Association Between Fertility Treatment and Cancer Risk in Children

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IMPORTANCE An increasing number of children worldwide are born after the use of fertility treatment, although it remains unclear whether the treatment affects the risk of childhood cancer and whether any associations observed are due to the use of specific drugs, the use of specific procedures, or the underlying infertility.

OBJECTIVE To examine the association between different types of fertility treatments and cancer risk in children.

DESIGN, SETTING, AND PARTICIPANTS A retrospective cohort study based on Danish population-based registry data and the Danish Infertility Cohort (individual record linkage) that included 1,085,172 children born in Denmark between January 1, 1996, and December 31, 2012, linked with parental information. There were a total of 22,17 children diagnosed with cancer (follow-up occurred during 1996-2015).

EXPOSURES Maternal fertility treatment during the index pregnancy, including the use of fertility drugs (clomiphene [n = 33,835], gonadotropins [n = 57,136], gonadotropin-releasing hormone analogs [n = 38,653], human chorionic gonadotropin [n = 68,181], progesterone [n = 41,628], and estrogen [n = 16,948]) and assisted reproductive technology (in vitro fertilization [n = 19,448], intracytoplasmic sperm injection [n = 13,417], and frozen embryo transfer [n = 3,356]). Each exposure was examined separately and compared with children born to fertile women.

MAIN OUTCOMES AND MEASURES Hazard ratios and incidence rate differences for childhood cancer.

RESULTS After 12.2 million person-years of follow-up (mean, 11.3 years), the incidence rate of childhood cancer was 17.5 per 100,000 for children born to fertile women (n = 910,291) and 44.4 per 100,000 for children born after the use of frozen embryo transfer (n = 3,356). Compared with children born to fertile women, the use of frozen embryo transfer was associated with an elevated risk of childhood cancer (14 cancer cases; hazard ratio, 2.43 [95% CI, 1.44 to 4.11]; incidence rate difference, 26.9 [95% CI, 2.8 to 51.0] per 100,000), mainly due to an increased risk of leukemia (5 cancer cases; incidence rate, 14.4 per 100,000; hazard ratio, 2.87 [95% CI, 1.19 to 6.93]; incidence rate difference, 10.1 [95% CI, −4.0 to 24.2] per 100,000) and sympathetic nervous system tumors (<5 cancer cases; hazard ratio, 7.82 [95% CI, 2.47 to 24.70]). There were no statistically significant associations with the use of the other types of fertility treatment examined.

CONCLUSIONS AND RELEVANCE Among children born in Denmark, the use of frozen embryo transfer, compared with children born to fertile women, was associated with a small but statistically significant increased risk of childhood cancer; this association was not found for the use of other types of fertility treatment examined.

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A n estimated 8 million children were born after the use of assisted reproductive technology (ART) between 1978 and 2018.1 In Denmark, a country with one of the world’s highest rates of ART,2 9.8% of all children were born after use of fertility treatment in 2018.3

Children born after use of fertility treatment have higher risks of adverse perinatal outcomes, including prematurity, low birth weight, and congenital malformations4,5; however, the long-term health consequences have been studied less frequently. It has been suggested that the use of fertility treatment increases the risk of cancer in children, possibly through epigenetic changes brought on by the use of fertility drugs, ART, or both,6 which has been supported by the finding of epigenetic changes in human oocytes, embryos, cord blood, and placentas.7,8 In addition, an unexpectedly large proportion of children conceived after the use of fertility treatment are born with rare imprinting disorders due to epigenetic changes.8

A recent systematic review and meta-analysis of 29 studies reported an increased cancer risk in children born after fertility treatment.9 However, many of the included studies were limited by small numbers of exposed cases and possible recall bias, and only a few studies10,11 could differentiate between associations with the treatment and the underlying infertility. Furthermore, only a few studies included information on specific fertility drugs11,12 or specific treatment procedures13-15 with potential differential effects on cancer risk. It therefore remains uncertain whether fertility treatment is associated with cancer risk in children and whether any observed associations are due to the use of specific drugs, the procedures, or the underlying infertility.

The risk for cancer among children born after the use of fertility treatment was examined in a large nationwide cohort of children born in Denmark between 1996 and 2012 that contained detailed registry-based information on maternal infertility, fertility drugs, and ART.

Methods

Cohort

From the Danish Medical Birth Registry, which contains information on all births in Denmark since 1973,16,17 we identified all children born alive in Denmark between January 1, 1996, and December 31, 2012, and their parents. Children were excluded from the cohort if (1) there was missing information on sex, maternal age at birth, or multiplicity; (2) there were missing or implausible data on gestational length; or (3) they died on the same day as being born. The unique personal identification number given to all residents of Denmark allowed us to link individual-level information on the children and their parents to nationwide registers and cohorts to identify maternal fertility problems, maternal use of fertility treatment, cases and types of cancer, and other information.

Vital status (date of emigration and death), year of birth, sex, and parental age at birth were identified from the Danish Civil Registration System and parental educational level was obtained from Statistics Denmark, which is the institution that hosts social and demographic registries. Both of these sources are based on administrative data and are considered highly reliable.18,19 Information on birth order, birth weight, gestational length, multiplicity, and maternal smoking was obtained from the Danish Medical Birth Registry. Basic information in the registry, such as identification of the mother for linkage, parity, gestational length, and birth weight, are virtually complete and of high validity (eg, information on gestational length is accurate within 1 week for 87% of records)16 because some of the information is drawn directly from the Danish Civil Registration System and it is mandatory to report other information after delivery.17 The study was approved by the Danish Health Data Board. According to Danish legislation, it is not necessary to seek informed consent from participants for studies based on data linkage.

Maternal Fertility Status and Fertility Treatment

To identify maternal fertility status and use of fertility treatment, we linked the study cohort to the Danish Infertility Cohort, which includes information from the Danish National Patient Register,20 the Danish National [in vitro fertilization] IVF Registry,21 the Danish National Prescription Registry,22 and local electronic databases, comprising virtually all women in Denmark evaluated for fertility problems during 1963 through 2012 (eTable 1 in the Supplement). The Danish National IVF Registry, which contains compulsory information (including information on fertility drugs) on all treatments involving ART in Denmark since January 1, 1994 (and intrauterine insemination since 2006), was used to identify children born during 1995 through 2012 after use of ART. The Danish National IVF Registry and the Danish National Prescription Registry, which contains information on all prescription drugs sold in Danish pharmacies since January 1, 1995, were used to identify maternal use of fertility drugs during 1995 through 2012 (eTable 1 in the Supplement).

The conception date of each child in the cohort was estimated by subtracting the gestational age from the date of birth and adding 14 days. We defined children conceived within 14 days of the treatment date recorded in the Danish National IVF Registry as children born after use of ART (14 days before conception to 14 days after), whereas a longer time frame (14 days before conception to 16 weeks after) was considered implausible because some of the information is drawn directly from the Danish Civil Registration System and it is mandatory to report other information after delivery.17
used for identifying use of fertility drugs during the index pregnancy because fertility drugs may be used several weeks into pregnancy. For fertility drugs identified in the Danish National Prescription Registry, we defined treatment during the index pregnancy as prescriptions filled from 12 weeks before to 16 weeks after conception because fertility drugs can be prescribed for 3 months.

**Childhood Cancer**

Children with cancer (diagnosed before reaching aged 20 years) were identified using the Danish Cancer Registry, a nationwide register of all incident cancer cases diagnosed in Denmark since 1943. This registry is estimated to be 95% to 98% complete and contains information on major diagnostic groups with an accuracy of up to 99%.23,24

**Statistical Methods**

Children contributed risk time from their date of birth until diagnosis of cancer, censoring (death, emigration, or 20th birthday), or December 31, 2015, whichever occurred first. Cox proportional hazards models were used to estimate hazard ratios (HRs) and 95% CIs for any type of cancer and for leukemia, lymphoma, sympathetic nervous system tumors, central nervous system neoplasms, and all other types of cancer. In all analyses, the first diagnosed cancer type was included for the majority of children. However, 5 children had 2 types of primary cancer diagnosed on the same date and were counted in the analyses for both cancer types.

Children’s risk of cancer was evaluated according to: (1) maternal fertility problems during or prior to the index pregnancy, (2) any use of fertility treatment during the index pregnancy, (3) any use of fertility drugs during the index pregnancy (clomiphene, gonadotropins [human menopausal gonadotrophins and follicle-stimulating hormone], gonadotropin-releasing hormone analogs, human chorionic gonadotropin, progesterone, estrogen, and other or unspecified drugs separately), and (4) any use of ART during the index pregnancy (IVF, intracytoplasmic sperm injection, frozen embryo transfer, and other or unspecified procedures) compared with children born to fertile women.

Each exposure was investigated separately. The types of ART use are mutually exclusive (ie, sums to 100%), whereas the types of fertility drug use are not mutually exclusive because several different hormones may be used during the same treatment cycle (with or without the use of ART). A partial likelihood test was performed to determine if the 4 ART treatments were statistically significantly different. To assess whether associations could be explained by the underlying infertility, analyses also were conducted comparing children born to women requiring fertility assistance (without receiving treatment during the index pregnancy).

The attained age of children was used as the underlying timescale and the year of birth (5-year intervals) was included as an a priori confounder in all models. Other potential confounders, including birth order (firstborn or born ≥second), maternal smoking during pregnancy (yes or no), maternal and paternal age at birth (<30, 30-34, or >34 years), maternal and paternal educational level (basic, vocational, or higher), and maternal cancer (yes or no) were investigated for their effect on the association between maternal infertility and cancer by complete case analysis (ie, excluding children with missing information for any of the potential confounders) and by multiple imputations of covariates using fully conditional specification for variables with missing values.25

Only year of birth (with no missing values) was included in further analyses because none of the other factors changed the risk estimate by more than 10%. Sex and selected perinatal factors (birth weight [<2500, 2500-4500, or >4500 g], gestational length [<37, 37-43, or >43 weeks], and multiplicity [yes or no]) were included in separate models defined a priori as possible intermediate factors.

We evaluated the proportional hazards assumption graphically based on scaled Schoenfeld residuals and detected no violation. To address the potential for unmeasured confounding explaining the results, a post hoc analysis of the E-value was performed for the association between use of frozen embryo transfer and any type of cancer.26 In addition, post hoc analyses of the association between the different fertility treatments and any type of cancer were performed, taking into account the effect of sibling clustering using robust standard errors to account for correlation among siblings. Because the effect of sibling clustering did not change the conclusions, estimates are reported without taking sibling clusters into account.

Adjusted incidence rates (cases per 100 000 person-years) and incidence rate differences (incidence rates in exposed children minus incidence rates in unexposed children) with 95% CIs were calculated using Poisson regression. All P values were 2-sided and a significance level of .05 was applied. Because no adjustment was made for multiple comparisons, the findings should be considered exploratory given the large number of treatment subgroups that are being compared. All analyses were performed using version 3.2.3 of the R survival package (R Foundation for Statistical Computing).

**Results**

Between 1996 and 2015, the 1 085 172 children included in the study accumulated 12.2 million person-years of follow-up (mean, 11.3 years; eFigure in the Supplement). During this period, cancer was diagnosed in 2217 children; 648 with leukemia (29.2%), 242 with lymphoma (10.9%), 541 with central nervous system neoplasms (24.3%), 130 with sympathetic nervous system tumors (5.9%), and 661 with other cancer types (29.7%). Most children (83.9%; n = 910 291) were born to fertile women, whereas 16.1% (n = 174 881) were born to women requiring fertility assistance; of whom 51.5% (n = 89 981) were born after use of fertility treatment and 48.5% (84 900) were born without use of fertility treatment.

The characteristics of the study cohort by maternal fertility status and treatment during the index pregnancy appear in Table 1. Compared with children born to fertile women, children born to women treated with ART or fertility drugs during the index pregnancy were more often born during later calendar years, had lower birth weights and gestational
Table 1. Baseline Characteristics of the Study Cohort by Maternal Fertility Status

<table>
<thead>
<tr>
<th>Characteristics of Children</th>
<th>Children Born to Women Requiring Fertility Assistance&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Children Born to Fertile Women (n = 910 291)&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Treated During Index Pregnancy (n = 89 981)</td>
<td>Not Treated During Index Pregnancy (n = 84 900)</td>
</tr>
<tr>
<td></td>
<td>1996-1999</td>
<td>21 386 (23.8)</td>
</tr>
<tr>
<td></td>
<td>2000-2003</td>
<td>20 583 (22.9)</td>
</tr>
<tr>
<td></td>
<td>2004-2007</td>
<td>23 299 (25.9)</td>
</tr>
<tr>
<td></td>
<td>2008-2012</td>
<td>24 713 (27.5)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>44 017 (48.9)</td>
<td>41 196 (48.5)</td>
</tr>
<tr>
<td>Male</td>
<td>45 964 (51.1)</td>
<td>43 704 (51.5)</td>
</tr>
<tr>
<td>Birth weight, median (IQR), g</td>
<td>3300 (2810-3720)</td>
<td>3550 (3200-3900)</td>
</tr>
<tr>
<td>Birth weight, No./Total No. (%)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12 662/85 486 (14.8)</td>
<td>3842/82 256 (4.7)</td>
</tr>
<tr>
<td></td>
<td>2500-4500 g</td>
<td>70 926/85 486 (83.0)</td>
</tr>
<tr>
<td></td>
<td>&gt;4500 g</td>
<td>1898/85 486 (2.2)</td>
</tr>
<tr>
<td>Multiplicity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>69 071 (76.8)</td>
<td>82 616 (97.3)</td>
</tr>
<tr>
<td>Yes</td>
<td>20 910 (23.2)</td>
<td>2284 (2.7)</td>
</tr>
<tr>
<td>Gestational age, median (IQR), wk</td>
<td>39.3 (37.9-40.6)</td>
<td>40.0 (38.7-40.9)</td>
</tr>
<tr>
<td></td>
<td>&lt;37</td>
<td>14 387 (16.0)</td>
</tr>
<tr>
<td></td>
<td>37-42</td>
<td>73 166 (81.3)</td>
</tr>
<tr>
<td>Birth order</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Firstborn</td>
<td>56 376 (62.7)</td>
<td>21 043 (24.8)</td>
</tr>
<tr>
<td>Born ≥ second</td>
<td>33 605 (37.3)</td>
<td>63 857 (75.2)</td>
</tr>
</tbody>
</table>

| Characteristics of Mothers  |                                                               |                                                    |                                                    |
| Smoking, No./Total No. (%)<sup>b</sup> | 73 875/83 070 (88.9)                                         | 65 739/80 210 (82.0)                                | 672 640/824 693 (81.6)                              |
|                             | 9195/83 070 (11.1)                                           | 14 471/80 210 (18.0)                                | 152 053/824 693 (18.4)                              |
| Age when gave birth, median (IQR), y | 32.6 (29.7-35.9)                                             | 32.5 (29.4-35.6)                                    | 29.9 (26.7-33.2)                                   |
|                             | <30                                                          | 24 680 (27.4)                                       | 25 206 (29.7)                                       | 461 640 (50.7)                                     |
|                             | 30-34                                                        | 37 534 (41.7)                                       | 34 820 (41.0)                                       | 313 611 (34.5)                                     |
| Educational level, No./Total No. (%)<sup>c</sup> | 18 129/88 767 (20.4)                                         | 23 535/83 682 (28.1)                                | 287 535/875 998 (32.8)                              |
|                             | Basic                                                        | 18 129/88 767 (20.4)                                | 23 535/83 682 (28.1)                                | 287 535/875 998 (32.8)                              |
|                             | Vocational                                                   | 34 289/88 767 (38.6)                                | 32 468/83 682 (38.8)                                | 313 569/875 998 (35.8)                              |
|                             | Higher                                                       | 36 349/88 767 (40.9)                                | 27 679/83 682 (33.1)                                | 274 894/875 998 (31.4)                              |
| Characteristics of Fathers  |                                                               |                                                    |                                                    |
| Age at birth, median (IQR), y | 34.6 (31.4-38.4)                                             | 34.5 (31.2-38.2)                                    | 32.1 (28.8-35.9)                                   |
| Age at birth, No./Total No. (%)<sup>c</sup> | 13 618/88 219 (15.4)                                         | 15 036/84 030 (17.9)                                | 299 121/899 094 (33.3)                              |
|                             | 30-34                                                        | 33 052/88 219 (37.5)                                 | 30 360/84 030 (36.1)                                | 330 389/899 094 (36.7)                              |
| Educational level, No./Total No. (%)<sup>c</sup> | 41 549/88 219 (47.1)                                         | 38 634/84 030 (46.0)                                | 269 584/899 094 (30.0)                              |
|                             | Basic                                                        | 18 657/86 482 (21.6)                                 | 21 584/81 820 (26.4)                                | 252 189/863 178 (29.2)                              |
|                             | Vocational                                                   | 42 212/86 482 (48.8)                                 | 40 670/81 820 (49.7)                                | 408 696/863 178 (47.3)                              |
|                             | Higher                                                       | 25 613/86 482 (29.6)                                 | 19 566/81 820 (23.9)                                | 202 293/863 178 (23.4)                              |

Abbreviation: IQR, interquartile range.

* Data are expressed as No. (%) unless otherwise indicated.

<sup>a</sup> Measured during first trimester of pregnancy.

<sup>b</sup> Basic indicates completed 9 years or less of school; vocational, completed 10 to 12 years of school or vocational training; higher, completed more than 12 years of school.
ages, were part of a multiple birth, were firstborns, their mothers did not smoke during pregnancy, and their parents were older and had higher educational levels. The characteristics of the study cohort by type of ART appear in eTable 2 in the Supplement.

Any Type of Cancer
Maternal infertility was not statistically significantly associated with the risk of any type of childhood cancer; there were 341 childhood cancer cases and the cancer incidence rate was 17.6 per 100 000 in women with infertility vs 17.5 per 100 000 in fertile women (HR, 1.02 [95% CI, 0.91 to 1.15]; incidence rate difference, 0.1 [95% CI, −2.0 to 2.2] per 100 000; Table 2). No statistically significant association was found with the use of any type of fertility treatment and risk of any type of childhood cancer; there were 178 cancer cases and the cancer incidence rate was 17.8 per 100 000 (HR, 1.03 [95% CI, 0.88 to 1.20]; incidence rate difference, 0.3 [95% CI, −2.5 to 3.2] per 100 000).

No statistically significant association was found with the use of any type of fertility drugs and risk of any type of childhood cancer; there were 173 cancer cases and the cancer incidence rate was 17.4 per 100 000 (HR, 1.01 [95% CI, 0.86 to 1.18]; incidence rate difference, −0.1 [95% CI, −2.9 to 2.8] per 100 000). No statistically significant association was found with the use of any type of ART and risk of any type of childhood cancer; there were less than 90 cancer cases and the cancer incidence rate was 21.0 per 100 000 (HR, 1.20 [95% CI, 0.96 to 1.49]; incidence rate difference, 3.5 [95% CI, −1.2 to 8.2] per 100 000).

In addition, no statistically significant association was observed with the use of specific fertility drugs. The partial likelihood test was statistically significant (P = .04), indicating the treatments using ART had different effects on cancer. Use of IVF or intracytoplasmic sperm injection during the index pregnancy was not statistically significantly associated with childhood cancer. In contrast, compared with children born to fertile women, the cancer risk increased among children born after the use of frozen embryo transfer; there were 14 cancer cases and the cancer incidence rate was 44.4 per 100 000 (HR, 2.43 [95% CI, 1.44-4.11]; incidence rate difference, 26.9 [95% CI, 2.8-51.0] per 100 000). Post hoc calculations of the E-value showed that for this finding to be explained by an unknown confounder, the confounder would have to be associated with both the exposure and outcome by a risk ratio of at least 4.29.

Specific Types of Cancer
The risk of leukemia, lymphoma, central nervous system neoplasms, sympathetic nervous system tumors, and other types of cancer was not statistically significantly associated with maternal infertility or use of any type of fertility treatment, fertility drug, or IVF or intracytoplasmic sperm injection during the index pregnancy compared with children born to fertile women (eTables 3-5 in the Supplement). However, children born after the use of frozen embryo transfer had a statistically significantly higher risk of leukemia (5 cases; incidence rate was 14.4 per 100 000; HR, 2.87 [95% CI, 1.19 to 6.93]; incidence rate difference, 10.1 [95% CI, −4.0 to 24.2] per 100 000) and sympathetic nervous system tumors (<5 cases; HR, 7.82 [95% CI, 2.47 to 24.70]) than children born to fertile women.

Sensitivity Analyses
Only minor changes were observed when sex and perinatal factors were included in the analyses (eTable 6 in the Supplement), when children born to women requiring fertility assistance (without receiving treatment during the index pregnancy) were used as the reference group instead of children born to fertile women (eTable 7-10 in the Supplement), and when sibling clustering was taken into account (eTable 11 in the Supplement).

Discussion
In this large nationwide Danish register-based cohort study, no statistically significant association was found between use of any fertility treatment, any fertility drugs, IVF, or intracytoplasmic sperm injection and cancer in children; however, children born after the use of frozen embryo transfer were at an increased risk, mainly driven by an increased risk of leukemia and sympathetic nervous system tumors.

A recent systematic review and meta-analysis on the use of any type of fertility treatment (including frozen embryo transfer) found increased risks for any type of cancer and leukemia in children born after the use of fertility treatment. In contrast to the findings from a previous meta-analysis, the most recent meta-analysis did not find a statistically significant association between the use of fertility treatment and the development of sympathetic nervous system tumors, which may be due to differences in the studies that were included.

Only 4 studies have reported on the association between use of frozen embryo transfer and childhood cancer. However, one study reported an elevated HR for any type of cancer (HR, 1.80 [95% CI, 0.65-4.95]) and another study reported an elevated HR for hepatoblastoma (HR, 5.24 [95% CI, 0.13-29.21]). The largest study to date found no statistically significant association between use of frozen embryo transfer and any type of cancer. However, that study was limited by short follow-up duration (mean, <5 years), loss to follow-up, and incomplete linkage with maternal fertility treatment and cancer, which could lead to an underestimation of the association. No previous study has investigated the incidence of leukemia and sympathetic nervous system tumors among children born after frozen embryo transfer.

Children born after the use of frozen embryo transfer have a higher risk of being large for gestational age and having a higher mean birth weight than children born after the use of fresh embryo transfer. This suggests that techniques involving cryopreservation induce changes in the developing embryo, potentially affecting intrauterine growth. Excessive fetal growth has been linked to increased childhood cancer risk, and epigenetic alterations have been proposed as a possible explanation. In the present study, only minor changes were
Table 2. Hazard Ratios, Incidence Rates, and Incidence Rate Differences for Any Childhood Cancer According to Maternal Infertility and Treatment

<table>
<thead>
<tr>
<th>Maternal infertility</th>
<th>Person-Years</th>
<th>No. of Children</th>
<th>No. of Cancer Cases</th>
<th>Hazard Ratio (95% CI)</th>
<th>Incidence Rate/100 000 Person-Years</th>
<th>Incidence Rate Difference/100 000 Person-Years (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>10385749</td>
<td>910291</td>
<td>1876</td>
<td>1 [Reference]</td>
<td>17.5</td>
<td>0 [Reference]</td>
</tr>
<tr>
<td>Yes</td>
<td>1824545</td>
<td>174881</td>
<td>341</td>
<td>1.02 (0.91 to 1.15)</td>
<td>17.6</td>
<td>0.1 (−2.0 to 2.2)</td>
</tr>
<tr>
<td>Any type of maternal fertility treatment</td>
<td>943199</td>
<td>89981</td>
<td>178</td>
<td>1.03 (0.88 to 1.20)</td>
<td>17.8</td>
<td>0.3 (−2.5 to 3.2)</td>
</tr>
<tr>
<td>Types of maternal fertility treatment</td>
<td>934946</td>
<td>89334</td>
<td>173</td>
<td>1.01 (0.86 to 1.18)</td>
<td>17.4</td>
<td>-0.1 (−2.9 to 2.8)</td>
</tr>
<tr>
<td>Any type of fertility drugs</td>
<td>372402</td>
<td>33835</td>
<td>64</td>
<td>0.95 (0.74 to 1.22)</td>
<td>16.7</td>
<td>-0.8 (−5.1 to 3.5)</td>
</tr>
<tr>
<td>Gonadotropins</td>
<td>572515</td>
<td>57136</td>
<td>112</td>
<td>1.06 (0.88 to 1.29)</td>
<td>18.6</td>
<td>1.1 (−2.6 to 4.7)</td>
</tr>
<tr>
<td>Human chorionic gonadotropin</td>
<td>682288</td>
<td>68181</td>
<td>129</td>
<td>1.02 (0.86 to 1.23)</td>
<td>17.7</td>
<td>0.2 (−2.1 to 3.5)</td>
</tr>
<tr>
<td>Gonadotropin-releasing hormone analogs</td>
<td>393199</td>
<td>38653</td>
<td>81</td>
<td>1.12 (0.90 to 1.40)</td>
<td>19.7</td>
<td>2.2 (−2.3 to 6.7)</td>
</tr>
<tr>
<td>Progesterone</td>
<td>429787</td>
<td>41628</td>
<td>85</td>
<td>1.08 (0.87 to 1.34)</td>
<td>18.6</td>
<td>1.1 (−3.1 to 5.3)</td>
</tr>
<tr>
<td>Estrogen</td>
<td>123958</td>
<td>16948</td>
<td>23</td>
<td>0.91 (0.60 to 1.38)</td>
<td>16.1</td>
<td>-1.4 (−9.0 to 6.2)</td>
</tr>
<tr>
<td>Other or unspecified</td>
<td>73129</td>
<td>8867</td>
<td>15</td>
<td>1.04 (0.63 to 1.74)</td>
<td>17.2</td>
<td>-0.3 (−10.3 to 9.7)</td>
</tr>
<tr>
<td>Any type of assisted reproductive technology</td>
<td>388681</td>
<td>37156</td>
<td>&lt;90g</td>
<td>1.20 (0.96 to 1.49)</td>
<td>21.0</td>
<td>3.5 (−1.2 to 8.2)</td>
</tr>
<tr>
<td>Types of assisted reproductive technology</td>
<td>220159</td>
<td>19448</td>
<td>38</td>
<td>0.96 (0.70 to 1.32)</td>
<td>17.1</td>
<td>-0.4 (−6.0 to 5.2)</td>
</tr>
<tr>
<td>In vitro fertilization</td>
<td>137076</td>
<td>13417</td>
<td>32</td>
<td>1.33 (0.94 to 1.89)</td>
<td>23.1</td>
<td>5.6 (−2.9 to 14.0)</td>
</tr>
<tr>
<td>Intracytoplasmic sperm injection</td>
<td>30260</td>
<td>3356</td>
<td>14</td>
<td>2.43 (1.44 to 4.11)</td>
<td>44.4</td>
<td>26.9 (2.8 to 51.0)</td>
</tr>
<tr>
<td>Other or unspecified</td>
<td>7536</td>
<td>935</td>
<td>&lt;5g</td>
<td>0.66 (0.09 to 4.70)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Adjusted for year of the birth.
- Each hazard ratio was generated from a separate analysis comparing the children with each individual exposure vs children born to fertile women.
- At any time before the birth.
- Defined as women requiring fertility assistance during index or prior pregnancy.
- Received treatment during index pregnancy and includes only the items listed below this row.
- Includes human menopausal gonadotropins and follicle-stimulating hormone.
- These data were not reported as whole numbers to protect patient confidentiality under the terms of the Danish Data Protection Law.
- The number of patients was too small to obtain a stable estimate.
observed in the estimates after adjustment for perinatal factors; hence, the associations found are unlikely to be explained by increased birth weight. Frozen embryo transfer differs from fresh embryo transfer because of the freezing and thawing of embryos, use of cryoprotectants, and dissimilar protocols for the use of fertility drugs.

Freezing and thawing per se and cryoprotectants have both been implicated in epigenetic changes in animal studies.30-32 In contrast to fresh embryo transfer (excluding egg donation), use of frozen embryo transfer can be performed in artificial (administration of estrogen and progesterone only) and spontaneous cycles.31 A Danish study found increased risks for both leukemia and sympathetic nervous system tumors in children born after the use of progesterone during fertility treatment32; however, no information on use of ART was available. Frozen embryo transfer is the only procedure in Denmark (excluding fresh egg donation) in which estrogen, an established carcinogen, is part of the treatment protocol, and progesterone, a drug classified as “reasonably anticipated to be a human carcinogen,”34 is used approximately 10 weeks into pregnancy.33,35 Although no increased risk was found to be associated with either of these hormonal fertility drugs, the duration or timing of exposure may be crucial for an effect.

The strengths of this study include individual-level linkage to several nationwide population-based registries that provided long-term prospective data and the limited concern regarding temporality, loss to follow-up, and recall bias. Furthermore, the fact that use of ART is free in Denmark until the age of 40 years (for the first child) reduces potential selection bias related to access to care. The exposure and outcome data are likely valid because information on fertility treatment was based on mandatory reporting to the Danish National IVF Registry and automatic transfer of information from the Danish National Prescription Registry; furthermore, the Danish Cancer Registry is complete.

Other strengths include individual-level information on several potential confounders, the population-based nationwide design, and the large numbers of person-years and types of cancer cases. In addition, information on children born to women requiring fertility assistance (without receiving treatment during the index pregnancy) allowed for estimating associations with treatment while minimizing potential effects of the underlying infertility. However, the underlying causes of infertility among women requiring fertility assistance (without receiving treatment during the index pregnancy) may have been less severe or different than among those who underwent treatment.

Limitations

This study has several limitations. First, childhood cancer is rare so few cases were available for the subgroup analyses, which limits the statistical precision of these estimates and the results should therefore be interpreted with caution. The data for specific types of cancer in particular are fragile.

Second, some women may not have used the prescribed fertility drugs or may have used them at a different time, resulting in some nondifferential misclassification and possible underestimation of the associations for use of fertility drugs. However, the effect on the results would likely be small because women who are trying to become pregnant are usually adherent to treatment.

Third, many potential confounders were examined, and although almost no risk factors for childhood cancer are established, the possibility of unknown or residual confounding cannot be ruled out. One potential confounder is infertility diagnosis, which is not available in the Danish Infertility Cohort. The findings for the use of frozen embryo transfer and overall childhood cancer risk may be explained by an unknown confounder; however, the unknown confounder would have needed to be associated with both the exposure and outcome by more than a 4-fold increase in risk.26

Conclusions

Among children born in Denmark, the use of frozen embryo transfer, compared with children born to fertile women, was associated with a small but statistically significant increased risk of childhood cancer; this association was not found for the use of other types of fertility treatment examined.

ARTICLE INFORMATION

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