Research Article

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A Cohort Study of Breast Cancer Risk after 20 Years of Follow-Up of Women Treated with Fertility Drugs

Sonia Guleria[1], Susanne K. Kjær[1,2], Vanna Albieri[3], Kirsten Frederiksen[3], and Allan Jensen[1]

Abstract

Background: Using a nationwide cohort of Danish women, we investigated the association between use of fertility drugs and risk of breast cancer.

Methods: The study cohort included women ages 20 to 44 years and living in Denmark between January 1, 1995 and December 31, 2011. Information on fertility status, use of fertility drugs, breast cancer, covariates, and vital status was obtained from the Danish Infertility Cohort and various Danish national registers. Cox proportional hazard regression models were applied to estimate hazard ratios (HR) and 95% confidence intervals (CI), adjusted for potential confounders.

Results: Of the 1,330,852 women included, 96,782 (7.3%) were infertile, and 20,567 (1.5%) were diagnosed with breast cancer during a median follow-up of 20.9 years. Compared with fertile women, infertile women who had used any fertility drugs did not have an increased hazard for breast cancer overall (HR = 1.02; 95% CI, 0.95–1.10), or for any of the histologic types (ductal, lobular, or mucinous) of breast cancer. Furthermore, no associations were observed between use of specific types of fertility drugs and breast cancer.

Conclusions: No convincing associations between use of fertility drugs and breast cancer were observed after two decades of follow-up.

Impact: Our results do not support a marked association between fertility drugs and breast cancer and are therefore reassuring for infertile women treated with fertility drugs.

Introduction

In 2018, 2.8 million new breast cancer cases were detected worldwide by the International Agency for Research on Cancer (http://gco.iarc.fr/today/home). Hence, breast cancer is the most frequent cancer among women across the world and accounts for one-fourth of all cancers in women. The etiology of breast cancer is multifactorial and important risk factors for breast cancer include nulliparity, older age at first child birth, early age at menarche, delayed menopause, use of oral contraceptives, and menopausal hormone therapy while late age at menarche, multiple child births, oophorectomy, and anti-estrogenic drugs such as tamoxifen decrease the risk (1).

Because of postponement of parenthood and increased availability of fertility treatment including assisted reproductive technologies (ART), the number of women undergoing fertility treatment has increased in most western countries and more than 7 million children have now been conceived following fertility treatment (2). Fertility drugs (e.g., clomiphene citrate, gonadotropins, and gonadotropin-releasing hormone analogues) are used in most treatment regimens and many epidemiologic studies have examined potential associations between fertility drugs and various hormone-sensitive cancers, including breast cancer (3). Excess endogenous estrogen may act as a carcinogen by stimulating proliferation of breast epithelial cells, by causing free-radical mediated DNA damage, or by altering the morphology of breast tissue (4–6). It can therefore be hypothesized that fertility drugs may increase the risk of breast cancer because fertility drugs can increase blood estrogen levels to a higher level than that observed during a normal menstrual cycle.

Many studies have examined the potential effect of fertility drugs on breast cancer risk, but the results have been inconclusive. Although most studies, including 2 meta-analyses (7, 8), report no marked associations (9–19), some studies show an increased risk of breast cancer among subgroups of users such as nulliparous women or women treated with specific types of fertility drugs (20–33) and some studies report a decreased risk of breast cancer (34–38). Many of the previous studies, however, have methodologic limitations including a low number of cancers, short or incomplete follow-up, or have not examined whether differences in risk according to histological type of breast cancer exist. Furthermore, most studies could not distinguish the possible effects of fertility drugs from the underlying causes of infertility, which could independently affect the risk of breast cancer.

Hence, using data from a large cohort consisting of all women in the reproductive age in Denmark between 1995 and 2011, the

[1]Virus, Lifestyle and Genes, Danish Cancer Society Research Center, Copenhagen, Denmark. [2]Statistics and Pharmacoepidemiology, Danish Cancer Society Research Center, Copenhagen, Denmark. [3]Department of Gynecology, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark.

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Corresponding Author: Allan Jensen, Danish Cancer Society Research Center, Strandboulevarden 49, Copenhagen 2100, Denmark. Phone: 45-3525-7693; Fax: 45-3525-7731; E-mail: allan@cancer.dk

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aim of this study was to further examine the association between use of fertility drugs and risk of breast cancer, both overall and according to histological type.

Material and Methods

For a description of the Danish national registers and the Danish Infertility cohort used in this study, please see the supplementary material.

From the Danish Civil Registration System, we included all women born during January 1, 1951 to December 31, 1991, and living in Denmark during January 1, 1995 to December 31, 2011 ($N = 1{,}467{,}287$). Accordingly, all women were between 20 and 44 years old when they entered the study cohort. We used the unique personal identification numbers assigned to all residents of Denmark to link the study cohort to the Danish Infertility Cohort and various Danish national registers to obtain information on fertility status, use of fertility drugs, breast cancer, covariates, and vital status.

The study cohort was linked to the Danish Infertility cohort to obtain information on the women's fertility status and use of fertility drugs. All women diagnosed with fertility problems and accordingly registered in the Danish Infertility cohort were defined as infertile women while the remaining women in the study cohort who were not registered in the Danish Infertility Cohort were defined as fertile women. The Danish Infertility Cohort contains information on fertility drug use from the Danish National Prescription Register and the Danish *In Vitro* Fertilization register. In this study, women were considered to have used fertility drugs if they had a registration of fertility drug use in Danish Infertility Cohort.

To identify all incident breast cancer cases, the study cohort was linked with the Danish Cancer Register using the International Classification of Diseases, tenth edition (ICD-10) code C50. Further, the International Classification of Disease for Oncology, 3rd edition (ICD-O-3) morphology codes were used to classify breast cancer cases into 3 main histologic types being ductal, lobular, and mucinous breast cancer and a common group consisting of all other histologic types of breast cancer encountered (all other ICD-O-3 codes). All covariates were selected *a priori* based on the knowledge of their influence on both the exposure and the outcome, albeit limited by their availability in the national Danish registers. Thus, we included calendar year of entry in cohort (from the Danish Civil Registration System), highest obtained level of education (from Statistics Denmark), pregnancy status (induced and spontaneous abortions from the Danish National Patient Register and births from the Danish Medical Birth Register), parity status and age at first child birth (from the Danish Medical Birth Register), and hormonal contraceptive use (from the Danish National Prescription Register).

We excluded all women with a cancer diagnosis (except nonmelanoma skin cancer) before study entry and with missing information on covariate (information was missing only for "highest obtained level of education"), leaving 1,330,852 women in the final study cohort for analyses. All women were followed from the date of entry in the study cohort to the date of diagnosis of breast cancer, any other cancer (except nonmelanoma skin cancer; $n = 55{,}200$), emigration ($n = 94{,}017$), death ($n = 17{,}758$), or end of follow-up (December 31, 2015), whichever occurred first.

Statistical analysis

We used Cox proportional hazard regression models to estimate hazard ratios (HR) and 95% confidence intervals (CI) for breast cancer overall and for histologic types of breast cancer with women's age as the underlying time-scale to ensure comparison of women of the same age and allowing for delayed entry. In the main analyses, fertile women were used as the reference group. For analyses, the following exposure groups were considered: ever use of any fertility drugs and ever use of specific types of fertility drugs being clomiphene citrate, gonadotropins (follicle-stimulating hormone and human menopausal gonadotropin), human chorionic gonadotropin, gonadotropin-releasing hormone analogues and progesterone. As most women in our study had received different combinations of fertility drugs, the rate estimates for the individual fertility drugs represent the effect of the specific fertility drug in combination with any other types of fertility drugs used. All associations were adjusted for women's calendar year of entry in the cohort (1995–1999, 2000–2004, 2005–2009, or 2010–2011), highest obtained level of education (basic, vocational, or higher), pregnancy status (ever or never), parity status (nulliparous or parous), age at first child birth (<30 years or ≥30 years), and hormonal contraceptive use (never or ever). To account for change in exposure and covariate status throughout the study period, all exposure variables and covariates were entered as time-varying variables. The covariate "highest obtained level of education" was entered as a time-varying variable in 3 age groups (20–24 years, 25–29 years, and ≥30 years), where each woman was assigned the highest obtained level of education she had achieved at the start of each age group.

To study if any observed association between use of any fertility drugs and breast cancer (overall and according to histological type) could be explained by factors related to underlying infertility, this analysis was also performed using infertile women not treated with fertility drugs as a reference group. Furthermore, to study whether parity status modified the risk of breast cancer, we examined whether the associations between ever use of any and specific types of fertility drugs and breast cancer overall were different among nulliparous and parous women. The interaction terms between parity status and the exposure variables were evaluated by testing the model with interaction term against the model with only the main exposure by means of likelihood ratio test. The proportional hazard assumption was evaluated with test and graphical support for the correlation between Schoenfeld residuals and survival time and no violation of the assumption was observed. All variables were evaluated using the Wald test and all *P* values were 2-sided with level of significance settled at 0.05. R version 3.5.1 ("survival" and "rms" packages) was used for all statistical analyses (https://www.r-project.org/).

Results

Of the 1,330,852 women included in the study cohort, 96,782 (7.3%) were infertile. During the study period, the majority of the infertile women were treated with fertility drugs ($n = 86{,}231$; 89.1%). The median age at end of follow-up was 44.6 years (IQR 33.8–54.8 years). The median age at infertility diagnosis was 30.7 years [IQR = 27.5–34.8 years] and at first fertility drug use was 31.0 years (IQR = 27.8–35.0 years). The women in the study cohort contributed with a total of 20,899,182 person-years of follow-up. During a median follow-up of 20.9 years (IQR 11.9–20.9 years), we observed a total of 20,567 (1.5%) breast cancers,

Table 1. Characteristics[a] of the 1,330,852 women included in the study cohort between 1995 to 2011 according to fertility status and use of fertility drugs at the end of the study period

		Infertile women	
Characteristics[a]	**Fertile women *n* (%)**	**Not treated with fertility drugs *n* (%)**	**Treated with fertility drugs (any) *n* (%)**
Total	1,234,070 (92.7)[b]	10,551 (0.8)[b]	86,231 (6.5)[b]
Calendar year of study entry			
1995–1999	912,724 (74.0)	8,653 (82.0)	71,103 (82.5)
2000–2004	120,629 (9.8)	1,328 (12.6)	11,117 (12.9)
2005–2009	137,838 (11.2)	534 (5.1)	3,750 (4.3)
2010–2011	62,879 (5.1)	36 (0.3)	261 (0.3)
Highest obtained level of education			
Basic	279,613 (22.7)	3,094 (29.3)	14,635 (17.0)
Vocational	609,352 (49.4)	4,752 (45.0)	40,295 (46.7)
Higher	345,105 (28.0)	2,705 (25.6)	31,301 (36.3)
Pregnancy status			
Never	290,778 (23.6)	1,593 (15.1)	7,922 (9.2)
Ever	943,292 (76.4)	8,958 (84.9)	78,309 (90.8)
Parity status			
Nulliparous	350,782 (28.4)	2,221 (21.1)	12,846 (14.9)
Parous	883,288 (71.6)	8,330 (78.9)	73,385 (85.1)
Age at first child birth			
Nulliparous	350,782 (28.4)	2,221 (21.1)	12,846 (14.9)
Parous			
<30 years	619,672 (50.2)	5,575 (52.8)	36,292 (42.1)
≥30 years	263,616 (21.4)	2,755 (26.1)	37,093 (43.0)
Hormonal contraceptive use			
Never	332,856 (27.0)	1,781 (16.9)	11,296 (13.1)
Ever	901,214 (73.0)	8,770 (83.1)	74,935 (86.9)

[a]All characteristics except calendar year of study entry were assessed at the end of the study period.
[b]Row percentages. All other percentages in the table are column percentages.

of which 19,725 (1.6%) were observed among fertile women and 743 (0.9%) were observed among infertile women treated with fertility drugs. Ductal breast cancer was the most common histologic type observed (17,648, 85.8%) followed by lobular (2,057, 10.0%) and mucinous breast cancer (231, 1.1%).

In Table 1, characteristics assessed at the end of the study period for all women in the study cohort are presented. Compared with the fertile women, infertile women who had used fertility drugs were more likely to have higher level of education, to have been pregnant, to have conceived the first child at 30 years of age or later, and to have used hormonal contraceptives.

In Table 2, associations between use of fertility drugs (any and for different types) and breast cancer (overall and histological types) are presented. Compared with fertile women, infertile women who had used any fertility drugs did not have an increased hazard for breast cancer overall (HR = 1.02; 95% CI, 0.95–1.10) compared with fertile women and none of the specific types of fertility drugs affected the overall hazard for breast cancer. Likewise, no marked associations between use of any and specific types of fertility drugs and histological types (ductal, lobular, and mucinous) of breast cancer were observed. We also assessed the associations between use of any fertility drugs and breast cancer (overall and according to histological type) using infertile women not treated with fertility drugs as a reference group; however, the results were virtually unchanged (data not shown).

Finally, we analyzed whether the associations between use of any fertility drugs and breast cancer varied according to parity status (Table 3). There was a weak indication of non-significantly increased breast cancer incidence rates in nulliparous women treated with gonadotropin-releasing hormone analogues (HR = 1.19; 95% CI, 0.97–1.45) and progesterone (HR = 1.18; 95% CI, 0.95–1.47) compared with parous women (gonadotropin-releasing hormone analogues: HR = 0.99; 95% CI, 0.85–1.15; progesterone: HR = 1.03; 95% CI, 0.88–1.47); however, none of the interactions terms were statistically significant (gonadotropin-releasing hormone analogues: $P = 0.06$; progesterone: $P = 0.30$). For the remaining drugs, breast cancer risk did not vary markedly with parity status.

Discussion

In this large population-based register study including 1.3 million Danish women followed up for a median period of 20 years, use of fertility drugs was not associated with an increased risk for breast cancer, neither overall nor for the different histologic types (ductal, lobular, and mucinous), when compared with fertile women. Similarly, the associations did not change when factors related to the underlying infertility were taken into account, that is when infertile women not treated with fertility drugs were used as a reference group. For most types of fertility drugs, the risk of breast cancer did not differ by parity status, but there was an indication of slight increase in breast cancer risk after use of gonadotropin-releasing hormone analogues and progesterone among nulliparous women.

Our finding of no association between use of fertility drugs and breast cancer is in line with the results from most previous studies (7, 9–19, 28). Short duration of follow-up and a low number of included cancer cases are important limitations of previous studies. In cohort studies with a long follow-up period (i.e. more than 10 years), however, the results are more inconclusive (Table 4). Although some of these studies have reported no associations (12, 14, 15, 17, 18), including 2

Table 2. Adjusted HRs and 95% CIs for breast cancer (overall and histologic types) according to use of any and specific types of fertility drugs compared with fertile women

	Overall breast cancer			Ductal breast cancer		Lobular breast cancer		Mucinous breast cancer	
Women's characteristics	**PY**	***n***	**HR (95% CI)[a]**	***n***	**HR (95% CI)[a]**	***n*[b]**	**HR (95% CI)[a]**	**n[b]**	**HR (95% CI)[a]**
Fertile	20,899,182	19,725	1	16,916	1	>5	1	>5	1
Use of any fertility drugs									
Infertile, not treated with fertility drugs	143,258	99	1.01 (0.83–1.23)	88	1.03 (0.83–1.27)	>5	0.73 (0.33–1.63)	≤5	1.73 (0.42–6.99)
Infertile, treated with fertility drugs	942,430	743	1.02 (0.95–1.10)	644	1.01 (0.93–1.10)	>5	1.12 (0.87–1.44)	>5	0.90 (0.43–1.87)
Clomiphene citrate									
Infertile, treated but not treated with clomiphene citrate[c]	280,278	249	1.02 (0.90–1.16)	211	1.00 (0.87–1.14)	≤5	1.29 (0.89–1.88)	≤5	1.02 (0.32–3.21)
Infertile, treated with clomiphene citrate	662,152	494	1.02 (0.93–1.12)	433	1.02 (0.93–1.13)	≤5	1.02 (0.74–1.41)	≤5	0.84 (0.34–2.10)
Gonadotropins[d]									
Infertile, treated but not treated with gonadotropins[c]	355,296	292	1.04 (0.92–1.16)	244	1.00 (0.88–1.13)	≤5	1.23 (0.86–1.76)	≤5	0.93 (0.30–1.92)
Infertile, treated with gonadotropins	587,134	451	1.01 (0.92–1.11)	400	1.03 (0.93–1.14)	≤5	1.04 (0.74–1.44)	≤5	0.89 (0.36–2.22)
hCG									
Infertile, treated but not treated with hCG[c]	215,157	190	1.06 (0.92–1.23)	157	1.01 (0.87–1.19)	≤5	1.41 (0.93–2.13)	≤5	1.49 (0.47–4.67)
Infertile, treated with hCG	727,273	553	1.01 (0.92–1.10)	487	1.01 (0.92–1.11)	≤5	1.01 (0.75–1.37)	≤5	0.72 (0.29–1.80)
GnRH									
Infertile, treated but not treated with GnRH[c]	533,611	378	1.00 (0.90–1.10)	326	0.99 (0.88–1.10)	≤5	1.10 (0.79–1.55)	≤5	0.91 (0.33–2.49)
Infertile, treated with GnRH	408,819	365	1.05 (0.94–1.16)	318	1.05 (0.93–1.17)	≤5	1.14 (0.80–1.61)	≤5	0.89 (0.32–2.46)
Progesterone									
Infertile, treated but not treated with progesterone[c]	533,275	391	0.98 (0.89–1.09)	343	0.99 (0.88–1.10)	≤5	0.98 (0.69–1.38)	≤5	0.43 (0.11–1.74)
Infertile, treated with progesterone	409,155	352	1.07 (0.96–1.19)	301	1.05 (0.93–1.18)	≤5	1.30 (0.93–1.82)	>5	1.45 (0.62–3.35)

Abbreviations: CI, confidence interval; GnRH, gonadotropin-releasing hormone analogues; hCG, human chorionic gonadotropin; HR, hazard ratio; PY, person-year.
[a]Adjusted for calendar year of study entry, highest obtained level of education, pregnancy status, parity status, age at first child, and hormonal contraceptive use.
[b]Due to the data security policy of Statistics Denmark, numbers <5 cannot be presented for protection of privacy.
[c]Women have received multiple combinations of drugs.
[d]Includes follicle-stimulating hormones and human menopausal gonadotropins.

studies with 30 years of follow-up (14, 33), some have reported an increased risk of breast cancer among subgroups of users such as women treated with specific types of fertility drugs (20, 22, 23, 27, 29, 31, 32), women treated with a high number of treatment cycles (27, 33), and among nulliparous or parous women only (32).

We also assessed whether use of fertility drugs increased the risk for different histologic types of breast cancer and found no

Table 3. Adjusted hazard ratios and 95% confidence intervals for breast cancer (overall) in nulliparous and parous women according to use of any and specific types of fertility drugs compared with fertile women

	Nulliparous			Parous			
Women's characteristics	**PY**	**Breast cancer**	**HR (95% CI)[a]**	**PY**	**Breast cancer**	**HR (95% CI)[a]**	**Interaction (*P* value)**
Fertile	6,667,490	2,674	1	14,231,692	17,051	1	
Use of any fertility drugs							
Infertile, not treated with fertility drugs	46,679	24	1.00 (0.67–1.50)	96,579	75	1.00 (0.78-1.27)	0.96
Infertile, treated with fertility drugs	224,238	142	1.00 (0.84–1.85)	718,192	601	1.02 (0.90-1.15)	
Clomiphene citrate							
Infertile, treated but not treated with clomiphene citrate[b]	64,688	36	0.79 (0.57–1.10)	215,590	213	1.06 (0.90-1.25)	0.30
Infertile, treated with clomiphene citrate	159,549	106	1.10 (0.90–1.33)	502,602	388	0.99 (0.87-1.14)	
Gonadotropins[c]							
Infertile, treated but not treated with gonadotropins[b]	69,574	36	0.91 (0.65–1.26)	285,723	256	1.05 (0.90-1.21)	0.85
Infertile, treated with gonadotropins	154,664	106	1.03 (0.85–1.26)	432,470	345	1.00 (0.86-1.15)	
hCG							
Infertile, treated but not treated with hCG[b]	39,619	23	0.98 (0.65–1.47)	175,539	167	1.07 (0.89-1.27)	0.98
Infertile, treated with hCG	184,619	119	1.00 (0.83–1.21)	542,654	434	1.00 (0.87-1.14)	
GnRH							
Infertile, treated but not treated with GnRH[b]	111,008	43	0.73 (0.54–0.99)	422,603	335	1.04 (0.90-1.20)	0.06
Infertile, treated with GnRH	113,230	99	1.19 (0.97–1.45)	295,589	266	0.99 (0.85-1.15)	
Progesterone							
Infertile, treated but not treated with progesterone[c]	122,172	57	0.81 (0.63–1.06)	411,103	334	1.01 (0.88-1.16)	0.30
Infertile, treated with progesterone	102,066	85	1.18 (0.95–1.47)	307,089	267	1.03 (0.88-1.20)	

Abbreviations: CI, confidence interval; GnRH, gonadotropin-releasing hormone analogue; hCG, human chorionic gonadotropin; HR, hazard ratio; PY, person-years.
[a]Adjusted for calendar year of study entry, highest obtained level of education, pregnancy status, parity status, age at first child, and hormonal contraceptive use.
[b]Women have received multiple combinations of drugs.
[c]Includes follicle-stimulating hormones and human menopausal gonadotropins.

Table 4. Review of cohort studies with more than 10 years of follow-up

Author, year, country	Study sample	Fertility drugs in the study	No. in study sample	No. exposed	No. of exposed with BC	Length of f/u (mean/median; years)	Age at end of f/u (mean; years)	Estimates (95% CI)[a]
Guleria, 2019, DK (this article)	Women ages 20–44 years, living in Denmark during 1995–2011	CC, gonadotropins, hCG, GnRH, progesterone	1,330,852	86,231	743	21 (median)	44	HR = 1.02 (0.95–1.10)
No association								
Modan and colleagues, 1998, Israel (15)	Women diagnosed with infertility during 1964–1974	CC, hMG	2,496	1,309	25	21 (mean)	50	SIR = 1.1 (0.7–1.6)
Doyle and colleagues, 2002, UK (12)	Women treated for infertility during 1975–1989	CC, gonadotropins, hCG, GnRH	5,556	4,188	55	15 (median)	46	RR = 0.95 (0.47–1.92)
Gauthier and colleagues, 2004, France (17)	Women ages 40–65 years during 1990–1991	CC, gonadotropins	92,555	6,602	183	10 (mean)	—	RR = 1.0 (0.8–1.1)
Lerner-Geva and colleagues, 2012, Israel (14)	Women treated for infertility during 1964–1974	CC, gonadotropins	2,431	2,431	153	34 (mean)	63	SIR = 1.20 (0.98–1.40)
Lundberg and colleagues, 2017, SW (18)	Women with first delivery during 1982–2012	CC, gonadotropins, GnRH	1,340,211	38,047	262	15 (mean)	—	HR = 0.8 (0.7–1.0)
No overall association but increased risk in specific subgroups								
Potashnik and colleagues, 1999, Israel (20)	Women treated for infertility during 1960–1984	CC, hMG	1,197	780	16	18 (mean)	44	SIR (CC, 1–2 cycles) = 2.60 (1.19–5.00) SIR (CC, ≤1,000 mg) = 2.52 (1.21–4.64)
Lerner-Geva and colleagues, 2006, Israel (23)	Women with ovulation induction during 1964–1984	CC, gonadotropins	5,788	3,076	73	21 (mean)	50	SIR (CC) = 1.40 (1.05–1.83)
Orgeas and colleagues, 2009, SW (27)	Women treated for subfertility during 1961–1976	CC, gonadotropins, hCG	1,135	1,135	54	26 (median)	53	SIR (CC, ≥4 cycles) = 1.9 (1.1–3.4)
Stewart and colleagues, 2012, Australia (31)	Infertile women ages 20–44 years during 1983–2002	IVF and/or fertility drugs	21,025	7,381	384	16 (mean)	48	HR (<24 years old at treatment) = 1.56 (1.0–2.4)
Brinton and colleagues, 2014, US (33)	Women treated for infertility during 1965–1988	CC, gonadotropins	12,193	9,892	749	30 (median)	NA	HR (CC, ≥ 2,251 mg, ≥ 6 cycles) = 1.27 (1.02–1.59) HR (≥35 years at treatment) = 1.31 (1.00–1.73)
Reigstad and colleagues, 2015, NO (29)	Women who delivered during 1984–2010	ART and/or fertility drugs	808,834	16,626	138	16 (median)	40	HR (parous women) = 1.20 (1.01–1.42) HR (IVF, >10 years f/u) = 1.35 (1.07–1.71)
Reigstad and colleagues, 2017, NO (32)	Women born during 1960–1996	CC	1,353,724	56,194	920	11 (median)	42	HR (CC, parous) = 1.3 (1.0–1.5)
Decreased risk								
Terry and colleagues, 2006, US (36)	Women registered as nurses during 1993–2001	CC, gonadotropins	4,000	—	71	12 (—)	NA	HR = 0.6 (0.4–0.9) HR (CC, use >10 months) = 0.3 (0.1–0.8)
van den Belt-Dusebout and colleagues, 2016, NL (38)	Women treated for infertility during 1983–1995	CC, gonadotropins, GnRH	25,108	19,158	839	21 (median)	54	HR = 1.0 (0.9–1.2) HR (>7 IVF cycles) = 0.6 (0.4–0.8)

Abbreviations: CC, clomiphene citrate; CI, confidence interval; f/u, follow-up; GnRH, gonadotropin-releasing hormone analogue; hCG, human chorionic gonadotropin; hMG, human menopausal gonadotropins; HR, hazard ratio; IVF, *in vitro* fertilization; NA, not available; No., number; RR, relative risk; SIR, standardized incidence ratio.

[a]These are overall estimates unless specified.

associations between use of fertility drugs and any of the major histologic types (ductal, lobular, and mucinous) of breast cancer. To our knowledge, only 2 studies have previously examined the associations between fertility drugs and breast cancer according to histologic type (21, 24). In a multicenter study that included approximately 4,000 breast cancer cases and an equal number of controls, Burkman and colleagues (21) also reported null associations but observed a statistically nonsignificant 60% increased risk for ductal breast cancer among women treated with human menopausal gonadotropin. In a case-cohort study based on 54,000 infertile Danish women, we observed a 4-fold increased risk for ductal breast cancers among progesterone users (24). However, the associations reported for ductal breast cancer in both previous studies were based on very few exposed women (32 and 8, respectively), whereas the associations observed in our study were based on a much larger number of women (644) with ductal breast cancer who had also used fertility drugs and may therefore be more precise.

Our study results showed a slight increase in breast cancer risk after use of gonadotropin-releasing hormone analogues and progesterone among nulliparous women. These results thus indicate that the use fertility drugs may impose a higher breast cancer risk if a woman does not succeed in having children and is supported by results from a few other studies that also examined the interaction between use of fertility drugs and parity status (24, 33). Possible explanations for a differentiated breast cancer risk according to parity status is not known but it can be suggested that nulliparous women may have more severe fertility problems which are associated with cancer risk, may have genetic susceptibility to both infertility and cancer, or may have received more intensive (i.e. more cycles and/or high doses) fertility treatment. Furthermore, it is also likely that the potential increase in breast cancer risk due to fertility drugs among nulliparous women was not offset by the protective effects of a pregnancy. However, the literature is conflicting as other studies have either not found any interaction between fertility treatment and parity status (18, 30), showed a reduced risk among nulliparous women (38), or observed an increased risk among parous women (29, 32), and this topic should clearly be further investigated.

Several strengths of our study are noteworthy. First, our study included 1.3 million women of whom 743 developed breast cancer after use of fertility drugs, which makes it one of the largest cohort studies to date. Our risk estimates are therefore more precise than most previous studies. Second, while our main analyses were performed with a reference group of fertile women, we additionally used a reference group of infertile women who had not used fertility drugs, which enabled us to minimize the potential effects of the underlying infertility. Third, the population-based design with inclusion of virtually all women diagnosed with infertility in Denmark makes our results generalizable to similar populations. Fourth, the unique Danish personal identification number enabled a precise linkage between the Danish Infertility Cohort and the different national registers and ensured virtually no loss to follow-up and prevented recall and selection bias. Finally, the use of the high-quality Danish Cancer Register ensured a complete ascertainment of breast cancer cases and the nationwide Danish In-Vitro Fertilization Register and the Danish National Prescription Register provided thorough and detailed information about the specific types of fertility drugs used in fertility treatment which enabled analyses on their potential differential associations with breast cancer.

Our study also has limitations. Even though we had longer follow-up period (median follow-up: 21 year) than most previous studies, the median age of breast cancer diagnosis in our study (50 years) was markedly lower than the median age at which most women in Denmark are diagnosed with breast cancer (62 years). Consequently, this study may not have been able to assess the true long-term association between use of fertility drugs and breast cancer and our results are therefore mostly applicable to premenopausal women. A further limitation of our study is that although we used various high-quality national registers to identify infertile women, we cannot rule out some misclassification of fertility status. This is due to the fact that some women in the general Danish population could have undiagnosed fertility problems and a few infertile women could be diagnosed only by private gynecologists who are not obliged to report to the national registers that were used in this study. Furthermore, even though we were able to examine the effect of the specific types of fertility drugs, we were not able to examine if breast cancer risk varied with number of treatment cycles or dose since this information is not complete in the used registers. Although, we were able to control for a number of important confounders, unmeasured and residual confounding may have been present as we could not adjust for some factors which are likely to influence the associations under study including comorbidity, age at menarche and menopause, a family history of breast cancer (including BRCA mutations), and body weight. Finally, we were not able to conduct analyses stratified by estrogen/progesterone receptor status or menopausal status as this information was not available for the women in the study cohort.

In conclusion, results from this large population-based cohort study showed that after 20 years of follow-up, infertile women who ever used fertility drugs had a similar risk for breast cancer compared with fertile women. Similarly, none of the specific types of fertility drugs affected the risk of breast cancer, except for an indication of an increased risk after use of gonadotropin-releasing hormone analogues and progesterone among nulliparous women. Our study thus adds to the existing literature and can help women and healthcare providers to make informed decisions on management of infertility.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: S. Guleria, S.K. Kjær, V. Albieri, K. Frederiksen, A. Jensen

Development of methodology: S. Guleria, S.K. Kjær, A. Jensen

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): S.K. Kjær, V. Albieri, A. Jensen

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): S. Guleria, S.K. Kjær, V. Albieri, K. Frederiksen, A. Jensen

Writing, review, and/or revision of the manuscript: S. Guleria, S.K. Kjær, V. Albieri, K. Frederiksen, A. Jensen

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): V. Albieri

Study supervision: S.K. Kjær, K. Frederiksen, A. Jensen

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