disentangle the effect of infertility from that of its treatment. Furthermore, we have no reason to suspect a selection bias of the cohort of the infertility women since infertility treatments in Israel are fully covered by the National Health Insurance to all women up to 45 years of age for first and second child.

The results of this study may be limited by several factors. Although the total follow-up period is long, the mean age at the end of follow-up (49.9 years) is not yet the peak age for breast cancer incidence in Israel [10]. Additionally, confounding by powerful breast cancer risk factors, such as family history, could not have been accounted for due to lack of available data in the medical records [31], but these risk factors were accounted for in the nested case–control study that used personal interviews. Unfortunately, women who were deceased at the time of interview were not included; thus, possibly compromising the representativeness of the cases.

Surveillance bias may be introduced to the cohort if the exposed (infertile) population is monitored more regularly over time as compared to the general population. However, the completeness of the National Cancer Registry reduces the possibility of such a bias.

Another limitation is related to the incompleteness of the data regarding ovulation induction treatments in the medical records, thus preventing the analysis of the treatment dosage. Unfortunately, this information could not be supplemented from the personal interviews.

In conclusion, this follow-up study of infertile women disclosed an excess risk for breast cancer in women exposed to CC treatment. These results are sufficient to cause concern, but they should be cautiously interpreted in light of the fact that participants did not yet reach the peak age for breast cancer incidence. Additional studies are needed to disentangle the potential carcinogenic effect of CC treatment in infertile women.

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