Incidence of retinoblastoma in Dutch children conceived by in vitro fertilization. An expanded study.

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Human Reproduction 2009; doi:10.1093/Humrep/dep335
Abstract

Background  In 2003, we reported an increased risk of retinoblastoma in children conceived by in vitro fertilization (IVF) between 1995 to 2002. However, population-based studies among children conceived by IVF did not find an elevated risk of retinoblastoma.

Methods  From nationwide estimates of numbers of live births conceived by IVF (n = 40,330), we estimated the expected numbers of retinoblastoma patients conceived by IVF in the period 1995-2007. The actual observed number of retinoblastoma diagnoses in children conceived by IVF was obtained by questionnaires sent to the parents of retinoblastoma patients diagnosed between 1995-2005. For non-responders and patients diagnosed after 2005, information was available through the medical files, in which information on fertility treatments of retinoblastoma patients is routinely recorded since 2000. The relative risk (RR) of retinoblastoma among children conceived by IVF was calculated for the total study period (1995-2007) and for the expanded study period (2002-2007).

Results  Of all eligible retinoblastoma patients (n = 162) diagnosed in the period 1995-2007, 7 retinoblastoma patients were conceived by IVF. In the total study period (1995-2007) the risk was statistically significantly elevated (RR = 2.54, 95% CI = 1.02-5.23). In the expanded study period (2002-2007), no significantly elevated risk (RR = 1.29, 95% CI = 0.16-4.66) was found.

Conclusion  We found a statistically significantly increased risk of retinoblastoma in children conceived by IVF in the period 1995-2007. However, the increased risk in the total study period was mostly based on the much stronger risk increase observed in our previous report for the period 1995 to 2002. Caution and awareness on the one hand and avoiding unnecessary worries on the other hand are important at this stage of our knowledge.
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Introduction

Retinoblastoma is a rare malignant tumour that arises in the retina. The disease is hereditary in 40% of the cases (mostly 2 eyes affected) and nonhereditary (always 1 eye affected) in 60% of the cases. In the Netherlands the incidence of retinoblastoma has been stable from 1945 onwards (1:17,000 live births)\(^1\). In 2003 we reported on an increased risk of retinoblastoma after in vitro fertilization (IVF), based on 5 newly diagnosed retinoblastoma patients born after IVF observed between 2000 and 2002\(^2,3\). In that study we estimated the relative risk (RR) assuming that the proportion of all children in the Netherlands conceived by IVF lay between 1.0-1.5%. The RR for retinoblastoma was significantly increased, and varied between 7.2 (95% Confidence Interval (CI) = 2.4-17.0) and 4.9 (95% CI = 1.6-11.3), respectively. We concluded that this indication of an increased risk of retinoblastoma after IVF required further research to confirm or refute the association. Therefore, we collected information about fertility treatments from parents of all retinoblastoma patients diagnosed in the Netherlands between 1995-2007.

Methods

Study design

From nationwide estimates of numbers of live births conceived by IVF from 1996-2007, we estimated the expected numbers of retinoblastoma patients conceived by IVF in the period 1995-2007. The actual observed number of children conceived by IVF among Dutch retinoblastoma patients was obtained by questionnaires sent to the parents of retinoblastoma patients and through medical files.

Our cohort of retinoblastoma patients has been described previously\(^4\). In total, we have data available of 1068 Dutch retinoblastoma patients diagnosed from 1862. The registry is estimated to have nationwide coverage since 1945\(^5\). For each cohort member data were collected concerning demography, family history of retinoblastoma, tumour laterality, treatment for retinoblastoma, second and subsequent cancers, and date and (underlying) cause of death.

Questionnaires sent to the parents of retinoblastoma patients diagnosed between 1995-2005 also included questions about number of pregnancies, infertility treatments, gestational age, pregnancy outcome and birthweight. When the child with retinoblastoma was conceived by IVF, further information on number of IVF cycles, cause of infertility and other fertility treatments was
collected and checked at the fertility centers concerned. Since the early 2000’s, parents of all newly diagnosed patients were asked whether the child was conceived by IVF or other fertility treatments, which was recorded into the medical file. For patients diagnosed after 2005 and the non-responders of the questionnaire, information on whether the child was conceived by IVF was obtained from these medical files. Information about the conception status recorded in the medical files and given in the questionnaires did not differ.

For this study we selected all retinoblastoma patients who were diagnosed between January 1, 1995 and December 31, 2007 (n = 165). We excluded one patient because she apparently had retinoma (a tumour with spontaneous growth arrest), and two patients were lost to follow-up. Finally, 162 (98%) retinoblastoma patients were eligible for this study.

This study was approved by the Medical Ethics Committees of all participating hospitals, and was conducted in accordance with the principles of the Helsinki declaration.

**DNA-mutation screening**

Since the early nineties DNA-mutation screening of the retinoblastoma (RB1) gene is performed in lymphocytes of all newly diagnosed retinoblastoma patients. All bilateral patients and patients with a family history of retinoblastoma were classified as hereditary. All unilateral patients without a RB1-mutation and a negative family history were classified as nonhereditary. If a RB1-mutation was detected in a child, parents were offered mutation testing.

DNA analysis included direct sequencing of exon 1, 15 and the RB1-promoter and Denaturing Gradient Gel Electrophoresis (DGGE) analysis of the other exons and flanking intronic sequences. To detect large deletions and duplications Multiplex Ligation-dependent Probe Amplification (MLPA) analysis was performed.

**Statistical analyses**

In the Netherlands nationwide numbers on ongoing pregnancies (an intrauterine pregnancy >10 weeks after embryo replacement confirmed by ultrasound) after IVF and ICSI are available since 1996 from the Dutch Society of Obstetrics and Gynaecology (NVOG) and the National Infertility Registry (LIR). All 13 certified IVF centres in the Netherlands provided their annual results from 1996-2007 to the NVOG (1996-2002) and the LIR (2003-2007). Numbers of live births after IVF are not available, however. Therefore we assumed that
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Each ongoing pregnancy resulted in a live born child, and that the number of ongoing pregnancies in 1995 was the same as in 1996. In total we estimated that 40,330 live births after IVF (including ICSI) had occurred in the period 1995-2007.

Subsequently, the expected number of retinoblastoma cases in children conceived by IVF in the period 1995-2007, was calculated using the number of births and the 1-year age-, sex-, and calendar year-specific mortality rates from Statistics Netherlands, and the age- and sex-specific retinoblastoma incidence rates from the Netherlands Cancer Registry.

The relative risk (RR) for the total study period (1995-2007) and the expanded study period (2002-2007), was calculated as the ratio of the observed and the expected number of retinoblastoma diagnosis among children born after IVF in the time period concerned, and a 95% CI was calculated based on the Poisson distribution. The observed number of retinoblastoma diagnosis was based on the results of our questionnaire survey. The absolute excess risk (AER) was calculated by subtracting the expected number of cases from the number observed, dividing by person-years at risk and multiplying by 10,000.

All analyses were processed with SPSS statistical software (SPSS, Chicago, IL).

Results

The Dutch retinoblastoma register contains a total of 165 retinoblastoma patients diagnosed between January 1, 1995 and December 31, 2007, of which 162 (98%) were eligible for this study. In total, 115 questionnaires were sent to the parents of retinoblastoma patients diagnosed between 1995-2005. Three retinoblastoma patients had emigrated and consequently did not receive a questionnaire. From 2005-2007, 44 new retinoblastoma patients were diagnosed. Of all questionnaires sent, 80% was filled in and returned. For all patients who did not respond to the questionnaire, emigrated or diagnosed after 2005, information on birth after IVF was obtained from medical files. Eighty one (50%) had hereditary retinoblastoma and 81 patients (50%) had nonhereditary retinoblastoma. Seven patients (4%) were conceived by IVF; 3 non-familial bilateral cases, and 4 non-familial unilateral cases without a detectable RB1-mutation.

A summary of the characteristics of these 7 retinoblastoma patients is given in Table 1. The patients were born between 1997 and 2005, and the diagnosis of retinoblastoma was made between 2000 and 2007. The family history of retinoblastoma was negative for all patients. Ophthalmological examination of the parents was unremarkable. The parents of the two patients that were
Table 1. Characteristics of retinoblastoma patients conceived by IVF

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Gender</th>
<th>Year of birth</th>
<th>Age at diagnosis</th>
<th>Affected eye</th>
<th>Birth weight</th>
<th>Gestational age (weeks)</th>
<th>Twin</th>
<th>Number of IVF cycles</th>
<th>Cause of infertility</th>
<th>RB1 analysis</th>
<th>Previous fertility treatment</th>
<th>AI=artificial insemination; ICSI=intracytoplasmic sperm injection.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Female</td>
<td>1997</td>
<td>38</td>
<td>both</td>
<td>3885</td>
<td>40</td>
<td>no</td>
<td>8</td>
<td>unexplained</td>
<td>g.59789A&gt;G</td>
<td>none</td>
<td>Initially, this was a twin pregnancy, but one foetus died because of a spontaneous abortion after 8 weeks’ gestation.</td>
</tr>
<tr>
<td>2</td>
<td>Male</td>
<td>1999</td>
<td>15</td>
<td>left</td>
<td>2005</td>
<td>36</td>
<td>yes</td>
<td>2</td>
<td>maternal cause</td>
<td>normal</td>
<td>6x clomid</td>
<td>AI=artificial insemination; ICSI=intracytoplasmic sperm injection.</td>
</tr>
<tr>
<td>3</td>
<td>Female</td>
<td>1998</td>
<td>34</td>
<td>right</td>
<td>2135</td>
<td>41</td>
<td>yes</td>
<td>1</td>
<td>unexplained</td>
<td>normal</td>
<td>none</td>
<td>AI=artificial insemination; ICSI=intracytoplasmic sperm injection.</td>
</tr>
<tr>
<td>4</td>
<td>Male</td>
<td>2001</td>
<td>8.5</td>
<td>both</td>
<td>2330</td>
<td>39</td>
<td>no</td>
<td>2</td>
<td>unexplained</td>
<td>normal</td>
<td>8x AI</td>
<td>AI=artificial insemination; ICSI=intracytoplasmic sperm injection.</td>
</tr>
<tr>
<td>5</td>
<td>Female</td>
<td>1999</td>
<td>32</td>
<td>left</td>
<td>4100</td>
<td>36</td>
<td>no</td>
<td>2</td>
<td>paternal cause</td>
<td>normal</td>
<td>none</td>
<td>AI=artificial insemination; ICSI=intracytoplasmic sperm injection.</td>
</tr>
<tr>
<td>6</td>
<td>Female</td>
<td>2005</td>
<td>23</td>
<td>right</td>
<td>2600</td>
<td>37</td>
<td>no</td>
<td>3</td>
<td>paternal cause</td>
<td>g.2162C&gt;T</td>
<td>6x AI; 1x ICSI</td>
<td>AI=artificial insemination; ICSI=intracytoplasmic sperm injection.</td>
</tr>
<tr>
<td>7</td>
<td>Female</td>
<td>2005</td>
<td>11</td>
<td>both</td>
<td>910</td>
<td>29</td>
<td>yes</td>
<td>1</td>
<td>maternal cause</td>
<td>none</td>
<td>none</td>
<td>AI=artificial insemination; ICSI=intracytoplasmic sperm injection.</td>
</tr>
</tbody>
</table>

found to carry a $RB1$-mutation tested negative for the mutation. Three patients were one of twins; the siblings of these three twins had no ocular abnormalities. Furthermore, two of the four singletons were born after an initial twin pregnancy in which one foetus died. In one case the foetus died because of a spontaneous abortion at 8 weeks of pregnancy; in the other case the foetus died because of an umbilical cord blood supply restriction after 30 weeks of pregnancy. The cause of infertility was unexplained in 3 of the 7 cases; male infertility was the cause in 3 cases, and maternal infertility in one case. The IVF technique (supplemented by ICSI in 2 cases) was performed in 5 different Dutch IVF Centres.

The affected eyes of the 4 patients with unilateral retinoblastoma were enucleated. Of all three bilaterally affected patients one eye was enucleated, the other eye was treated with a radioactive ruthenium plaque in one patient and with external beam radiation therapy (EBRT) in the two other patients. Two patients needed 6 cycles of preventive chemotherapy because tumour extension into the optic nerve past the lamina cribrosa was found by pathology examination. All patients are currently alive, free of disease, and had a median follow-up of 6.1 years (range = 0.5–7.1).

For the expanded study period 2002-2007, the expected number of retinoblastoma cases conceived by IVF was estimated at 1.55 cases. With 2 observed retinoblastoma cases conceived by IVF, the RR was 1.29 (95% CI = 0.16–4.66). For the total study period (1995-2007) we estimated an expected number of 2.76 retinoblastoma cases among children conceived by IVF. With 7 observed cases, the RR was 2.54 (95% CI = 1.02–5.23). The absolute excess risk (AER) of retinoblastoma among children conceived by IVF in the total study period was 1.05 per 10,000 person-years.
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Figure 1 gives the percentages of expected and observed retinoblastoma cases in the general Dutch population, and the percentage of observed retinoblastoma patients conceived by IVF per year.

Figure 1. Percentage ($10^{-3}$) retinoblastoma patients by year

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Gender</th>
<th>Year of birth</th>
<th>Age at diagnosis (months)</th>
<th>Affected eye</th>
<th>Birth weight (g)</th>
<th>Gestational age (weeks)</th>
<th>Twin</th>
<th>Number of IVF cycles</th>
<th>Cause of infertility</th>
<th>RB1 analysis (lymphocytes)</th>
<th>Previous fertility treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Female</td>
<td>1997</td>
<td>38</td>
<td>both</td>
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<td>no</td>
<td>2</td>
<td>unexplained</td>
<td>no pathogenic mutation$^a$</td>
<td>8x AI</td>
</tr>
<tr>
<td>5</td>
<td>Female</td>
<td>1999</td>
<td>32</td>
<td>left</td>
<td>4100</td>
<td>41</td>
<td>no</td>
<td>2</td>
<td>paternal cause</td>
<td>normal</td>
<td>1x ICSI</td>
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<td>6</td>
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<td>2600</td>
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<td>paternal cause</td>
<td>g.2162C&gt;T</td>
<td>6x AI</td>
</tr>
</tbody>
</table>

AI = artificial insemination, ICSI = intracytoplasmic sperm injection.

$^a$ Initially, this was a twin pregnancy, but one foetus died because of a spontaneous abortion after 8 weeks’ gestation.

$^b$ IVS3+10C>G, known polymorphism.

$^c$ Initially, this was a twin pregnancy, but one foetus died because of an umbilical cord blood supply restriction.
Discussion

In the total study period (1995-2007), the risk of retinoblastoma among children conceived by IVF was statistically significantly elevated. In the expanded period (2002-2007), however, the risk of retinoblastoma was not significantly increased. The increased risk in the total study period was mostly based on the much stronger risk increase observed in our previous report for the period 1995 to 2002.

The first report on retinoblastoma occurring in a child conceived by IVF was published in 2001. In 2003 we added another 5 cases from the Netherlands, since then only Lee et al. reported one additional case from the United States. However, two IVF register-based studies did not find an indication of an increased risk of retinoblastoma after IVF. Bradbury and Jick used the United Kingdom-based General Practice Research Database to identify all live births, cases of retinoblastoma and IVF procedures occurring between January 1989 and December 2001. They found no cases of retinoblastoma among the 176 children conceived by IVF. As BenEzra stated in his letter to the editor; the power of this study was very low since the incidence of retinoblastoma worldwide is around one case per 15,000-20,000 live births. The other study, that compared the frequency of imprinting diseases in children conceived by IVF (n = 6,052) with the incidence in naturally conceived children (n = 442,349), no children with cancer (including retinoblastoma) were found in the IVF group, whereas 5 retinoblastoma cases were found in the non-IVF group. This study covers a 7-year study period (1995-2001) with 4.5 years follow-up in the Danish National Register of Patients and the Central Register of Psychiatric Diseases (including all diagnosis from somatic and psychiatric hospitals/clinics). They found fewer specific imprinting diagnosis in both IVF and non-IVF children than expected. This cohort, however, was also too small to find at least one retinoblastoma. Therefore, we recommend larger extended follow-up studies of children conceived by IVF to have adequate power to examine the association between IVF and retinoblastoma risk.

Based on the literature, only in the Netherlands an elevated risk of retinoblastoma was found among children conceived by IVF during the period 1995-2001. In the present study, no significantly elevated risk was found for the period 2002-2007. Previous research on women undergoing IVF in the Netherlands from 1980-1995 revealed no cases of retinoblastoma in the offspring.

An association between retinoblastoma and IVF is difficult to explain. An explanation might be that, as suggested before, the association between retino-
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Retinoblastoma and IVF is an example of clustering or a chance finding, which is supported by the fact that the association was not found before 1996 and not confirmed in the period 2002-2007. However, there are many other possible explanations for the observed elevated risk. Since 2002 reports have suggested an association between ART and imprinting disorders, specifically Beckwith-Wiedemann syndrome, and Angelman syndrome. Animal studies have demonstrated alterations in gene imprinting of embryos cultured in vitro. The association of retinoblastoma, IVF and imprinting is suggested, because 10-12% of the mutations in the retinoblastoma tumour are caused by hypermethylation of the \textit{RB1} promoter. In the Netherlands 7 retinoblastoma patients conceived by IVF were observed in the period 1995-2007. In two patients a causative de novo \textit{RB1} germline mutation was found. Whether the second hit was caused by promoter hypermethylation of the other allele, is currently not known. In the third bilaterally affected patient and the four isolated unilaterally affected retinoblastoma patients, no germline \textit{RB1}-mutation could be detected by current screening techniques.

Another explanation for the elevated risk could be that the same genetic factors are involved in infertility and the occurrence of retinoblastoma. This has also been suggested for Beckwith-Wiedemann syndrome, Angelman syndrome, and Prader-Willi syndrome. The authors found an increased risk for all three syndromes among children conceived by assisted reproduction technology (ART). They also demonstrated that after correction for impaired fertility, the incidence was not increased. It was concluded that ART does not seem to have a direct effect on the increase of imprinting diseases, but that the increased risk can be explained by maternal or paternal subfertility causes. Several other studies have implied subfertility itself as a opposed to ART, as an increased risk for an imprinting defect or congenital malformations.

Yet another explanation might be sought in changes in the IVF procedure itself or in changes of the IVF procedure. It is known that in 1999 the Dutch IVF centres stopped using human chorionic gonadotrophin (HCG) and started using recombinant follicle stimulating hormone (FSH).

This study focused on the risk of retinoblastoma among children conceived by IVF and provides information on a cohort of retinoblastoma patients. Nevertheless, a number of study limitations should be considered when interpreting the results. Unfortunately the IVF registration from the Dutch Society of Obstetrics and Gynecology (NVOG) and the National Infertility Registry (LIR) has some restrictions. In short, the IVF registry is based on retrospec-
tive data obtained from the centers; no validation studies have been done. Relevant data, like numbers of embryos per transfer, complications, number of live births, congenital abnormalities, and health of the child are not registered. Therefore, we assumed that each ongoing pregnancy resulted in one live born child. Despite these limitations, it is the best available information on IVF in the Netherlands at this moment. Some assumptions were made to estimate the risk of retinoblastoma and overall percentage of live births among children conceived by IVF. Therefore, it is possible that we have slightly under- or over-estimated the risk.

Unfortunately, this study has not resolved the issue of a possible association between IVF and the occurrence of retinoblastoma. Whether treatment with ovarian stimulating regimens increases the risk of childhood cancer remains an important question, especially with the increasing numbers of women undergoing treatment for infertility. Future, larger studies in children conceived by IVF have to consider the number of IVF treatments, other fertility drugs administered prior to IVF. It should be taken into account that serious disorders in children conceived by IVF may be diagnosed earlier through close medical surveillance.

In conclusion, we found a statistically significantly increased risk of retinoblastoma in children conceived by IVF in the period 1995-2007. However, the increased risk in the total study period was mostly based on the much stronger risk increase observed in our previous report for the period 1995 to 2002. In the expanded period 2002-2007 no statistically significantly risk elevation was observed (RR = 1.29), but numbers were small (n = 2). These findings require further research in larger patient numbers to explore a possible causal mechanism. Caution and awareness on the one hand and avoiding unnecessary worries on the other hand are important at this stage of our knowledge.

Acknowledgements
We thank A.H. van der Hout for performing the DNA analysis.
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Reference List

