

A multi-centre cohort study of the physical health of 5-year-old children conceived after intracytoplasmic sperm injection, *in vitro* fertilization and natural conception

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BACKGROUND: Over a million children have been born from assisted conception worldwide. Newer techniques being introduced appear less and less ‘natural’, such as intracytoplasmic sperm injection (ICSI), but there is little information on these children beyond the neonatal period. **METHODS:** 540 ICSI conceived 5-year-old children from five European countries were comprehensively assessed, along with 538 matched naturally conceived children and 437 children conceived with standard IVF. **RESULTS:** Of the 540 ICSI children examined, 63 (4.2%) had experienced a major congenital malformation. Compared with naturally conceived children, the odds of a major malformation were 2.77 (95% CI 1.41–5.46) for ICSI children and 1.80 (95% CI 0.85–3.81) for IVF children; these estimates were little affected by adjustment for socio-demographic factors. The higher rate observed in the ICSI group was due partially to an excess of malformations in the (boys’) urogenital system. In addition, ICSI and IVF children were more likely than naturally conceived children to have had a significant childhood illness, to have had a surgical operation, to require medical therapy and to be admitted to hospital. A detailed physical examination revealed no further substantial differences between the groups, however. **CONCLUSIONS:** Singleton ICSI and IVF 5-year-olds are more likely to need health care resources than naturally conceived children. Assessment of singleton ICSI and IVF children at 5 years of age was generally reassuring, however, we found that ICSI children presented with more major congenital malformations and both ICSI and IVF children were more likely to need health care resources than naturally conceived children. Ongoing monitoring of these children is therefore required.

Key words: birth defects/ICSI/IVF

Introduction

Intracytoplasmic sperm injection (ICSI) was developed over 10 years ago and is now used worldwide for treating male factor infertility. However, concerns remain about the long-

term prospects for the children conceived with this technique and indeed of children conceived from ‘standard’ IVF (Hawkins *et al.*, 1999; Givens, 2000; Tournaye, 2003). Initial studies have investigated perinatal outcome, congenital anomalies and early neurodevelopmental ability (up to age 5 years) but there has been little to comprehensively assess these childrens’ physical well-being at later stages (Van Steirteghem *et al.*, 2002; Kurinczuk, 2003b).

We aimed to perform a detailed assessment of 5-year-old children and their families in a collaborative study, the International Collaborative Study of ICSI–Child and Family Outcomes (ICSI–CFO.) One of the primary objectives of the study was to assess whether ICSI is associated with

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significant health problems at age 5 years. Our initial report from ICSI–CFO (Barnes *et al.*, 2004) described our findings regarding family functioning and children's socio-emotional development. Here we report findings surrounding growth, morbidity and physical defects in the 540 5-year-old ICSI conceived children compared with similar numbers of naturally conceived children and an IVF conceived group.

Materials and methods

Study design

This was a European five-nation controlled cohort study. This involved a cross-sectional evaluation of three groups of children who were recruited at age 5 years according to their mode of conception. We recruited equivalent sized groups of ICSI, IVF and naturally conceived children (NC) according to centre size and number of ICSI cycles performed per year with targets of: 175 children per group in the UK/Belgium (UK/Be); 66 children per group in Denmark/Sweden (Dk/Sw); and 50 children per group in Greece (Gr). The study was conducted over a 24-month period, commencing November 2000.

ICSI

In the UK and Belgium, ICSI conceived children were recruited mainly from established cohorts already assessed in their second year (Sutcliffe *et al.*, 2001; Bonduelle *et al.*, 2002b). Additional children (~10%) were recruited from major fertility clinics. In Sweden, ICSI children were recruited from complete cohorts of ICSI children, conceived after treatment at the two fertility clinics in Gothenburg. In Denmark, most ICSI children were recruited from one clinic but additional children were recruited from three other clinics. In Greece the children were recruited from several clinics.

IVF comparison group

Due to differing national laws and quality of child health records, recruitment of comparison groups differed. Children born after IVF were recruited from participating fertility clinics in all countries via letters, which were sent to the families whose child fulfilled the matching criteria (listed below) for the study. In the UK, Belgium and Greece this involved five clinics, in Denmark one and in Sweden two clinics.

Naturally conceived controls

Local school and nurseries were used to recruit a matched comparison group of naturally conceived children for the UK, Belgium and Greece. In Sweden, NC children were recruited from the medical birth registry selecting the children of the same age, matching for maternal age and sex and for the closest matching age. Five NC children were recruited for each ICSI child. Only children born in Western Sweden were chosen. In Denmark, a similar recruitment was performed from the participating hospital's birth registry.

Criteria for inclusion and exclusion

We recruited ICSI and IVF conceived children between 4.5 and 5.5 years. Children were eligible if they were singleton, Caucasian, born at least 32 weeks gestation, first or second born and whose mother tongue was either English, Dutch, Danish, Swedish or Greek. Naturally conceived controls were selected according to the above criteria and were matched for age, sex, maternal education and parental socio-economic status.

Participation rates

In the UK, 91% of the ICSI cohort assessed at age 18 months participated (Sutcliffe *et al.*, 2001), leading to the examination of 189/201 of the original ICSI cohort. Due to ethical restrictions it was not possible to ascertain the response rate in the IVF or NC groups. In Belgium, 45% of eligible children participated for ICSI and IVF groups and in the NC group 54% of those contacted responded. In Sweden, 96% of ICSI, 96% of IVF and 78% of NC children contacted participated. In Denmark, 68% of ICSI, 56% of IVF and 34% of NC contacted participated. In Greece, 25% of ICSI, 25% of IVF and 100% of NC children contacted participated. In all countries the ICSI conceived children were consecutive births.

Study protocol

The children were assessed in a child friendly environment by trained paediatricians using an identical protocol. In the UK and Belgium two consecutive paediatricians (in each country) saw all children and in Denmark, Sweden and Greece children in each country were assessed by single paediatricians. The paediatrician was blinded for conception mode in Sweden, but not in the other countries, due to logistical settings. In all the centres, type of assisted reproductive technology (ICSI or IVF) was not revealed to the paediatrician.

A full history was taken from the parent(s) using a standard proforma. Socioeconomic status was classified according to the British system (Classification of Occupations. London: HM Stationery Office, 1970 and revised 1995) and equivalencies between the five participant countries were established for parental educational levels. The mother's health in pregnancy was recorded, including chronic maternal illnesses, maternal smoking/drinking and pregnancy complications.

Neonatal history included birthweight, gestational age, neonatal unit admission and treatments, and infant feeding details. Additional information was obtained from routine child health records for validation.

Significant childhood illnesses were recorded, including operations and periods in hospital. Illnesses described by the parent and considered significant by the paediatrician but not involving hospital admission/operation were also noted. Later these were coded by ICD10 (World Health Organization, 1992) and checked by the senior paediatrician (AGS, blind to mode of conception). Illnesses considered minor were not recorded. A general physical examination was conducted with special attention to the detection of malformations. Height and weight were measured using standard auxiological equipment. Pure tone audiometry (across standard screening frequencies down to 25dB), distance visual acuity (using Snellen charts) and stereotactic vision were assessed (Cooper *et al.*, 1979).

Congenital anomalies were classified according to the International Classification of Diseases 10th revision using Q codes (Q00–Q99). (World Health Organization, 1992). Malformations having a Q code were classified into major and minor by the geneticist (M.B., blind to mode of conception). Major malformations were defined as malformations that generally cause functional impairment or require surgical correction. (Smith, 1975). The remaining malformations were defined as minor. Minor malformations not having a Q code were also recorded according to a checklist containing 242 items (Bonduelle *et al.*, 2002a; Aase, 2004) used in previous studies.

Statistical analysis

The study was designed to have 80% power (at a 5% significance level) to detect a 2-fold difference in congenital anomaly rates at

Table I. Parental socio-demographic characteristics and maternal health during pregnancy*

	ICSI (<i>n</i> = 540)	IVF (<i>n</i> = 437)	NC (<i>n</i> = 538)	<i>P</i> -value
Maternal characteristics				
Age, years	33 ± 4	34 ± 4	31 ± 5	<0.001
Married, <i>n</i> (%)	491 (91)	370 (86)	357 (75)	<0.001
Current smoker, <i>n</i> (%)	97 (18)	96 (22)	85 (24)	0.079
Current drinker, <i>n</i> (%)	289 (55)	254 (60)	206 (53)	0.106
Manual social class <i>n</i> (%)	121 (23)	100 (23)	87 (18)	0.147
Education (university entry exam or higher), <i>n</i> (%)	278 (52)	219 (51)	271 (58)	0.055
Paternal characteristics				
Age, years	35 ± 5	35 ± 5	32 ± 5	<0.001
Current smoker, <i>n</i> (%)	132 (25)	96 (22)	107 (31)	0.018
Current drinker, <i>n</i> (%)	381 (71)	306 (71)	254 (74)	0.584
Manual social class, <i>n</i> (%)	157 (29)	127 (30)	123 (26)	0.417
Education (university entry exam or higher), <i>n</i> (%)	267 (49)	196 (45)	251 (47)	0.346
Maternal health during pregnancy				
Smoking while pregnant, <i>n</i> (%)	47 (9)	49 (11)	58 (13)	0.142
Drinking while pregnant, <i>n</i> (%)	38 (7)	42 (10)	54 (11)	0.129
Maternal illnesses				
Any chronic illness, <i>n</i> (%) ^a	42 (8)	52 (12)	36 (7)	0.011
Pregnancy complications, <i>n</i> (%) ^b	77 (14)	67 (15)	28 (5)	<0.001
Labor onset				
Induced, <i>n</i> (%)	139 (26)	108 (25)	110 (25)	0.055 ^c
Planned caesarean section, <i>n</i> (%)	75 (14)	49 (11)	38 (8)	
Mode of delivery				
Vaginal (including forceps/ventouse), <i>n</i> (%)	377 (71)	316 (73)	368 (79)	0.006 ^c
Caesarean section, <i>n</i> (%)	155 (29)	119 (27)	95 (21)	

*± values are means ± SD. P-values are for the comparisons between the three subgroups;

^aasthma, diabetes mellitus, endocrine, hypertension, renal disease, inflammatory bowel disease, epilepsy or other neurological disease;

^bplacental abruption, placenta previa, gestational diabetes, gestational hypertension/ preeclampsia;

^cglobal test for difference across different types of labour onset (resp. mode of delivery).

NC, naturally conceived.

age 5 years. Differences in continuous measurements between the three study groups were assessed by analysis of variance; differences in categorical outcomes were assessed by Pearson's chi square test or, if cell counts were low (<5), by Fisher's exact test. If significant differences were observed between the three study groups ($P < 0.05$), *post hoc* tests were employed to compare ICSI and IVF children separately to the NC group. Logistic regression (with adjustment for child's age and country) was used for dichotomous outcomes to compare ICSI and IVF children separately to the NC group, and to assess whether any observed differences could be explained by parental socio-demographic factors or oligozoospermia (<20 million/ml). All statistical tests were two-sided.

Role of the funding source

The protocol was approved by the ethics committee of each institution in accordance with national regulations in each country. The European Union 5th framework quality of life programme contract QL4-CT-2000-00545 paid for this project, entitled 'An International Collaborative Study of ICSI: Child and Family Outcomes (ICSI-CFO)'. The funding source had no responsibility for study design or interpretation of data.

Results

Recruitment and matching

In total, 1515 children were recruited into the study between November 2000 and November 2002. Recruitment targets were achieved with the exception of Greece (the smallest participant); matching was close and participating children's

parents were similar across a range of sociodemographic factors (Table I).

Antenatal/perinatal characteristics

Table I shows the antenatal characteristics of the study population. Both maternal and paternal age was higher in the ICSI and IVF groups compared with the NC group ($P < 0.001$). A history of chronic illness in mothers was more likely in those conceived with IVF (12%) than with ICSI (8%) or naturally conceived (7%). ICSI and IVF mothers were more likely to experience pregnancy complications. There was a higher rate of caesarean section delivery amongst ICSI and IVF children (29% ICSI, 27% IVF, 21% NC) mainly due to planned caesarean section.

The perinatal characteristics of the study population are shown in Table II. ICSI and IVF children were more likely to be born slightly less mature; mean gestational age was 39.2 weeks for ICSI children, 39.3 weeks for IVF and 39.7 weeks for NC children. ICSI and IVF conceived babies were more likely to be admitted to a neonatal unit and were more likely to have been admitted for longer than 7 days.

Illness and morbidity up to age 5 years (Table III)

A number of differences between the three study groups were observed; 74% of ICSI children and 77% of IVF children experienced significant childhood illness compared with only 57% of NC children ($P < 0.001$). Hospital admissions

Table II. Perinatal characteristics

Characteristic	ICSI (n = 540)	IVF (n = 437)	NC (n = 538)	P-value
Male, n (%)	293 (54)	230 (53)	280 (52)	0.754
Gestational age, weeks	39.2 ± 1.7	39.3 ± 1.9	39.7 ± 1.6	<0.001
Birth weight, kgs	3.3 ± 0.6	3.3 ± 0.5	3.4 ± 0.5	0.081
Resuscitation required, n (%)	23 (4)	18 (4)	23 (4)	0.992
Neonatal admission required, n (%)	77 (14)	69 (16)	52 (10)	0.011
Neonatal admission (>7 days) required, n (%)	39 (7)	29 (7)	18 (3)	0.013
Ventilation required, n (%)	8 (2)	5 (1)	6 (1)	0.839
Exclusively breastfed, n (%)	178 (38)	117 (32)	115 (41)	0.040

*± values are means ± SD. P-values are for the comparisons among the three subgroups.

Table III. Medical history at age 4.5 to 5.5 years*

	ICSI (n = 540)	IVF (n = 437)	NC (n = 538)	P-value
Age, years	5.0 ± 0.3	5.1 ± 0.3	5.1 ± 0.3	0.086
Height, cms	111 ± 6	111 ± 5	111 ± 5	0.857
Weight, kgs	19.5 ± 3.2	19.3 ± 2.8	19.7 ± 3.0	0.149
Head circumference, cms	51.6 ± 1.6	51.8 ± 1.4	51.5 ± 1.4	0.057
Medications, n (%)				
Asthmatic medication	53 (10)	44 (10)	34 (10)	0.959
Any medication	71 (13)	52 (12)	41 (11)	0.727
Admitted to hospital, n (%)	168 (31)	124 (28)	105 (20)	<0.001
Any therapy, n (%)	62 (11)	41 (9)	26 (5)	<0.001
Physiotherapy	11 (2)	6 (1)	2 (0)	0.032
Speech/language	37 (7)	30 (7)	19 (4)	0.028
Psychological	4 (1)	2 (0)	1 (0)	0.406
Any illness, n (%)	398 (74)	337 (77)	307 (57)	<0.001
Upper respiratory tract	167 (31)	122 (28)	122 (23)	0.009
Lower respiratory tract	108 (20)	92 (21)	71 (13)	0.002
Dermatological	69 (13)	72 (16)	59 (11)	0.038
Gastrointestinal	61 (11)	49 (11)	39 (7)	0.043
Other infections	174 (32)	146 (33)	154 (29)	0.234
Any surgery, n (%)	128 (24)	95 (22)	73 (14)	<0.001
Circumcision	15 (3)	13 (3)	8 (1)	0.236
Other GU surgery	27 (5)	15 (3)	8 (1)	0.005

*± values are means ± SD. P-values are for the comparisons among the three subgroups.

NC, naturally conceived; GU, genitourinary.

were increased for ICSI and IVF children and these children were more likely to have had medical therapies (e.g. physiotherapy, speech therapy). Furthermore, a higher proportion of ICSI and IVF children required surgery (24% ICSI, 22% IVF, 14% NC; $P < 0.001$), particularly genitourinary surgery other than circumcision (5% ICSI, 3% IVF, 1% NC; $P = 0.005$).

Physical examination at age 5 years

A thorough physical examination showed all children to have few problems. Audiological and vision measurements showed no differences between the three groups (data not shown). Height, weight and head circumference at 5 years of age were similar (Table III). After adjustment for maternal height, birth weight, gestational age and age of child at examination, the odds ratio for being above the 97th centile for height at 5 years (compared with NC children) was 0.98 (95% CI 0.44–2.21; $P = 0.97$) for ICSI children and 0.34 (95% CI 0.11–1.00; $P = 0.05$) for IVF children.

Table IV shows the prevalence of detected malformations. Compared with NC children, the age and country adjusted

odds of major malformation was 2.77 (95% CI 1.41–5.46) for ICSI children and 1.80 (95% CI 0.85–3.81) for IVF children. After adjustment for socio-demographic differences (maternal age, educational level, social class, maternal smoking and drinking, number of previous pregnancies), these odds ratios were attenuated only slightly (OR = 2.54, 95% CI 1.13–5.71 for ICSI children and OR = 1.66, 95% CI 0.70–3.95 for IVF children).

In the neonatal history, ICSI, IVF and NC children had comparable rates of previously observed major malformations. However, when re-examined in our study more major malformations became apparent either from the ICSI children's history of illness experience during their first 5 years (e.g. a history of corrective surgery or positive medical investigations) or as a result of our physical check. When considering organ specific major malformations it was noted that the increase in ICSI was partially due to increased defects in the urogenital system (3.7% ICSI, 2.1% IVF, 0.6% NC; $P < 0.001$). Furthermore, the increase in major malformations was mainly due to a higher malformation rate in ICSI boys (8.2%) compared to ICSI girls (3.6%), compared

Table IV. Prevalence of congenital malformations, *n* (%) counted per child for the items (a) to (g)

Type	ICSI (<i>n</i> = 540)	IVF (<i>n</i> = 437)	NC (<i>n</i> = 538)	<i>P</i> -value
(a) Neonatal major malformations ^a	18 (3)	9 (2)	10 (2)	0.242
(b) Neonatal minor malformations	83 (15)	66 (15)	43 (8)	<0.001
(c) Major malformations detected after neonatal period (up to 5 years) ^a	16 (3)	10 (2)	2 (0)	0.002
(d) Minor malformations detected after neonatal period (up to 5 years)	86 (16)	80 (18)	72 (13)	0.108
(e) Total major malformation (a or c) ^{a,b}	33 (6)	18 (4)	12 (2)	0.006
(f) Total minor malformation (b or d)	154 (29)	137 (31)	109 (20)	<0.001
(g) Overall malformations (e or f) ^b	178 (33)	146 (33)	117 (22)	<0.001
Major malformations by organ type ^{a,d}				
Cardiac	4 (1)	1 (0)	2 (0)	0.637
Eyes, ears, face	2 (0)	2 (0)	1 (0)	0.861
Uro-genital ^c	20 (4)	9 (2)	3 (1)	<0.001
Gastrointestinal	4 (1)	2 (0)	2 (0)	0.751
Musculoskeletal	5 (1)	2 (0)	1 (0)	0.237
Skin	0 (0)	3 (1)	4 (1)	0.098

^aAll cases were allocated Q codes from ICD-10.

^bThese figures do not necessarily equal the sum of their components as some children will have had both types of malformation.

^cUro-genital anomalies were: congenital pelviureteric junction obstruction (two cases) bilateral congenital vesico-uretero-renal reflux (two cases), unilateral duplication of ureter (one), urethral stenosis (one), cervical cyst (one), hydrocele (one), hypospadias (three cases) labial cyst (one), other specified congenital malformations of male genital organs (one), undescended testicle, unilateral (one).

^dMalformations are counted per organ type and not per child.
NC, naturally conceived.

to their respective NC groups. Oligozoospermia (<20 million/ml) did not influence the presence of major or minor malformations.

Conversely, more minor malformations had already been detected in the neonatal period for ICSI and IVF children compared with the NC children (15% in both ICSI and IVF children compared with 8% in NC children; $P < 0.001$). Similarly, by age 5 years minor malformations were still more frequent in ICSI and IVF children (29% ICSI, 31% IVF, 20% NC; $P < 0.001$).

Discussion

Our detailed study of 1515 children, which is the biggest cohort of ICSI and IVF children studied at the age of 5 years, showed that rates of major malformations were higher in ICSI compared to the NC group and were little affected by adjustment for socio-demographic differences. IVF children had a similar pattern of increased congenital anomalies, but this did not reach statistical significance. Bearing in mind that only children born at ≥ 32 weeks of gestation were included in the study, the ICSI/IVF children were nevertheless still a little less mature (Table II), a finding that is consistent with previous studies (Schieve *et al.*, 2002). Although these children were born at ≥ 32 weeks of gestation they still experienced greater neonatal morbidity. We did not include infants born less mature, to avoid possible confounding effects from extreme prematurity. We also excluded twins and higher order births for the same reason.

As most of the ICSI children were first born and rarely second born (as a consequence of the recent introduction of the ICSI technique) we limited the selection of the IVF and NC children to first and second born, trying to avoid a bias

in the interpretation of the results of delivery and birth parameters and in the study on psycho-emotional development and family functioning, which was done in parallel with this study on the same group of children.

Contrary to a previous report (Leslie *et al.*, 2003) we found that ICSI and IVF children appeared to experience higher illness morbidity compared with NC children in the first 5 years (Table III). It might be suggested that a higher recorded history of medical illnesses reflected excessive parental concern, but this would not explain why these children have had significantly more surgical interventions, especially genitourinary surgery, because of congenital malformations or higher rates of hospitalization overall (Table III). Parental concern might be expected to manifest as higher use of medication, which there was not.

On detailed physical examination, which included non-routine items such as vision and hearing assessments, children born after ICSI and IVF were reassuringly similar to the NC group (with the exception of congenital malformations; see Table IV). We did not detect any significant neurological findings, despite previous reports suggesting higher rates of cerebral palsy (Stromberg *et al.*, 2002) and fine motor defects (Sutcliffe *et al.*, 2001). We might, however, have missed cerebral palsy in children born > 32 weeks since the power of this study to detect such rare events was limited. One strength of our study was that each child was examined; we did not rely on proxy measures or birth registry data. We did not detect an excess of tall children after ICSI conception. This is of interest in view of recent reports (Cox *et al.*, 2002; DeBaun *et al.*, 2003; Maher *et al.*, 2003) suggesting an increase in Beckwith Wiedemann syndrome (BWS) after IVF/ICSI. It is recognised that BWS is a spectrum and large size is one of those features. Our measurements are the first

documented data on the long-term growth of IVF/ICSI children.

Our study finding of increased risk of congenital malformations is in agreement with previous retrospective studies of Hansen *et al.* (2002b) and Wennerholm *et al.* (2000b). One prospective study on ICSI children ($n = 3372$) (including a pre- and postnatal period up to 8 weeks) compared to a selected NC group out of the general population ($n = 8016$) resulted in an initial relative risk (RR) of 1.44 (95% CI: 1.25–1.65) (Katalinic *et al.*, 2004). After adjustment for parental malformations, previous malformed child and maternal age the RR declined to 1.24 (95% CI: 1.02–1.50) suggesting that parental background played a role in the apparent increase of congenital malformations in ICSI. We examined our study children at the age of 5 years and did not have access to genetic data such as family history of malformations or number of miscarriages, which might influence the relative risk of malformations to ICSI children.

Major malformations were found to be present more often in the ICSI group, in particular in ICSI boys (data not shown), beyond the neonatal period and were due to an excess in uro-genital malformations. Some authors have already shown an excess risk of uro-genital malformations in ICSI compared to the general population (Wennerholm *et al.*, 2000a; Ericson and Kallen, 2001). Our data suggest a slight increase in major malformations in IVF children which is consistent with the literature, however, we had a smaller sample size than some previous reports (Westergaard *et al.*, 1999; Anthony *et al.*, 2002; Hansen *et al.*, 2002a). It is recognised that at least 30% of congenital anomalies are missed at birth and so the higher rates of anomalies at 5 years are unsurprising. The higher rates of genitourinary defects in the ICSI conceived boys (see Table IV) with a corresponding increased rate of genitourinary surgery are more likely to reflect paternal genetic factors than the ICSI procedure itself.

One limitation of our study is the potential for survivor bias to have been introduced by the recruitment of children who had already survived to the age of 5 years, as children with severe malformations and/or illnesses would have had a greater risk of infant death than children without such malformations. However, it is likely that any biases would have resulted in us underestimating, rather than overestimating, the true relative risks of severe congenital malformations associated with ICSI and IVF.

On the other hand, selection of the NC group from local schools and nurseries may have led to underestimation of the risk of severe malformations and/or illnesses in this group (since naturally conceived children with severe malformations may be less likely to attend normal school facilities). Our NC group could therefore have been healthier than average, leading to a false interpretation of the increase of congenital malformation associated with the ICSI/IVF groups. However, the observation that none of the children in the ICSI or IVF groups attended special schools or other institutions indicates that this is unlikely to have provided a major source of bias.

The main limitation of this study is the rather high rate of non-participation ('not reached and refusals') for ICSI

children (in two of the centres) and for IVF children (in three of the centres). Non-participation was especially high in Greece, where cultural influences led to a higher level of secrecy around the fertility treatment (and hence a lower participation rate among ICSI and IVF children) than was observed in other countries. Our non-participation rates were generally in accordance with other long-term follow-up studies, however (Bowen *et al.*, 1998). Despite the inter country differences in participation rate, results were nevertheless comparable between countries, with no country bias. For example, Sweden had virtually complete data and comparison of these data with other countries revealed no differences. Furthermore, our data showed internal consistency; IVF mothers, for example, experienced greater morbidity from chronic illnesses than ICSI mothers, which would be expected in view of the greater use of ICSI for male factor problems. In addition, after matching of cases, comparison and NC children, the three groups were similar for a broad range of sociodemographic factors (Table I). These observations therefore suggest that our findings are unlikely to have been greatly influenced by participation bias.

As discussed, cross-sectional recruitment of a proportion of a cohort has limitations when assessing the incidence of congenital anomalies, but is probably the most appropriate approach for assessing other outcomes, such as general health and development. As multiple outcomes were the focus of this study, it was felt that such compromises in study design were therefore necessary.

Overall, our study provides reassuring information about the physical health of children conceived after ICSI and standard IVF. On the other hand, it also shows the need for attentive health care of the growing-up ART children since more birth defects in ICSI and more hospital admissions, surgery and medical therapies were recorded in ICSI and IVF singletons compared to a NC comparative group. It also highlights the need for continuing monitoring of children conceived after assisted reproductive therapies, particularly regarding longer term issues such as a possible higher risk of imprinting disorders, cancers and reduced future fertility (Kurinczuk, 2003a). However the main risk (beyond the scope of this study) to couples having assisted reproductive therapies remains the morbidity and mortality associated with prematurity and multiple births.

Conflict of interest statement

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References

- Aase JM (2004) *Diagnostic Dysmorphology*. Textbook Plenum Medical Book Company, New York & London.
- Anthony S, Buitendijk SE, Dorrepaal CA et al. (2002) Congenital malformations in 4224 children conceived after IVF. *Hum Reprod* 17, 2089–2095.
- Barnes J, Sutcliffe AG, Kristoffersen I et al. (2004) The influence of assisted reproduction on family functioning and children's socio-emotional development: results from a European study. *Hum Reprod* 19, 1480–1487.
- Bonduelle M, Liebaers I, Deketelaere V et al. (2002a) Neonatal data on a cohort of 2889 infants born after ICSI (1991–1999) and of 2995 infants born after IVF (1983–1999). *Hum Reprod* 17, 671–694.
- Bowen JR, Gibson FL, Leslie GI et al. (1998) Medical and developmental outcome at 1 year for children conceived by intracytoplasmic sperm injection. *Lancet* 351, 1529–1534.
- Cooper J, Feldman J and Medlin D (1979) Comparing stereoscopic performance of children using the Titmus, TNO and Randot stereo tests. *J Am Optom Assoc* 50, 821–825.
- Cox GF, Burger J, Lip V et al. (2002) Intracytoplasmic sperm injection may increase the risk of imprinting defects. *Am J Hum Genet* 71, 162–164.
- DeBaun MR, Niemitz EL and Feinberg AP (2003) Association of in vitro fertilization with Beckwith–Wiedemann syndrome and epigenetic alterations of LIT1 and H19. *Am J Hum Genet* 72, 156–160.
- Ericson A and Kallen B (2001) Congenital malformations in infants born after IVF: a population-based study. *Hum Reprod* 16, 504–509.
- Givens CR (2000) Intracytoplasmic sperm injection: what are the risks? *Obstet Gynecol Surv* 55, 58–62.
- Hansen M, Kurinczuk JJ, Bower C et al. (2002a) The risk of major birth defects after intracytoplasmic sperm injection and in vitro fertilization. *N Engl J Med* 346, 725–730.
- Hawkins MM, Barratt CL, Sutcliffe AG et al. (1999) Male infertility and increased risk of diseases in future generations. *Lancet* 354, 1906–1907.
- Katalinic A, Rosch C and Ludwig M (2004) Pregnancy course and outcome after intracytoplasmic sperm injection (ICSI)—a controlled, prospective cohort study. *Fertil Steril*. In press.
- Kurinczuk JJ (2003a) Safety issues in assisted reproduction technology. From theory to reality—just what are the data telling us about ICSI offspring health and future fertility and should we be concerned? *Hum Reprod* 18, 925–931.
- Leslie GI, Gibson FL, McMahon C et al. (2003) Children conceived using ICSI do not have an increased risk of delayed mental development at 5 years of age. *Hum Reprod* 18, 2067–2072.
- Mahe ER, Afnan M and Barratt CL (2003) Epigenetic risks related to assisted reproductive technologies: epigenetics, imprinting, ART and icebergs? *Hum Reprod* 18, 2508–2511.
- Schieve LA, Meikle SF, Ferre C et al. (2002) Low and very low birth weight in infants conceived with use of assisted reproductive technology. *N Engl J Med* 346, 731–737.
- Smith WD (1975) Classification, nomenclature and naming of morphologic defects. *J Pediatr* 87, 162–163.
- Stromberg B, Dahlquist G, Ericson A et al. (2002) Neurological sequelae in children born after in-vitro fertilisation: a population-based study. *Lancet* 359, 461–465.
- Sutcliffe AG, Taylor B, Saunders K et al. (2001) Outcome in the second year of life after in-vitro fertilisation by intracytoplasmic sperm injection: a UK case-control study. *Lancet* 357, 2080–2084.
- Tourmaye H (2003) ICSI: a technique too far? *Int J Androl* 26, 63–69.
- Van Steirteghem A, Bonduelle M, Liebaers I et al. (2002) Children born after assisted reproductive technology. *Am J Perinatol* 19, 59–65.
- Wennerholm UB, Bergh C, Hamberger L et al. (2000a) Incidence of congenital malformations in children born after ICSI. *Hum Reprod* 15, 944–948.
- Westergaard HB, Johansen AM, Erb K et al. (1999) Danish National In-Vitro Fertilization Registry 1994 and 1995: a controlled study of births, malformations and cytogenetic findings. *Hum Reprod* 14, 1896–1902.
- World Health Organization (1992) *Manual of the International Statistical Classification of Diseases, Injuries and Causes of Death (ICD)*, Geneva.

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