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Imprinting diseases and IVF: Danish National IVF cohort study

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BACKGROUND: The aim of this study was to compare the frequency of imprinting diseases in children born after IVF with the incidence in naturally conceived children. METHODS: All singleton children born in Denmark from January 1, 1995 through December 31, 2001 were stratified into children born without and after IVF, and were followed from birth until the end of 2002 in the National Register of Patients and the Central Register of Psychiatric Diseases, which include all discharge diagnoses from somatic and psychiatric hospitals/clinics, respectively. Included in the study were malignancies, mental, behavioural and neurological diseases, congenital syndromes, and developmental disturbances. Only diagnosis codes potentially relevant for imprinting diseases were included. RESULTS: During the 7-year study period, 442 349 singleton non-IVF and 6052 IVF children were born. Mean follow-up time was 4.5 and 4.1 years for the two groups, respectively, corresponding to 2 million and 25 000 follow-up years. In the IVF/non-IVF cohort, we detected 0/72 children with cancer, 47/3766 with mental diseases, 72/3654 neurological diseases, 4/287 congenital syndromes and 96/6727 developmental disturbances, in a total of 219/14 506 clinical outcomes. The number of children with specific imprinting diseases in the non-IVF group was 54: 44 kidney cancers, five retinoblastoma, three Prader–Willi syndrome and two Russel–Silver syndrome. Anticipating the same occurrence in IVF children, the total expected number was calculated to be 0.74. The observed number in the IVF group was 0. We found a significantly increased risk of cerebral palsy in the IVF group, with a rate ratio (RR) (IVF:non-IVF) of 1.8 [95% confidence interval (CI) 1.2–2.8; $P < 0.01$], and of sleeping disturbances, with an RR 2.0 (95% CI 1.2–3.3). The incidence rate of childhood cancers, mental diseases, congenital syndromes and developmental disturbances was equal in the two groups. CONCLUSIONS: We found no indication of an increased risk of imprinting diseases after IVF, but an 80% increased risk of cerebral palsy. We observed equal frequencies of childhood cancers, mental diseases, congenital syndromes and developmental disturbances in the two groups. Danish register data do not support reports of an increased risk of imprinting diseases after IVF.

Key words: cerebral palsy/imprinting/infertility/IVF

Introduction

Imprinting is an epigenetic modification of the genome by which only genes in one of two parental alleles are expressed. At present, 75 human imprinted genes have been identified. In the wake of this knowledge, a number of diseases caused by inadequate imprinting of specific genes have been detected.

Five recent reports have suggested an increased risk of imprinting diseases after IVF (Cox *et al.*, 2002; DeBaun *et al.*, 2003; Gicquel *et al.*, 2003; Maher *et al.*, 2003; Moll *et al.*, 2003), and experimental studies have demonstrated that the culture media used in IVF may influence the imprinting process in animals (Khosla *et al.*, 2001; Young *et al.*, 2001).

Although the specific known imprinting diseases are all rare, they have serious consequences for the children.

In Denmark, the National IVF Registry was established in 1994 (Andersen *et al.*, 1999). All clinics and hospitals, public as well as private, are by law obliged to report all treatment cycles to this register, including the personal identification number of the involved patient. Personal identification numbers of all children born after IVF with or without simultaneous ICSI can be identified by record-linkage of the IVF Registry with the National Birth Registry.

Since 1977, all discharge diagnoses from hospitals have been recorded in the National Register of Patients, and all psychiatric discharge diagnoses from clinics and hospitals in the National Register of Psychiatric Diseases (NRPD). These discharge diagnoses are recorded together with personal identification numbers of the patients. Since January 1, 1994, the WHO International Classification of Diseases, 10th revision, has been used in Denmark.

The aims of this study were to (i) assess the incidence rate of imprinting diseases in children born after and without IVF, and (ii) establish a cohort of Danish IVF and non-IVF singletons for regular follow-up.

Materials and methods

All Danish children born from January 1, 1995 to December 31, 2001 were included in the cohort. As twins have a substantially increased risk of neonatal and neurological diseases, primarily due

Table I. Included diagnosis codes according the ICD-10, and the number of children diagnosed with these diseases in non-IVF and IVF cohort

			All neurological diseases		
Code	Rank	Diagnosis	Non-IVF	IVF	Rate ratio
DC64	2	Kidney cancer	44	0	0.0
DC692A	1	Retinoblastoma	5	0	0.0
DC920	3	Acute myeloblastic leukaemia	23	0	0.0
DC total		Specified cancer diseases total	72	0	0.0
DF68	6	Disturbances in personality or behaviour	2	0	0.0
DF69	6	Disturbances unspecified in personality	0	0	–
DF70	4	Mental retardation, mild degree	254	4	1.2
DF71	3	Mental retardation, moderate degree	111	0	0.0
DF72	2	Mental retardation, severe degree	45	0	0.0
DF73	1	Mental retardation, most severe degree	22	0	0.0
DF78	5	Mental retardation other	16	0	0.0
DF79	5	Mental retardation, unspecified	368	5	1.0
DF80	9	Specific speech disturbances	245	3	0.9
DF81	9	Specific cognitive disturbances	11	0	0.0
DF82	9	Specific motor developmental disturbances	76	1	1.0
DF83	9	Mixed developmental disturbances	107	2	1.4
DF84	7	Severe mental disturbance	762	13	1.2
DF88	8	Other psychic developmental disturbances	21	0	0.0
DF89	8	Unspecified psychical developmental disturbances	39	0	0.0
DF90	8	Hyperkinetic syndrome	340	1	0.2
DF91	8	Behavioural disturbances	184	1	0.4
DF92	8	Mixed behavioural and sensory disturbance	76	0	0.0
DF98	8	Other behavioural and sensory disturbances	986	17	1.3
DF99	8	Non-specified psychical disease	101	0	0.0
DF total		Specified mental disturbances	3766	47	0.9
DG40	4	Epilepsy	2013	30	1.1
DG47	3	Sleeping disturbances	579	16	2.0*
DG80	1	Cerebral palsy	819	20	1.8*
DG81	2	Hemiparesis	87	3	2.5
DG82	2	Paraparesis	86	2	1.7
DG83	2	Other syndromes with paresis	40	0	0.0
DG90	2	Diseases in autonomous nervous system	30	1	2.4
DG total		Specified neurological diseases	3654	72	1.4
DQ871	2	Syndromes with dwarf growth	60	2	2.4
DQ871E	1	Prader–Willi syndrome	3	0	0.0
DQ871G	1	Russel–Silver syndrome	2	0	0.0
DQ872	3	Malformation of extremities	44	0	0.0
DQ873	2	Malformations with early length growth	22	0	0.0
DQ873A	1	Beckwith–Wiedemann syndrome	0	0	–
DQ874	6	Marfans syndrome	17	0	0.0
DQ875	4	Other skeletal malformations	12	0	0.0
DQ878	5	Other syndromes with malformations	127	2	1.2
DQ total		Congenital malformation syndromes	287	4	1.0
DR62	3	Delayed physiological development	0	0	0.0
DR620	2	Retarded psycho-motor development	2557	39	1.1
DR620A	1	Speech retardation	7	0	0.0
DR620B	1	Language retardation	12	0	0.0
DR620C	1	Motor retardation	26	1	2.8
DR628	8	Other forms of abnormal physiological development	3092	40	0.9
DR628A	7	Impaired development	392	6	1.1
DR628B	7	Unspecified infantility	5	0	0.0
DR628C	7	Growth retardation	37	0	0.0
DR629	8	Delayed physiological development	206	4	1.4
DR63	6	Food or drinking disturbances	212	3	1.0
DR630	5	Anorexia	57	2	2.6
DR631	5	Polydipsy	25	0	0.0
DR632	5	Polyphagy	55	1	1.3
DR633	4	Other eating disturbances	44	0	0.0
DR total		Specified developmental disturbances	6727	96	1.0
Total		All included diagnoses	14 506	219	1.1

*$P < 0.01$.
ICD-10 = WHO International Classification of Diseases, 10th revision.

to premature delivery, we included only singletons in the present study. Data from twins are currently under separate analysis.

Women treated with IVF were identified in the IVF Registry. Subsequently, all established pregnancies were traced in the Birth Registry, which includes information on gestational age, and the personal identification numbers of mothers and children born. To approve a pregnancy as being a result of IVF treatment, the gestational age in the IVF Registry should match, within 2 weeks, the gestational age in the Birth Registry. For the year 2001, only children born after IVF initiated in the 2000 were included, because the registration in the IVF Registry for 2001 was not completed. Therefore, ~300 IVF children born during the last 3 months of 2001 were misclassified as non-IVF children; however, they constitute less than one in 1000 of the control children.

All specific diagnosis codes for known imprinting diseases were included, as well as codes for diseases that might have been used in children with clinical symptoms but who had not been diagnosed with the specific imprinting disorder. The specific codes included in the study are indicated in Table I. In- as well as out-patients were included, and primary diagnoses as well as secondary diagnosis codes were included. Not all imprinting diseases had a specific diagnosis code during the study period, the most important omission being for Angelman syndrome.

All children were followed by their personal identification number in the National Register of Patients and the NRPD until December 2002.

Each child was allowed to be counted only once with a diagnosis in each of the five main diagnosis groups (Table I). In case of more diagnoses within the same main diagnosis group, a priority according to the specificity for imprinting diseases was established (Table I). This arbitrary 1–9 ranking (1 had highest specificity) of diseases was performed prior to data retrieval.

Incidence rates of main diagnosis groups and of each specific diagnosis included were calculated for IVF and non-IVF children. Secondly, incidence rate ratios (IRR) between IVF and non-IVF children were established. Ninety-five per cent confidence intervals (95% CI) were calculated for these IRRs.

Permission to link data in the IVF registry, the National Register of Patients and the Central Register of Psychiatric Diseases was achieved by 'Datatilsynet' (Board of Registers = Danish Data Protection Agency), j. No. 2003-41-3048.

Results

During the 7-year study period, 442 349 singleton non-IVF and 6052 IVF were born. The mean follow-up time for the two groups was 4.5 and 4.1 years, respectively, due to an increasing number of IVF children born during the study period. The corresponding follow-up years were 2 million in the non-IVF group and 25 000 in the IVF group. Of 6052 IVF children, 1680 (28%) were conceived with and 4372 (72%) without ICSI.

In the non-IVF cohort, the number of detected cancers was 72, specified mental diseases 3766, neurological diseases 3654, congenital malformations/syndromes 287 and developmental disturbances 6727, in a total of 14 506 clinical outcomes (Table I). The corresponding number of diseases in the IVF cohort was 0, 47, 72, 4 and 96, in a total of 219 clinical outcomes (Table I).

The incidence of specific diagnoses in the two cohorts and the corresponding IRRs between the IVF and the non-IVF cohort are indicated in Table I. The absolute number of cases with each diagnosis in the IVF cohort and calculated IRRs are indicated in Figure 1.

In total, 54 children with anticipated imprinting diseases were identified in the non-IVF cohort: 44 had kidney cancer (DC54), five retinoblastoma (DC692A), three Prader–Willis syndrome (DQ871E) and two Russel–Silver syndrome (DQ871G); none were diagnosed with Beckwith–Wiedemann syndrome (DQ873A). Anticipating the same occurrence of imprinting diseases in IVF children as in non-IVF children, the expected number of imprinting diseases was calculated to be 0.74. We observed no children in the IVF cohort with specific imprinting diseases. Hence, we were not able to stratify our analysis into IVF with and without ICSI.

Based on 20/819 cases in the IVF/non-IVF cohorts, we found a significantly increased risk of cerebral palsy after IVF; 3.3 versus 1.9 per 1000, and an IRR of 1.8 (95% CI 1.2–2.8). Sleeping disturbances were also detected more frequently in the IVF cohort: IRR 2.0 (1.2–3.3).

The incidence rates of the included main diagnosis groups of childhood cancers (DC), mental diseases (DF), congenital

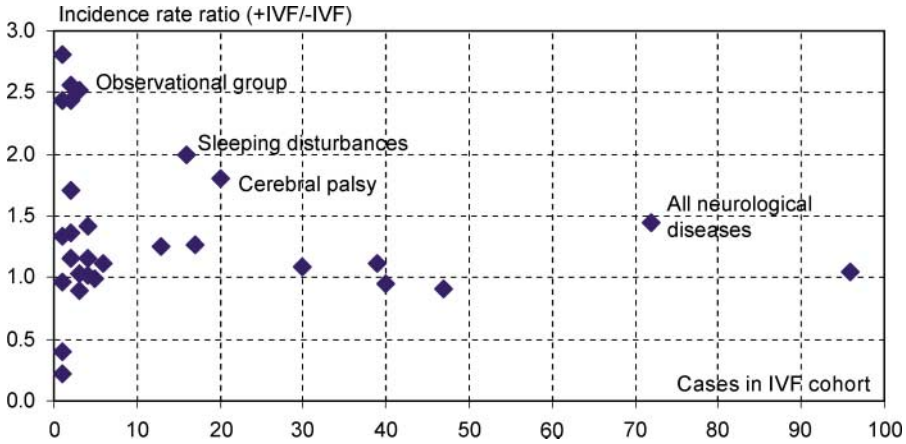

Figure 1. Number of cases with specific diagnoses in the IVF cohort and the IRR between the IVF and non-IVF cohort. Number of children in IVF cohort, 6052; number of cases, 219.

syndromes (DQ) and developmental disturbances (DR) were equal in the two cohorts.

Five diseases had statistically non-significant increased IRRs, ranging from 2 to 3 (Table I and Figure 1), each with few cases in the IVF cohort. These diseases were hemiparesis (IRR 2.0), diseases of the autonomous nervous system (IRR 2.4), syndromes with dwarf growth (IRR 2.4), motor retardation (IRR 2.8) and anorexia (IRR 2.6), and they constitute an 'observational group' that will be paid special attention in our future follow-up of these cohorts.

Discussion

To our knowledge, this is the first systematic historical follow-up study on IVF children and a control group of children without prior IVF specifically focusing on imprinting diseases.

The diagnosis of imprinting diseases, however, is difficult. Many of the syndromes have a broad clinical spectrum, different molecular pathogenesis, and the infant has to reach a certain age before these diseases become clinically detectable. It was therefore supposed that many children with these diseases were not recorded with the specific diagnosis code for these syndromes.

With 54 imprinting diseases among 442 349 singleton children born without prior IVF, or 12 per 100 000 children, the expected number of these syndromes in the IVF cohort was below one, anticipating the same frequency in the two cohorts. This finding first of all indicates that a significant number of imprinting diseases are misclassified during the first years of children's life. It is not very likely, however, that the misclassification of these diagnoses occurred differentially in the IVF and non-IVF cohort. The fact that we did not find any children with these diagnoses in the IVF group within a follow-up period of 4.1 years is an encouraging indication that these diseases do not occur more frequent among IVF children than among children born without IVF. Larger multicentre or transnational studies need to confirm this finding. Although some authors have questioned whether these cancers are or could be imprinting diseases, a single study reported an association between retinoblastoma and IVF (Moll *et al.*, 2003). Whether an imprinting disease or not, our data were not able to exclude an increased risk of this specific cancer, due to the rarity of this disease.

An increased risk of cerebral palsy in IVF singletons was also demonstrated in a Swedish follow-up of 3183 IVF singletons by Strömberg *et al.* (2002). They found an IRR of 2.8 (95% CI 1.3–5.8), and no differential risk after stratification for maternal age. We have not found other assessments of the frequency of cerebral palsy in IVF singletons versus controls.

Although some imprinting syndromes might be misclassified with the diagnosis of cerebral palsy, especially during the first years of life, such a misclassification hardly explains the 80% demonstrated increased risk of this disease. IVF mothers are on average 3–4 years older than the mothers of non-IVF children. Lidegaard (2004) found, in a Danish Register study, a slightly increasing frequency of cerebral palsy with increasing age. After stratification for parity, this increase was only detectable in primiparous women >35 years old. As IVF mothers constitute 20–25% of all primiparous women >35 years old, an increased risk of cerebral palsy after IVF might fully explain this age-trend in primiparous women.

Premature birth (before week 37) in IVF singletons is slightly more frequent (7.3%) than in non-IVF singletons (5.3%) (Westergaard *et al.*, 1999). This difference, however, could only explain about one-fifth of the increased risk of cerebral palsy in our IVF singletons, and the rate ratio of cerebral palsy among IVF singletons was of same magnitude in mature (≥ 37 weeks) and in premature (<37 weeks) children.

Pharoah and Adi (2000) demonstrated a 40-fold increased risk of cerebral palsy in a twin surviving death (mainly in the third trimester) of the other twin *in utero*, so called vanishing twins. The risk of cerebral palsy in vanishing twins due to death in first or second trimester has not been assessed.

In a review, Landy and Keith (1998) concluded that $\sim$30% of all twin pregnancies ultimately result in singletons. In our IVF clinic, over the last 3 years, we had 723 clinical pregnancies at 7 weeks of gestation, 519 (72%) singletons, 43 (6%) vanishing twins and 161 (22%) twins. If Landy and Keith's estimate is correct, according to our data, $(43 + 161 \times 0.30)/(519 + 43 + 48)$, or $\sim$15%, of all singleton children born after IVF are vanishing twins. If the risk of cerebral palsy among vanishing twins established in the first or second trimester is not 40-fold but, for example, four times increased, that would increase the incidence rate of cerebral palsy by $\sim$45% in IVF singletons.

If vanishing twins are the only explanation of the increased risk of cerebral palsy in IVF singletons, we would not expect an increased risk of cerebral palsy in IVF twins as compared with age-matched non-IVF twins. In the study by Strömberg *et al.* (2002) and in a recent Danish study (Pinborg *et al.*, 2004), no increased risk of cerebral palsy was demonstrated in IVF twins as compared with non-IVF twins, supporting that vanishing twins could be a contributing explanation of the increased risk of cerebral palsy found in singletons after IVF treatment.

The doubled frequency of sleeping disturbances could be influenced by the older parents in the IVF children. Older parents probably have a lower threshold for referring their child to investigation in case of sleeping disturbances. We have not found similar results in other published follow-up data.

Regular follow-up of this National Danish IVF cohort will be carried out in the Danish registers during the coming years.

In conclusion, we found fewer specific imprinting diagnoses in both IVF and non-IVF children than expected, and with this limitation, no indication was found of an increased risk of imprinting diseases in children born after IVF as compared with children born without prior IVF. We observed equal frequencies of childhood cancers, mental diseases, congenital syndromes and developmental disturbances in the two cohorts, but an 80% increased risk of cerebral palsy in singletons born after IVF.

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