Risk of Breast Cancer After Exposure to Fertility Drugs: Results from a Large Danish Cohort Study

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Abstract

Background: **Few epidemiologic studies have examined the association between fertility drugs and breast cancer risk, and results have been contradicting. Using data from the largest cohort of infertile women to date, the aim of this study was to examine the effects of fertility drugs on breast cancer risk overall and according to histologic subtypes.**

Method: **A cohort of 54,362 women with infertility problems referred to all Danish fertility clinics between 1963 and 1998 was established. A detailed data collection, including information of type and amount of treatment, was conducted. We used case-cohort techniques to calculate rate ratios (RR) of breast cancer associated with use of five groups of fertility drugs, after adjustment for parity status.**

Results: **Three hundred thirty-one invasive breast cancers were identified in the cohort during follow-up through 1998. Analyses within cohort showed no overall increased breast cancer risk after use of gonadotrophins, clomiphene, human chorionic gonadotrophin, or gonadotrophin-releasing hormone, whereas use of progesterone increased breast cancer risk (RR, 3.36; 95% confidence interval, 1.3-8.6). For all groups of fertility drugs, no relationships with number of cycles of use or years since first use of fertility drug were found. However, gonadotrophins may have a stronger effect on breast cancer risk among nulliparous women (RR, 1.69; 95% confidence interval, 1.03-2.77). Similar risk patterns were present for ductal, lobular, and tumors of other histologies, indicating identical etiologies.**

Conclusion: **The results showed no strong association between breast cancer risk and use of fertility drugs. Follow-up is, however, needed to assess long-term breast cancer risk after use of progesterone and among nulliparous women exposed to gonadotrophins.** (Cancer Epidemiol Biomarkers Prev 2007;16(7):1400–7)

Introduction

The etiology of breast cancer is multifactorial where both endogenous and exogenous hormones have an important role. Concerning the endogenic hormones, influence is well recognized for factors such as nulliparity, late age at first birth, early menarche, and late menopause (1-4), and the influence of different groups of exogenous hormones (i.e., hormonal contraceptives and hormone replacement therapy) on the risk of breast cancer has also been widely studied (5, 6).

In contrast, less is known about the influence of fertility hormonal drugs on the risk of breast cancer, despite the well-known effect on ovulation and endogenous hormone production of this group of exogenous hormones. Experimentally, it has been shown that ovarian hormones play a role in the development of breast cancer (7) and also some clinical findings (8-10) have indicated that fertility drugs may increase the risk of developing breast cancer. The use of fertility drugs has held an important place in infertility treatment during the last ~30 years, with a large and constantly growing numbers of women seeking advice for infertility treatments (e.g., *in vitro* fertilization). In combination with the high incidence of breast cancer found in most Western countries, the question of whether use of fertility drugs increases the risk of breast cancer is therefore a matter of great public health concern.

However, only a limited number of epidemiologic studies have examined the possible association between use of fertility drugs and risk of breast cancer. Results, thus far, have been contradicting, as most studies found no association between use of fertility drugs and risk of breast cancer (11-22), two studies found that fertility drugs increase the risk of breast cancer (23, 24), whereas two other studies found that use of fertility drugs decrease the risk of developing breast cancer (25, 26). Many of the previous studies have faced various methodologic limitations, for example, low statistical power due to a low number of breast cancer cases and inability to control for potential confounders. However, the most recent cohort study by Brinton et al. (22), which involved a large number of cases and had the ability to control for potential confounders, reported no overall increase in breast cancer after exposure to clomiphene and gonadotrophins, but found a significantly elevated risk of breast cancer after 20 years of follow-up since first use of clomiphene.

We have established a cohort including 54,362 Danish women with infertility problems in the period 1963 to 1998. The cohort involves a large number of breast cancer cases and includes extensive information about drug history and information about reproductive factors. In a previous analysis,[3] we found that women in this infertility cohort had an 8% higher breast cancer risk than women in the general Danish population, even when adjusted for nulliparity that is a recognized risk factor for breast cancer. This result indicates that the increased risk of breast cancer may not only be due to a lower parity in the cohort of infertile women, but may also be related to other factors, such as use of fertility drugs. In this article, we did a case-cohort study to evaluate the effects of different types of fertility drugs on the risk of breast cancer after adjustment for reproductive factors.

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[3] A. Jensen, et al. Risk of breast cancer and gynaecological cancers in a large population of nearly 50,000 infertile Danish women, personal communication.

Materials and Methods

Cohort Identification and Data Collection. A cohort of women with infertility problems referred to Danish hospitals or private fertility clinics in the period 1965 to 1998 was established. All gynecological departments and all private fertility clinics in Denmark were included. Patients were identified from medical files, microfilms, or index cards. In addition, we included patients with an infertility diagnosis (ICD-8 code 628; ICD-10 code DN97) recorded in the National Patient Registry, a nationwide registry of virtually all somatic discharges in Danish Hospitals since 1977. Both women with primary and secondary infertility were included in the study. A total of 54,379 women were included in the infertility cohort.

All data were edited and merged into a single database with a record for each woman with an infertility diagnosis. Each record included the Danish unique personal identification number, name of fertility clinic, and first date of infertility evaluation. To verify the personal identification number and to determine eventual migration date or date of death, the cohort of infertile women was linked with the Civil Registration System using the personal identification number. The computerized population-based Civil Registration System was founded on April 1, 1968, under the Ministry of the Interior, when all citizens were assigned the Danish unique personal identification number. The registry includes information about current and former addresses, migration dates, and date of death on all persons ever living in Denmark since April 1, 1968, and is updated weekly. Of the 54,379 infertile women, all but 17 were found to have a valid personal identification number in the Civil Registration System. The study was approved by the Scientific Ethical Committee and the Data Protection Board in Denmark.

Identification of Cases. To determine breast cancer status after enrollment in the study, the cohort was linked to the Danish Cancer Registry. Established in 1943, the Danish Cancer Registry contains nationwide information about all incident invasive cancer cases from hospital departments, practicing specialists, and autopsy reports from pathology departments. It is supplemented by linkages to The Causes of Death Registry and The National Patient Registry to ensure a complete registry. The cohort of infertile women were followed for breast cancer occurrence from the first date of infertility evaluation until date of emigration, date of death, or December 31, 1998, whichever came first. At the time of the linkage, a total of 372 women were diagnosed with breast cancer in the follow-up period.

Identification of the Subcohort. In a case-cohort design, the experience of all cases is compared with the experience of a randomly selected subcohort (27). In the present study, a subcohort of 1,360 women were randomly selected from the cohort in four strata for the age at entry to the infertility cohort (18-26, 27-30, 31-36, and $\geq$37 years) and five strata for the year of entering the infertility cohort (1965-1977, 1978-1984, 1985-1989, 1990-1996, and 1997-1998), equaling 20 strata.

Ascertainment of Exposure and Potential Confounders. For all infertile women developing breast cancer and the members of the subcohort, we collected hospital files and medical records on all available infertility-related medical visits. For 31 cases, the records could not be found, and for 10 cases the cause of infertility was found to be previous sterilization, leaving 331 cases (89%) for analysis. In the subcohort, we had to exclude 93 women for whom the records could not be found, 8 women where the infertility diagnosis could not be confirmed, and 33 women where the cause of infertility was previous sterilization, leaving 1,226 women in the subcohort (90%). Of the 1,226 women in the subcohort, 24 women were diagnosed with breast cancer in the follow-up period. These women are therefore included both as cases and as members of the subcohort in the analyses.

Information was abstracted on surgical and medical interventions for infertility, including the types of fertility drugs prescribed and the number of treatment cycles. For each treatment cycle, dates of starting and stopping were abstracted to define the windows of exposure to the drugs. Dosage information was also abstracted, although, in many instances, this information was not recorded. In addition, we intended to abstract information about causes of infertility, ever use of oral contraceptives, and body mass index from the medical records; however, the information was, unfortunately, not available for most women. Trained abstractors entered all these data into computers, using standardized software.

To obtain information about reproductive history, the cohort of infertile women was linked to the Civil Registration System and the Danish National Birth Registry using the personal identification numbers as key identifiers. The population-based Danish National birth registry contains information about all births in Denmark since 1973. From 1973 and onward, information about reproductive history was obtained from this registry, whereas reproductive history before 1973 was obtained from the Civil Registration System, as this registry includes ways to link parents and children. Information about number of births and age at births was obtained for all infertile women with breast cancer and for all the members of the subcohort.

Statistical Analysis. According to the sampling strategy, the unweighted case-cohort approach (28, 29) was used to estimate rate ratios (RR) for breast cancer using a Cox proportional hazard regression model stratified according to the sampling strata (age and year of enrollment). Age was used as time scale to ensure that the estimation was based on comparisons of women of the same age. The analysis was corrected for delayed entry such that women were considered at risk only from the age at first date for infertility evaluation. The estimation of the RRs was done as suggested by Prentice (27), in which all the women in the subcohort contribute to all the relevant risk sets until end of the follow-up period due to cancer diagnosis, death, migration, or censoring, whereas case women outside the subcohort only enter their own risk set. The 95% confidence intervals (95% CI) were based on robust estimates of the variance-covariance matrix of the Cox regression variables.

Using this Cox model, we evaluated the effect of the following fertility drugs: follicle-stimulating hormone (FSH), human menopausal gonadotrophins (hMG), clomiphene, human chorionic gonadotrophins (hCG), gonadotrophin-releasing hormone (GnRH), and progesterone, all measured as (*a*) ever use and (*b*) number of cycles prescribed. In the analyses, however, we pooled the two gonadotrophins, follicle-stimulating hormone (FSH) and human menopausal gonadotrophin (hMG), into one group called gonadotrophins, as they have identical modes of operation. The women in the cohort were also treated with a large number of other types of fertility drugs, such as estradiol and diethyl stilbestrol. The effect of these heterogeneous fertility drugs will not be analyzed, however, as each type of drugs was only provided to a very low number of women. Potential confounding factors investigated included parity (ever childbirth), number of additional births, age at first birth, and age at last birth. All variables were entered as time-dependent covariates, changing values at the specific ages where a new event happens (e.g., birth of a child or start of a new treatment cycle). Information about dosage of fertility drugs, causes of infertility, and ever use of oral contraceptives was not available for most women and will therefore not be included as potential confounders in the analyses.

Table 1. Calendar year and age at initial clinic evaluation among 54,362 Danish women evaluated for infertility in the period 1965 to 1998

Calendar year	Age (y)				In total (%)
	18-26	27-30	31-36	≥37	
1963-1977	1,837	1,981	1,345	182	5,345 (9.8)
1978-1984	4,538	4,857	3,917	773	14,085 (25.9)
1985-1989	1,789	2,747	2,816	635	7,987 (14.7)
1990-1996	3,734	7,893	9,383	3,038	24,048 (44.2)
1997-1998	420	814	1,219	444	2,897 (5.3)
In total (%)	12,318 (22.7)	18,292 (33.6)	18,680 (34.4)	5,072 (9.3)	54,362 (100.0)

Results

A total of 54,362 women were included in the infertility cohort. The distribution of entry period and age at entry in the cohort is shown in Table 1. The median year and age at first infertility evaluation were 1989 and 30 years, respectively, whereas the median age at the end of follow-up was 40 years. The median length of follow-up was 8.8 years (range 0.0-35.2 years), with 25% followed up for more than 16 years. In total, the 54,362 women contributed 564,971 person-years of observation. Due to the sampling strategy, there were no marked differences in the distribution of the demographic variables (median year and age at cohort entry, median age at the end of the study, and median length of follow-up) between the subjects in the subcohort and the subjects of the total infertility cohort.

The combinations of fertility drugs use within single treatment periods (where one treatment period is defined as a continuous sequence of treatment cycles) for women with breast cancer and subcohort members are shown in Table 2. Out of the 331 women with breast cancer who were included in the study, 140 women (42%) used any fertility drug in 247 treatment periods during the follow-up period. Among the 1,226 subcohort members, 599 subcohort members (49%) used any fertility drug in 1,089 treatment periods during the follow-up period. In total, the most frequently used fertility drugs were by far clomiphene (cases 31%; subcohort 33%) and hCG

Table 2. Combined use of fertility drugs within single treatment periods for (*a*) women with breast cancer and (*b*) subcohort members from the Danish infertility cohort in the period 1963 to 1998

Combinations of fertility drugs	No. treatments periods (%)	
	Cases	Subcohort
Clomiphene	27 (10.9)	154 (14.1)
Clomiphene, gonadotrophins	12 (4.9)	18 (1.7)
Clomiphene, gonadotrophins, hCG	14 (5.7)	76 (7.0)
Clomiphene, gonadotrophins, hCG, GnRH	4 (1.6)	51 (4.7)
Clomiphene, gonadotrophins, hCG, GnRH, progesterone	1 (0.4)	1 (0.1)
Clomiphene, gonadotrophins, hCG, GnRH, other types	0 (0.0)	3 (0.3)
Clomiphene, gonadotrophins, hCG, progesterone	1 (0.4)	2 (0.2)
Clomiphene, gonadotrophins, hCG, other types	2 (0.8)	17 (1.6)
Clomiphene, gonadotrophins, GnRH	4 (1.6)	10 (0.9)
Clomiphene, gonadotrophins, GnRH, progesterone	1 (0.4)	0 (0.0)
Clomiphene, gonadotrophins, GnRH, progesterone, other types	1 (0.4)	0 (0.0)
Clomiphene, gonadotrophins, GnRH, other types	0 (0.0)	2 (0.2)
Clomiphene, gonadotrophins, other types	0 (0.0)	1 (0.1)
Clomiphene, hCG	63 (25.5)	246 (22.6)
Clomiphene, hCG, GnRH	0 (0.0)	1 (0.1)
Clomiphene, hCG, progesterone	1 (0.4)	2 (0.2)
Clomiphene, hCG, progesterone, other types	1 (0.4)	0 (0.0)
Clomiphene, hCG, other types	16 (6.5)	74 (6.8)
Clomiphene, progesterone	1 (0.4)	1 (0.1)
Clomiphene, other types	14 (5.7)	50 (4.6)
Gonadotrophins	1 (0.4)	2 (0.2)
Gonadotrophins, hCG	3 (1.2)	14 (1.3)
Gonadotrophins, hCG, GnRH	5 (2.0)	39 (3.6)
Gonadotrophins, hCG, GnRH, other types	1 (0.4)	4 (0.4)
Gonadotrophins, hCG, other types	0 (0.0)	5 (0.5)
Gonadotrophins, hCG, GnRH, progesterone	0 (0.0)	1 (0.1)
Gonadotrophins, hCG, GnRH, progesterone, other types	1 (0.4)	0 (0.0)
Gonadotrophins, GnRH	4 (1.6)	23 (2.1)
Gonadotrophins, GnRH, progesterone	1 (0.4)	1 (0.1)
Gonadotrophins, GnRH, other types	0 (0.0)	2 (0.2)
hCG	24 (9.7)	98 (9.0)
hCG, progesterone, other types	1 (0.4)	1 (0.1)
hCG, other types	10 (4.1)	24 (2.2)
GnRH	0 (0.0)	4 (0.4)
GnRH, progesterone, other types	0 (0.0)	1 (0.1)
GnRH, other types	0 (0.0)	4 (0.4)
Progesterone	0 (0.0)	3 (0.3)
Other types	33 (13.4)	154 (14.1)
In total	247 (100.0)	1,089 (100.0)

NOTE: One treatment period is defined as a continuous sequence of treatment cycles.

(cases 28%; subcohort 32%) followed by gonadotrophins (cases 11%; subcohort 13%), GnRH (cases 5%; subcohort 8%), and progesterone (cases 2%; subcohort 1%). The most frequently used combinations of fertility drugs were (*a*) clomiphene + hCG (cases 26%; subcohort 23%), (*b*) clomiphene + gonadotrophins + hCG (cases 6%; subcohort 7%), (*c*) clomiphene + gonadotrophins + hCG + GnRH (cases 2%; subcohort 5%), (*d*) gonadotrophins + hCG + GnRH (cases 2%; subcohort 4%), and (*e*) gonadotrophins + GnRH (cases 2%; subcohort 2%). The relative use of fertility drugs within the subcohort during the follow-up period is presented in Fig. 1. In 1963 to 1972, clomiphene was used in 29.2% of all treatment periods, increased steadily to 84.6% in 1988 to 1992, but fell to 62.1% in 1993 to 1998. Use of gonadotrophins, the other major group of ovulation-stimulating drugs, was low until the mid-1980s but then increased steadily from 7.1% in 1983 to 1987 to 70.9% in 1993 to 1998. Likewise, GnRH was not used until the mid-1980s but then increased steadily from 18.7% in 1988 to 1992 to 48.8% in 1993 to 1998. Use of hCG increased continuously during the period from 50.8% in 1963 to 1972 to 69.9% in 1993 to 1998, whereas use of the group of other fertility drugs declined steadily from 56.9% in 1963 to 1972 to 16.0% in 1993 to 1998. Use of progesterone was low during the whole period, but increased slightly to 2.9% in 1993 to 1998. The median lengths of follow-up after first use of each of the five fertility drugs were as follows: gonadotrophins, 6.3 years; clomiphene, 13.0 years; hCG, 13.9 years; GnRH, 4.8 years; and progesterone, 7.6 years.

A total of 331 women with an invasive breast cancer diagnosis during the follow-up period was included in the study. Median time from cohort entry until cancer diagnosis was 14.2 years (range 0.01-34.7 years) and women ranged in age from 23 to 62 years at the time of diagnosis (median, 44 years). Age at first and last birth did not affect the risk of breast cancer significantly. In contrast, a notably lower breast cancer risk was associated with parity (childbirth ever/never) and with increasing number of births among parous women (Table 3).

After adjustment for ever childbirth and number of additional births, none among gonadotrophin (RR, 1.20; 95% CI, 0.82-1.78), clomiphene (RR, 1.08; 95% CI, 0.85-1.39), hCG (RR, 0.94; 95% CI, 0.73-1.21), or GnRH (RR, 1.28; 95% CI, 0.75-2.19) significantly affected the risk of breast cancer (Table 4). In addition, there was no substantial difference in risk according to number of cycles of use. In contrast, use of progesterone increased the risk of subsequent breast cancer in infertile women (RR, 3.36; 95% CI, 1.60-7.07). However, the increased risk for progesterone was based on few exposed cancers (eight cases). Years since first use of fertility drugs (latency) did not markedly affect the risk of breast cancer among infertile women.

To estimate whether the breast cancer risk associated with use of fertility hormones varied according to parity, we estimated separate effects of hormone use according to parity status (Table 5). For gonadotrophins, a significantly higher breast cancer risk was observed among nulliparous women (RR, 1.69; 95% CI, 1.03-2.77), whereas the risk of breast cancer among parous women was not increased (RR, 0.84; 95% CI, 0.46-1.54). However, the interaction term was only marginally significant ($P = 0.07$). For all other fertility drugs, the risk of breast cancer was not markedly affected by parity status.

The breast cancer risk of different histologic types was also analyzed (Table 6). Histologic types of cancers were as follows: infiltrating ductal carcinoma, 260; lobular carcinoma, 26; tubular adenocarcinoma, 8; mucinous adenocarcinoma, 6; apocrine adenocarcinoma, 4; comedocarcinoma, 7; medullary carcinoma, 6; solid carcinoma, 3; papillary carcinoma, 2; carcinoma, 2; squamous cell carcinoma, 1; adenocarcinoma, 1; scirrhous adenocarcinoma, 1; Paget's disease 1; invasive malignancy, unknown histology, 3. For the analysis, we classified the tumors into three histologic groups: ductal (260 breast cancer cases), lobular (26 breast cancer cases), and other tumors (45 breast cancer cases). In general, we observed similar risk estimates for ductal, lobular, and other tumors as for all invasive breast cancer tumors. The effect of progesterone on the risk of lobular and other tumors could not be analyzed, as women who ever used progesterone only developed ductal tumors.

Last, RRs for the most prevalent combinations of fertility drugs were analyzed. However, none of five most frequently used combinations of fertility drugs significantly affected the risk of breast cancer (clomiphene + hCG: RR, 1.00; 95% CI, 0.70-1.43; clomiphene + gonadotrophins + hCG: RR, 1.00; 95% CI, 0.55-1.81; clomiphene + gonadotrophins + hCG + GnRH: RR, 0.95; 95% CI, 0.41-2.19; gonadotrophins + hCG + GnRH: RR, 0.30; 95% CI, 0.03-2.68; gonadotrophins + GnRH: RR, 1.62; 95% CI, 0.18-14.67).

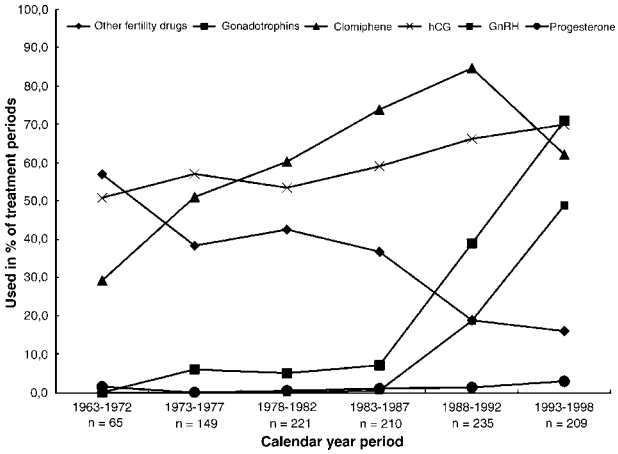

Figure 1. Relative use of fertility drugs within treatment periods in the subcohort of infertile Danish women from 1963 to 1998. One treatment period is defined as a continuous sequence of treatment cycles.

Discussion

In general, our results indicated that treatment with fertility drugs is not related to breast cancer risk, a result that is in line with the main findings from most previous studies (11-22). An exception, however, was the finding of a ~4-fold increased risk of ductal breast cancer after use of progesterone. We found no substantial difference in breast cancer risk according to number of cycles or length of follow-up and almost similar risk patterns were present for ductal, lobular, and tumors of other histologies, indicating homogeneous etiologies. No clear pattern concerning breast cancer risk and parity status was found, except for a seemingly higher breast cancer risk associated with use of gonadotrophins among women who remain nulliparous.

The mechanisms for a potential association between fertility drugs and breast cancer risk are not completely clear. Gonadotrophins have no direct effect on breast tissue, but may increase the estrogen levels during the follicular phase of ovulation induction cycles. Combined with a high progesterone level produced during the simultaneous ovulation of multiple follicles, this may expose infertile women to an environment that favors the development of breast cancer (30). However, only a single study by Burkman et al. (23) found an increased risk of breast cancer after use of gonadotrophins, and the results from our study, which is in line with result from previous studies (12, 15, 19, 22-24), found no overall support

Table 3. RRs of breast cancer according to reproductive factors

Determinant	No. cases/no. in subcohort	RR* (95% CI)
Childbirth		
Never	141/430	1.00
Ever	190/796	0.75 (0.60-0.94)
No. births		
1	111/390	0.86 (0.67-1.11)
2	63/310	0.65 (0.48-0.88)
≥3	16/96	0.58 (0.34-1.00)
Per additional birth among parous women		0.81 (0.65-1.00)
Age at first birth (y)		
<25	57/245	0.77 (0.56-1.05)
25-29	43/232	0.59 (0.42-0.84)
≥30	90/319	0.85 (0.65-1.11)
Per 5 y among parous women		1.02 (0.91-1.16)
Age at last birth (y)		
<30	67/276	0.80 (0.60-1.08)
30-35	76/317	0.74 (0.56-0.99)
≥35	47/203	0.70 (0.50-0.99)
Per 5 y among parous women		1.01 (0.90-1.14)

NOTE: All analyses were stratified according to calendar year (in categories) and age at start of follow-up (in categories).
*All RRs (except childbirth) were adjusted for childbirth (ever/never) and number of additional births (linear).

for the adverse effect of gonadotrophins on breast cancer risk. We did find, however, a higher breast cancer risk associated with use of gonadotrophins among women who remain nulliparous. It is possible, however, that chance might have played a role because the increased risk was based on a relatively small number of cases. However, this finding might indicate that gonadotrophins might possess a higher breast cancer risk in nulliparous women compared with parous women, and is in line with results from a study by Brinton et al. (22) who also found a higher breast cancer risk associated with use of gonadotrophins among nulliparous women. Whether the seemingly higher risk of breast cancer observed in nulliparous women is due to a shared genetic susceptibility to both breast cancer and infertility, or a special biological susceptibility when exposed to fertility drugs, is not yet understood, and this subgroup finding will require assessment in future investigations.

Clomiphene is structurally similar to tamoxifen, which is used in the treatment of breast cancer, and clomiphene could therefore potentially reduce the risk of breast cancer. However, only a single study by Rossing et al. (26) has actually found a reduced risk of breast cancer after clomiphene use. Our study, however, in line with most previous studies, found no support for the supposed chemopreventive effect of clomiphene as breast cancer risk was not associated with use of clomiphene (12, 22, 23). In contrast, the most recent cohort study that focused on 5,788 infertile women (19) found an increased breast cancer risk after treatment with clomiphene (OR, 2.7; 95% CI, 1.3-5.7). The authors suggested that this finding might be caused by the fact that the direct antiestrogenic effects on the breast are overridden by the elevated estradiol levels induced by clomiphene in women of reproductive age.

We found an ~4-fold increased risk of ductal breast cancer after use of progesterone. Our study is the first to analyze the association between progesterone in infertility treatment and the risk of breast cancer. In Denmark, progesterone is mainly used as a routine treatment in most *in vitro* fertilization/intracytoplasmic sperm injection protocols to enhance implantation of the fertilized eggs since it increases thickening of the endometrial lining. As *in vitro* fertilization/intracytoplasmic sperm injection protocols often involve a regimen of multiple fertility drugs, it is therefore common that women treated with progesterone also receive other types of fertility drugs. In the present study, the eight progesterone-exposed cases had also used between two to four other fertility drugs, and we could therefore not establish a potential independent effect of progesterone. It is therefore possible that the excess breast cancer risk associated with progesterone use might be explained by the additional use of the other types of fertility drugs and their combined effect. It is possible, however, that progesterone itself may be a risk factor for breast cancer as our result is in line with previous findings concerning the association between use of hormone replacement therapy regimens with progestin (a group name of compounds that have effects similar to progesterone), where most papers found an increased risk of breast cancer after use of hormone

Table 4. RRs of breast cancer according to usage of fertility drugs

Fertility drug	No. cases/no. in subcohort					Adjusted RR* (95% CI)				
	Gonadotrophins†	Clomiphene	hCG	GnRH	Progesterone	Gonadotrophins†	Clomiphene	hCG	GnRH	Progesterone
Use of fertility drugs										
Never	295/1,061	229/82	237/831	313/1,128	323/1,213	1.00	1.00	1.00	1.00	1.00
Ever	36/165	102/405	94/395	18/98	8/13	1.20 (0.82-1.78)	1.08 (0.85-1.39)	0.94 (0.73-1.21)	1.28 (0.75-2.19)	3.36 (1.60-7.07)
No. cycles										
1-4	25/122	64/216	53/227	16/91	8/13	1.15 (0.73-1.81)	1.26 (0.95-1.69)	0.89 (0.66-1.22)	1.20 (0.68-2.13)	3.36 (1.60-7.07)
5-9	11/38	26/118	30/112	2/7	0/0	1.67 (0.89-3.12)	0.89 (0.58-1.34)	1.10 (0.74-1.62)	2.32 (0.55-9.79)	—
≥10	0/5	12/71	11/56	0/0	0/0	—	0.83 (0.46-1.50)	0.81 (0.44-1.49)	—	—
Year since first use										
≤4	21/48	25/31	19/42	14/47	2/3	1.22 (0.70-2.12)	1.14 (0.69-1.87)	0.72 (0.42-1.24)	1.22 (0.61-2.44)	1.75 (0.31-9.93)
5-9	13/80	31/93	25/91	4/45	4/6	1.96 (1.06-3.64)	1.31 (0.86-2.00)	1.11 (0.69-1.78)	1.55 (0.56-4.31)	6.28 (1.87-21.09)
10-14	1/18	17/102	16/70	0/6	1/3	0.42 (0.06-3.03)	0.75 (0.43-1.28)	0.76 (0.44-1.31)	—	3.48 (0.52-23.56)
≥15	1/19	29/179	34/192	0/0	1/1	0.35 (0.04-2.72)	1.14 (0.73-1.80)	1.11 (0.72-1.72)	—	2.84 (0.19-42-44)

NOTE: All analyses were stratified according to calendar year (in categories) and age at start of follow-up (in categories).
*RRs adjusted for childbirth (ever/never) and number of additional births (linear).
†Follicle-stimulating hormone and human menopausal gonadotrophin.

Table 5. RRs of breast cancer in nulliparous, respectively parous women, according to use of fertility drugs

Fertility drug	Nulliparous women		Parous women		Interaction *P*
	No. cases/no. in subcohort	RR* (95% CI)	No. cases/no. in subcohort	RR* (95% CI)	
Gonadotrophins †					
Never	118/353	1.00	177/708	1.00	
Ever	23/77	1.69 (1.03-2.77)	13/88	0.84 (0.46-1.54)	0.07
Clomiphene					
Never	92/276	1.00	137/545	1.00	
Ever	49/154	1.18 (0.82-1.68)	53/251	1.05 (0.75-1.45)	0.63
hCG					
Never	97/285	1.00	140/546	1.00	
Ever	44/145	1.07 (0.74-1.54)	50/250	0.88 (0.63-1.22)	0.42
GnRH					
Never	131/374	1.00	182/754	1.00	
Ever	10/56	1.12 (0.56-2.23)	8/42	1.67 (0.75-3.70)	0.44
Progesterone					
Never	137/424	1.00	186/789	1.00	
Ever	4/6	3.24 (1.13-9.35)	4/7	3.49 (1.25-9.77)	0.92

NOTE: All analyses were stratified according to calendar year (in categories) and age at start of follow-up (in categories).
*RRs adjusted for childbirth (ever/never), and number of additional births (linear).
†Follicle-stimulating hormone and human menopausal gonadotrophin.

replacement therapy (6, 31, 32). However, the effect of progesterone with respect to breast cancer is complicated and the specific role of progesterone as a promoter of breast cell proliferation is debated, as certain progestogens are able to either induce proliferation or inhibit growth of benign or malignant human breast epithelial cells (7, 33-35). Additional, larger studies are clearly needed to reject or confirm the finding from our study.

Our study found no association between the number of cycles for any type of fertility drugs and the risk of breast cancer. This results is in line with previous findings from Potashnik et al. (24), Rossing et al. (26), and Brinton et al. (22) who found no substantial association with breast cancer risk and number of clomiphene cycles. Only a single study by Burkman et al. (23) found an increased breast cancer risk with RRs ranging from 2.7 to 3.8 in women using gonadotrophins for at least six cycles. However, in a comment to the study by Burkman et al. (23), Healy and Venn (36) doubt whether the minimal increase in hormones that would be associated with more than six cycles is sufficient to affect subsequent breast cancer risk substantially, and suggest that the statistically significant increases in breast cancer risk observed in women treated with gonadotrophins may have occurred by chance or that the risk observed is distorted by residual confounding that has not been controlled for in the analysis.

No increased breast cancer risk with increased follow-up time for any of the five fertility drugs examined was found in our study. Brinton et al. (22) is the only other study that have earlier examined this topic, and they found a statistically significant increased breast cancer risk after use of clomiphene when followed-up for more than 20 years, thus suggesting that the effect of clomiphene have long latency effects on breast cancer risk. However, both our results and the results from Brinton et al. (22) are weakened by the fact that only a small proportion of women in the cohorts were followed for more than 20 years. In our study, especially women exposed to gonadotrophins, GnRH, and progesterone had short follow-up time, as only <5% of these women were followed for more than 20 years. Longer follow-up time is therefore needed to further study latency effects on breast cancer risk after exposure to fertility drugs.

The detailed information contained in the Danish Cancer Registry enabled us to differentiate our analyses between different histologic types of breast cancer. In general, however, we observed similar risk estimates for ductal tumors, lobular tumors, and other tumors for all fertility drugs. Only Burkman et al. (23) have earlier evaluated the association between use of

Table 6. RRs of histologic subgroups of breast cancer according to usage of fertility drugs

Fertility drug	No. cases/no. in subcohort			Adjusted RR* (95% CI)		
	Ductal	Lobular	Other	Ductal	Lobular	Other
Gonadotrophins †						
Never	231/1,061	22/1,061	42/1,061	1.00	1.00	1.00
Ever	29/165	4/165	3/165	1.13 (0.71-1.81)	1.96 (0.66-5.82)	0.70 (0.22-2.22)
Clomiphene						
Never	180/821	18/821	31/821	1.00	1.00	1.00
Ever	80/405	8/405	14/405	1.06 (0.79-1.43)	0.91 (0.47-1.80)	1.10 (0.62-1.95)
hCG						
Never	187/831	18/831	32/831	1.00	1.00	1.00
Ever	73/395	8/395	13/395	0.92 (0.67-1.25)	1.26 (0.66-2.41)	1.08 (0.61-1.91)
GnRH						
Never	243/1,128	25/1,128	45/1,128	1.00	1.00	1.00
Ever	17/98	1/98	0/98	1.43 (0.75-2.70)	1.44 (0.11-18.45)	—
Progesterone						
Never	252/1,213	26/1,213	45/1,213	1.00	1.00	1.00
Ever	8/13	0/13	0/13	4.09 (1.62-10.37)	—	—

NOTE: All analyses were stratified according to calendar year (in categories) and age at start of follow-up (in categories).
*RRs adjusted for childbirth (ever/never), and number of additional births (linear).
†Follicle-stimulating hormone and human menopausal gonadotrophin.

fertility drugs and risk of different histologic types of breast cancer. Using a case-control design, Burkman et al. (23) found no association between use of fertility drugs and the risk of ductal, lobular, or other type breast cancer tumors, except for a greater risk of ductal breast tumors after use of human menopausal gonadotrophins. Based on the sparse literature, there is thus no major indication of differences in the risk profile between the different histologic types of breast cancer when exposed to fertility drugs.

Our study has several strengths. First, our study has a high precision of the risk estimates as our study includes 331 women with breast cancer, which is by far the largest number of cases ever included in a cohort study examining the association between breast cancer risk and the use of fertility drugs. Most previous cohort studies have been limited by a small number of cases, ranging from 5 to 243 cases (11, 12, 14, 15, 17-19, 22, 24, 26), with only two studies including more than 100 breast cancer cases: the study by Venn et al. (12) with 143 cases and the study by Brinton et al. (22) with 243 invasive breast cancer cases. Second, due to the unique nature of the Danish personal identification number, which enables a precise linkage between our infertility cohort and the Danish population–based registries, practically no women were lost to follow-up, thus removing selection bias, and allowing a precise estimation of the numbers of person-years of risk. Third, we had a complete ascertainment of breast cancer diagnoses through linkage with the Danish Cancer Registry. Fourth, we had extensive information about the different types of fertility drugs described, and the numbers of cycles used. Only a few of the previous follow-up studies (12, 19, 22, 23, 26) have been able to assess the specific effect of the different types of fertility drugs, which is of great importance provided their possible different effects. Furthermore, use of fertility drugs in our infertility cohort may resemble the actual use among Danish women in the period, as patterns of use for most fertility drugs in our subcohort are in good concordance with results published by Mosgaard et al. (37), about the use of fertility drugs in the general Danish population for the period 1973 to 1993 and sales statistics from the Danish Medicines Agency for the period 1994 to 1998 (38). Only the use of progesterone in our cohort seems to be somewhat underestimated compared with the actual use among Danish women (37, 38).

Our study also had some limitations. We have a relatively short follow-up period and the median age at the end of follow-up (40 years) and median age at cancer diagnosis (44 years) was not yet the peak age of breast cancer in Denmark (1999: 62 years), which might weaken our estimates. Potentially important risk factors such as cause of infertility and use of oral contraceptives were only registered for a small minority of the women and could therefore not be included in the main analyses. However, we did perform analyses on the subsets of women who had information about causes of infertility or oral contraceptive use, but these adjustments did not change the overall estimates, indicating that these risk factors are not confounders in the association between the use of fertility drugs and the risk of breast cancer in our study. In addition, most previous studies that have examined the association between fertility drugs, causes of infertility, and breast cancer risk (22, 24, 26, 39), found that adjustment for causes of infertility did not change the risk estimates associated with use of fertility drugs. However, both our results and the results from previous studies may partly reflect methodologic differences and weaknesses in the assessment of cause of infertility, for example, no knowledge of to what extent the woman in question has, in fact, been evaluated for all causes of infertility or not, and this topic clearly need further assessment in future well-conducted studies.

In summary, the results from our large nationwide study were generally assuring that treatment with fertility drugs do not cause breast cancer, as risk was not related to neither ever use, number of cycles of use, or follow-up time for most fertility drugs, except for use of progesterone that increased the risk of ductal breast cancer. In addition, we found an indication of a stronger effect of gonadotrophins on breast cancer risk among women remaining nulliparous. However, it is possible that chance might have played a role because these increased risks were based on a small number of cases, and additional long-term follow-up studies should be done to confirm or refute the findings from the present study.

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