Retinoblastoma

Dommering, C.J.

2015


CHAPTER

INCIDENCE OF RETINOBLASTOMA IN DUTCH CHILDREN CONCEIVED BY IN VITRO FERTILIZATION. AN EXPANDED STUDY

7

Tamara Marees, Charlotte J. Dommering, Saskia M. Imhof, Wijnanda A. Kors, Peter J. Ringens, Flora E. van Leeuwen, Annette C. Moll

Hum Reprod. 2009;24(12):3220-4

ABSTRACT

Background

In 2003, we reported an increased risk of retinoblastoma in children conceived by in vitro fertilization (IVF) between 1995 and 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 and 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 patients with retinoblastoma (n=162) diagnosed in the period 1995-2007, seven 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.

Conclusions

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

INTRODUCTION

Retinoblastoma is a rare malignant tumour that arises in the retina. The disease is hereditary in 40% of the cases (mostly two eyes effected) and nonhereditary (always one 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 five 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 and 2007.

METHODS

Study design

From nationwide estimates of numbers of live births conceived by IVF from 1996 to 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 from data in medical files.

Our cohort of patients with retinoblastoma has been described previously.[4] In total, we have data available for 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 and 2005 also included questions about number of pregnancies, infertility treatments, gestational age, pregnancy outcome and birth weight. 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 cross-checked at the fertility centres concerned. Since the early 2000s, parents of all newly diagnosed patients with retinoblastoma 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 patients with retinoblastoma who were diagnosed between 1 January 1995 and 31 December 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 1990s, DNA-mutation screening of the retinoblastoma (*RB1*)-gene has been performed in lymphocytes of all newly diagnosed patients with retinoblastoma. All patients with bilateral tumours and patients with a family history of retinoblastoma were classified as hereditary. All patients with unilateral tumour without an *RB1*-mutation and a negative family history were classified as non-hereditary. If an *RB1*-mutation was detected in a child, parents were offered mutation testing.
DNA analysis included direct sequencing of exon 1, exon 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 data 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 to 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 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 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.[6]
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 patients with retinoblastoma diagnosed between 1 January 1995 and 31 December 2007, of which 162 (98%) were eligible for this study. In total, 115 questionnaires were sent to the parents of patients with retinoblastoma diagnosed between 1995 and 2005. Three patients had emigrated and consequently did not receive a questionnaire. From 2005 to 2007, 44 new retinoblastoma patients were diagnosed. Of all questionnaires send, 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 non-hereditary 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 seven 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 found to carry an *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 three of the seven cases; male infertility was the cause in three cases, and maternal infertility in one case. The IVF technique (supplemented by ICSI in two cases) was performed in five different Dutch IVF Centres.

Table 1. Characteristics of patients with retinoblastoma who were conceived by IVF and born between 1997 and 2005 in the Netherlands

Patient No.	Gender	Year of birth	Age at diagnosis (months)	Affected eye(s)	Birth weight (g)	Gestational age (weeks)	Twin	Number of IVF cycles	Cause of Infertility	*RB1* analysis (lymphocytes)	Previous fertility treatment
1	Female	1997	38	Both	3885	40	No	8	Unexplained	g.59789A>G	6x Clomid
2	Male	1999	15	Left	2005	36	Yes	2	Maternal cause	Normal	None
3	Female	1998	34	Right	2135	41	Yes	1	Unexplained	Normal	None
4	Male	2001	8.5	Both	2330	39	No[a]	2	Unexplained	No pathogenic mutation[b]	8x AI
5	Female	1999	32	Left	4100	41	No	2	Paternal cause	Normal	1x ICSI
6	Female	2005	23	Right	2600	37	No[c]	3	Paternal cause	Normal	6x AI, 1x ICSI
7	Female	2005	11	Both	910	29	Yes	1	Paternal cause	g.2162C>T	6x AI

AI = Artificial insemination; Clomid: Clomiphene citrate

[a]Initially, this was a twin pregnancy, but one fetus died because of a spontaneous miscarriage after 8 weeks 'gestation.

[b]Intervening sequence 3+10C>G, known polymorphism.

[c]Initially, this was a twin pregnancy, but one fetus died because of an umbilical cord blood supply restriction

The affected eyes of the four patients with unilateral retinoblastoma were enucleated. For 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 in the two other patients. Two patients needed six 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 two 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 seven observed cases, the RR was 2.54 (95% CI= 1.02-5.23). The AER of retinoblastoma among children conceived by IVF in the total study period was 1.05 per 10,000 person-years.

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.

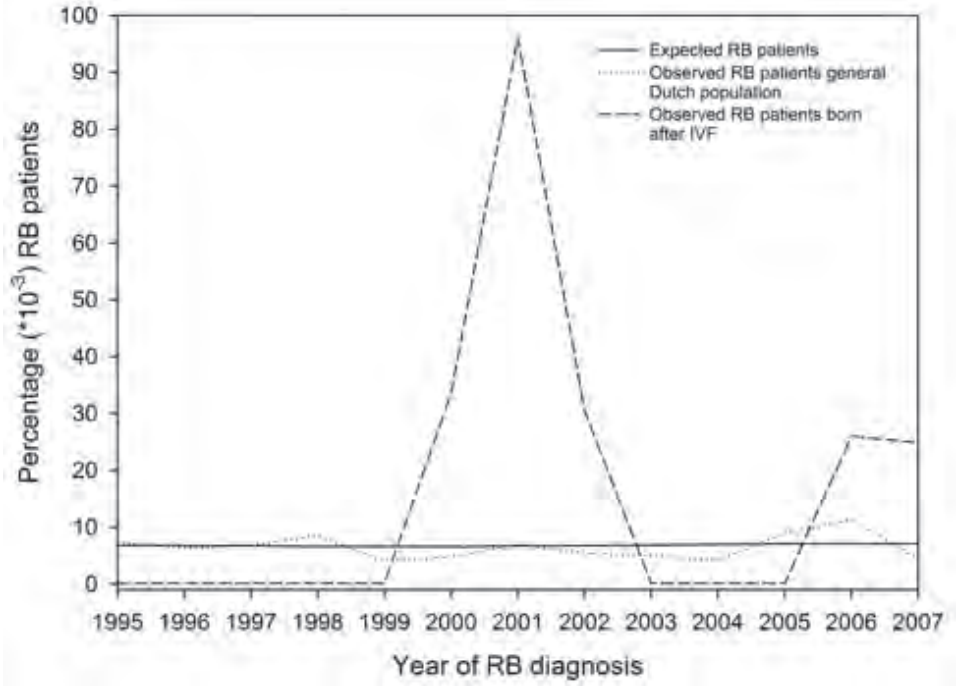

Figure 1. Percentage (*10-3) retinoblastoma patients by year

DISCUSSION

In the total study period (1995-2007), the risk of retinoblastoma among children conceived by IVF was 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 1995-2002.

The first report on retinoblastoma occurring in a child conceived by IVF was published in 2001.[7] In 2003, we added another five cases from the Netherlands[3,8], and since then only Lee *et al.*[9] 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.[10,11] Bradbury and Jick[10] 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[12] 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. In the other study, that compared the frequency of diseases linked to genetic imprinting 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 five retinoblastoma cases were found in the non-IVF group.[11] 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). Lidegaard et al.[11] found fewer specific imprinting diagnosis in both IVF and non-IVF children than expected. However, this cohort was also too small to find at least one retinoblastoma. Therefore, we recommend larger extended follow-up studies of children conceived by IVF to provide adequate power to examine the association between IVF and retinoblastoma risk.

On the basis of 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.[13]

An association between retinoblastoma and IVF is difficult to explain. An explanation might be that, as suggested before, the association between retinoblastoma and IVF is an example of clustering or a chance finding[14], 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[15,16], and Angelman syndrome.[17] Animal studies have

demonstrated alterations in gene imprinting of embryos cultured *in vitro*.[18] 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 *RB1* promoter.[19] In the Netherlands seven retinoblastoma patients conceived by IVF were observed in the period 1995-2007. In two patients a causative *de novo 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 *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[20], and an increased risk for all three syndromes among children conceived by ART was initially observed. However, the authors also demonstrated that, after correction of data 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.[20] Several other studies have implied that subfertility itself, as opposed to ART, is the factor which may increase risk of imprinting defects[21,22] or congenital malformations.[23]

Yet another explanation might be sought in the IVF procedure itself[24-26] or in changes of the IVF protocol. It is known that in 1999 the Dutch IVF centres stopped using human chorionic gonadotrophin (HCG) and started using recombinant follicle stimulating hormone (recFSH).[27] However, a causal link between use of recFSH and retinoblastoma is unlikely as recFSH was also used after 2002, when the incidence of retinoblastoma is low. Any suggestion of adverse effects resulting from changes in hormone use is purely speculative at this stage.

Our 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 NVOG and the LIR has some restrictions.[27] In short, the IVF registry is based on retrospective data obtained from the centres; 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 the overall percentage of live births among children conceived by IVF. Therefore, it is possible that we have slightly under- or overestimated the risk.

Unfortunately, the present study has not resolved the issue of a possible association between IVF and the occurrence of retinoblastoma. Whether treatment using 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 confirm that further research in larger patient numbers is required 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.

FUNDING

The study was supported by award VU 2004-3046 from the Dutch Cancer Society. The sponsor of the study had no role in the design and conduct of the study, the data collection, data analysis, data interpretation, or writing of the report.

REFERENCES

1 Moll AC, Kuik DJ, Bouter LM et al.: Incidence and survival of retinoblastoma in The Netherlands: a register based study 1862-1995. Br J Ophthalmol 1997; 81: 559-562.

2 Moll AC, Imhof SM, Cruysberg JR, Schouten-van Meeteren AY, Boers M, van Leeuwen FE: Incidence of retinoblastoma in children born after in-vitro fertilisation. Lancet 2003; 361: 309-310.

3 Moll AC, Imhof SM, Schouten-van Meeteren AY, van Leeuwen FE: In-vitro fertilisation and retinoblastoma. Lancet 2003; 361: 1392

4 Marees T, Moll AC, Imhof SM, de Boer MR, Ringens PJ, van Leeuwen FE: Risk of Second Malignancies in Survivors of Retinoblastoma: More Than 40 Years of Follow-up. J Natl Cancer Inst 2008; 100: 1771-1779.

5 DerKinderen DJ, Koten JW, Tan KE, Beemer FA, Van Romunde LK, Den OW: Parental age in sporadic hereditary retinoblastoma. Am J Ophthalmol 1990; 110: 605-609.

6 Pearson ES, Hartley HO, editors: Biometrika tables for statisticians. 1976;

7 Anteby I, Cohen E, Anteby E, BenEzra D: Ocular manifestations in children born after in vitro fertilization. Arch Ophthalmol 2001; 119: 1525-1529.

8 Cruysberg JR, Moll AC, Imhof SM: Bilateral sporadic retinoblastoma in a child born after in vitro fertilization. Arch Ophthalmol 2002; 120: 1773-1774.

9 Lee I, Finger PT, Grifo JA, Rausen AR, Rebarber A, Barad DH: Retinoblastoma in a child conceived by in vitro fertilisation. Br J Ophthalmol 2004; 88: 1098-1099.

10 Bradbury BD, Jick H: In vitro fertilization and childhood retinoblastoma. Br J Clin Pharmacol 2004; 58: 209-211.

11 Lidegaard O, Pinborg A, Andersen AN: Imprinting diseases and IVF: Danish National IVF cohort study. Hum Reprod 2005; 20: 950-954.

12 BenEzra D: IVF and retinoblastoma. Br J Ophthalmol 2005; 89: 393

13 Klip H, Burger CW, de Kraker J, van Leeuwen FE: Risk of cancer in the offspring of women who underwent ovarian stimulation for IVF. Hum Reprod 2001; 16: 2451-2458.

14 BenEzra D: In-vitro fertilisation and retinoblastoma. Lancet 2003; 361: 273-274.

15 DeBaun MR, Niemitz EL, Feinberg AP: Association of in vitro fertilization with Beckwith-Wiedemann syndrome and epigenetic alterations of LIT1 and H19. Am J Hum Genet 2003; 72: 156-160.

16 Maher ER, Brueton LA, Bowdin SC et al.: Beckwith-Wiedemann syndrome and assisted reproduction technology (ART). J Med Genet 2003; 40: 62-64.

17 Cox GF, Burger J, Lip V et al.: Intracytoplasmic sperm injection may increase the risk of imprinting defects. Am J Hum Genet 2002; 71: 162-164.

18 Khosla S, Dean W, Brown D, Reik W, Feil R: Culture of preimplantation mouse embryos affects fetal development and the expression of imprinted genes. Biol Reprod 2001; 64: 918-926.

19 Zeschnigk M, Bohringer S, Price EA, Onadim Z, Masshofer L, Lohmann DR: A novel real-time PCR assay for quantitative analysis of methylated alleles (QAMA): analysis of the retinoblastoma locus. Nucleic Acids Res 2004; 32: e125

20 Doornbos ME, Maas SM, McDonnell J, Vermeiden JP, Hennekam RC: Infertility, assisted reproduction technologies and imprinting disturbances: a Dutch study. Hum Reprod 2007; 22: 2476-2480.

21 Ludwig M, Katalinic A, Gross S, Sutcliffe A, Varon R, Horsthemke B: Increased prevalence of imprinting defects in patients with Angelman syndrome born to subfertile couples. J Med Genet 2005; 42: 289-291.
22 Kobayashi H, Hiura H, John RM et al.: DNA methylation errors at imprinted loci after assisted conception originate in the parental sperm. Eur J Hum Genet 2009; 17: 1582-1591.
23 Zhu JL, Basso O, Obel C, Bille C, Olsen J: Infertility, infertility treatment, and congenital malformations: Danish national birth cohort. BMJ 2006; 333: 679
24 Sato A, Hiura H, Okae H et al.: Assessing loss of imprint methylation in sperm from subfertile men using novel methylation polymerase chain reaction Luminex analysis
77. Fertil Steril 2011; 95: 129-34, 134.
25 Fortier AL, Lopes FL, Darricarrere N, Martel J, Trasler JM: Superovulation alters the expression of imprinted genes in the midgestation mouse placenta. Hum Mol Genet 2008; 17: 1653-1665.
26 Rivera RM, Stein P, Weaver JR, Mager J, Schultz RM, Bartolomei MS: Manipulations of mouse embryos prior to implantation result in aberrant expression of imprinted genes on day 9.5 of development
23. Hum Mol Genet 2008; 17: 1-14.
27 Kremer JA, Bots RS, Cohlen B et al.: [Ten years of results of in-vitro fertilisation in the Netherlands 1996-2005]. Ned Tijdschr Geneeskd 2008; 152: 146-152.