Article

Medical follow-up study of 5-year-old ICSI children



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Abstract

Children born after intracytoplasmic sperm injection (ICSI) are still a matter of concern. The purposes of the present study were to investigate the physical outcome in 5-year-old children born after ICSI and compare them with children born after spontaneous conception. Three hundred singleton children from Belgium, Sweden and the USA, born after ICSI, were matched by maternal age, child age and gender. In one centre, matching was also performed for maternal education. The main end-point was growth. Secondary end-points were general health, e.g. common diseases, chronic illnesses, surgical interventions and physical/neurological examinations. Standard deviation scores assessed growth. Growth assessed as stature at follow-up was similar in the two groups, despite a higher rate of preterm birth and low birth weight in the ICSI children. Common diseases and chronic illnesses occurred at similar rates in both groups. More ICSI children underwent surgical interventions and required other therapy e.g. physiotherapy and dietary therapy. Physical/neurological examinations revealed few abnormalities in either group. In conclusion, infertility treatment by ICSI does not adversely affect growth during childhood. The children's general health seems satisfactory.

Keywords: *abnormalities, children, follow-up, growth, ICSI*

Introduction

Intracytoplasmic sperm injection (ICSI) was introduced in 1992 as a treatment for male infertility. Since ICSI has been performed for only 10 years, long-term outcomes and risks to the offspring remain largely unknown. Some studies conclude that ICSI causes a small but statistically significant increase in congenital anomalies at birth (Wennerholm *et al*., 2000; Ludwig and Katalinic, 2002), compared with the general population, but not compared with conventional IVF (Bonduelle *et al*., 2002). A higher risk of prenatal chromosomal anomalies has been documented (Bonduelle *et al*., 2002). Only a few studies have examined ICSI children beyond the neonatal period and documented a higher risk of congenital malformations (Hansen *et al*., 2002). ICSI might also affect the epigenetics of early embryogenesis, leading to birth defects or imprinting disorders not always detectable at birth (DeBaun *et al*., 2003). There is still an ongoing debate regarding the developmental long-term outcome (Bowen *et al*., 1998; Sutcliffe *et al*., 2001; Bonduelle *et al*., 2003).

In order to study the long-term effects of ICSI, a collaborative study in three countries was performed in which physical and psychological outcome at age 5 years in ICSI children was

examined and compared with spontaneously conceived children. The medical data from the study, including details on diseases, growth, physical development and morphology, are presented in this article. Data on psychological outcome are reported elsewhere.

Materials and methods

Study participants

Singleton children born after ICSI were compared with singleton children born after spontaneous conception (SC) at age 5 (±9 months) in a multicentre controlled study. ICSI children were born using ejaculated, epididymal or testicular spermatozoa, fresh or cryopreserved spermatozoa. Male factor infertility was defined as $<0.8 \times 10^6$ motile sperm cells after preparation. Donor spermatozoa were not used.

Eligible ICSI children were consecutively recruited from the Centre for Reproductive Medicine, at the Dutch-Speaking Free University of Brussels (BRU), from the Fertility Centre Scandinavia at Carlanderska Hospital and from the Department of Reproductive Medicine at Sahlgrenska University Hospital of Göteborg (GOT), and from the Center for Reproductive Medicine and Infertility at the Weill Medical College of Cornell University in New York (CNY). In BRU, SC children were recruited from and examined at their schools. In GOT, SC children were recruited from the Swedish Medical Birth Registry and thereby representing a sample of the general population. They were examined at Sahlgrenska University Hospital. In CNY, SC children were recruited by advertisements at the Cornell Medical Center and in parent magazines in the tri-state area (New York, New Jersey and Connecticut), the area in which the ICSI families lived and where the children were examined.

Study protocol

Matching was performed for child gender, child age (±3 months per child in GOT and group matching ±9 months in BRU and CNY) and maternal age (±3 years in GOT and group matching in BRU and CNY) in the three centres. In GOT, child age and maternal age were case-control matched from the Swedish Medical Birth Registry; in BRU, maternal educational level was group-matched and only primiparous women were included. Education was divided into six levels, according to length and type. Exclusion criteria were multiple birth, birth <32 weeks of pregnancy (two children, one from BRU and one from GOT) and maternal or child language different from the national language (Dutch, Swedish and English respectively). Collection of data was accomplished through written questionnaires in all centres and through medical records in addition to the questionnaires in GOT. Questions on demographic parameters, maternal health, pregnancy, birth and childhood history up to age 5 years were asked. Chronic illness was defined as a disorder of at least 3 months duration during the last year that interfered with daily functioning and/or required treatment (medicine, physiotherapy, special diet or special aids). Indications for ICSI were retrieved from the original data in the three fertility centres.

At the age of 5 years, a paediatrician performed a medical examination, including growth parameters and neurological examination in a standardized way. The neurological examination included tone, cranial nerve status, tendon reflexes, walking, running and jumping. Malformations were classified according to the Manual of the International Statistical Classification of Diseases, Injuries and Causes of Death (ICD), based on the 10th Revision Conference (WHO, 1992). Only malformations listed in Chapter XVII on congenital malformations, deformities and chromosomal aberrations (codes Q00–Q99) were collected. A major malformation was defined as a malformation causing functional impairment and/or requiring surgical correction. Malformations with a Q code in the ICD were classified as major or minor by one blinded geneticist for all centres. Classification was based on information from the physical examination and medical records (in GOT) and/or based on the questionnaires on the medical history up to age 5 years filled in by parents at all centres. Additional information was collected in the individual centres: on hearing in BRU and on vision in GOT. Hearing was tested by performing a pure tone audiometry; a hearing loss of >30 dB was considered as abnormal. Visual acuity was tested monocularly with Lea Symbols Chart at 3 m distance (Hered *et al*., 1997). If wearing glasses, the children were tested with their glasses on. Data on prenatal or postnatal karyotypes were collected when available.

Growth was evaluated by size at birth (birth weight) and by height and weight at age 5 years. In order to adjust for gender and differences in age at birth and at follow-up, one reference was used for evaluation of birth weight standard deviation scores (BW SDS) (Marsal *et al*., 1996) and another reference for evaluation of height SDS and weight SDS at follow-up (Albertsson-Wikland *et al*., 2002). SDS is calculated as individual value minus mean value for reference population (given gender and age) divided with the SD for the reference population. Mean reference (in SDS) is therefore zero with 1 SD = 1. Since the growth curve is probably similar in different countries, the same reference was used for all three centres (adjusting for gender and age differences). Although SDS mean level may vary somewhat between countries, the SD is close to 1 and the same references have therefore been used for comparison between ICSI and controls for the combined groups.

In the GOT group, mean parental height was calculated using the following formulae: mother's height SDS = [mother's height (cm) – 166.5/6.3], father's height SDS = [father's height (cm) – 179.7/6.5] and mean parental height SDS = (mother's height SDS + father's height SDS)/2. The GOT children's height SDS was then adjusted for mean parental height SDS according to the Swedish reference system (Albertsson-Wikland *et al*., 2002).

In GOT, the parent accompanying the child was first asked to give his/her own and the other parent's height and then measured with a Stadiometer, used for all parents and children. Sometimes both parents were available. Measured values for parents were used if available; otherwise, the reported figure was used. Weight was measured (in underwear) using the same digital scale for all children.

In GOT, investigators were blinded for the mode of conception. In Brussels and New York, blinding was not

possible due to examination of the control children in schools and ICSI children at hospitals.

The main end-point at follow-up was growth, assessed as stature. Secondary end-points were general health and morphology.

Statistical methods

Descriptive statistics are given with median and range or mean and standard deviation (SD). Differences between groups within each centre were analysed with Fisher's exact test for dichotomous variables and with the Mann–Whitney *U*-test for ordered and continuous variables. When all centres were analysed together, adjustment for centre was made with the Van Elteren test (Van Elteren, 1960) for ordered and continuous variables and the Cochran Mantel–Haenszel test (Landis *et al*., 1978) for dichotomous variables. Odds ratio with 95% confidence interval was given when appropriate.

For comparison between groups within centres, means and differences between means with 95% confidence intervals were given for BW SDS, height SDS and weight SDS at the age of 5 years.

All significance tests were two-tailed and conducted at the 5% significance level.

With a sample size of 266 children in each group it is possible to detect a difference in height of 1.2 cm (assuming an SD of 4.7 cm) and a weight difference of 0.61 kg (assuming an SD of 2.5 kg) with an alpha error 0.05 and a power of 80% (Albertsson-Wikland *et al*., 2002).

Ethics

The study was approved by the ethical committees of the three university hospitals (BRU, GOT, CNY) and written informed consent was obtained from the parents.

Role of the funding source

The sponsors of the study had no role in study design, data collection, data analysis, data interpretation or writing of the report.

Results

Demographics

A total of 300 children born after ICSI (100 children from BRU, 98 children from GOT and 102 children from CNY) and 266 children born after SC (100 children from BRU, 111 children from GOT and 55 children from CNY) were examined. Dropout rates due to 'not reached' among ICSI children were 35/150 (23.3%) in BRU, 0/112 in GOT and 88/345 (25.5%) in CNY, and refusals were 15/150 (10.0%) in BRU, 14/112 (12.5%) in GOT and 155/345 (44.9%) in CNY. Among SC children, refusals were 46/146 (31.5%) in BRU, 32/143 (22.3%) in GOT and not possible to calculate due to the methodology used in CNY.

All children from BRU and GOT were of Caucasian origin; in CNY four ICSI and seven controls were non-Caucasian. Dutch, Swedish or English was the main language of all but three children, the latter from CNY. Gender distribution in the ICSI group was 146 males (48.7%), 154 females and 130 males (48.9%) and 136 females in the control group. The indication for ICSI was male-factor infertility in 84% and non-male-factor infertility, e.g. failed IVF, in 16%. Further maternal, paternal and neonatal characteristics are given in **Tables 1** and **2**. Both maternal and paternal age was significantly higher in the ICSI parents. Median educational level of mothers and fathers was similar in the ICSI and SC groups. The rate of pre-term birth as well as the rate of low birth weight was significantly higher and median birth weight was significantly lower in the ICSI group.

Paediatrics

The median (range) age of the children at follow-up was 5.2 (4.0–6.3) for the ICSI group and 5.4 (4.3–6.1) for the controls ($P < 0.0001$). About one-third of the children in both groups had common diseases, e.g. allergy and recurrent infections. Chronic illnesses occurred in 24 ICSI (8%) and 18 (6.8%) controls ($P = 0.493$) (**Table 3**). The number of children undergoing one or more surgical interventions was higher in the ICSI group, 69 (23%), compared to the control group, 44 (16.5%) ($P = 0.019$) (**Table 4**). Other therapy, e.g. physiotherapy, speech, orthoptic, dietary and psychological therapy, was required more often in ICSI children [27 (13.6%)], than in SC children [13 (6%)] ($P = 0.012$; information retrieved from BRU and GOT). Other therapy limited to speech therapy and psychological therapy was required to a similar extent in ICSI children, 19/198 (9.6%), as in SC children, 12/211 (5.7%) ($P = 0.191$).

Growth

At birth, ICSI children were found to have lower BW SDS than SC children (**Table 2**). The mean BW SDS in ICSI children was lower than the reference used. Parental height had no influence on BW SDS in the GOT group.

At age 5, the ICSI children were found to have a higher SDS for height at all centres combined and in GOT (**Tables 5**, **6** and **7**). The difference was, however, not significant after correction for parental height which could be analysed for the Swedish children. Graphical presentation of confidence intervals for all children (**Figure 1a**) as well as individual values for height SDS by mode of conception and country is given in **Figure 1b**, **c** and **d**. Weight SDS at follow-up was found to be comparable between the ICSI and the SC group in the three centres and in all centres combined. Height SDS and weight SDS were above the reference used in all groups except for the control children in the CNY group.

Physical examination (**Table 8**)

Cardiac anomaly was registered in three ICSI children (one child each with aortic stenosis, dextrocardia and tetralogy of Fallot). A situs inversus was detected in one control child.

The median systolic and diastolic blood pressure were 103 mm and 56 mm respectively in the ICSI group and 103 and 56

Table 1. Demographic and neonatal characteristics. LBW = low birth weight, VLBW = very low birth weight, NICU = neonatal intensive care unit, BW SDS = birth weight standard deviation score (Marsal *et al*., 1996).

	ICSI (n = 300)	*Range*	*Spontaneous conception (n = 266)*	*Range*	P-*value*
Mothers					
Age (median; years)	33.0	24.1–44.0	31.0	19.0–44.5	<0.001
Educational level[b] (median)	3.0	1–6	3.0	1–6	0.128
Level 1 (%)	8.4	–	9.6	–	–
Level 2 (%)	37.0	–	32.1	–	–
Level 3 (%)	22.6	–	28.1	–	–
Level 4 (%)	18.9	–	16.5	–	–
Level 5 (%)	10.8	–	11.2	–	–
Level 6 (%)	2.4	–	2.4	–	–
Smoking during pregnancy (%)	5.0	–	8.4	–	0.255
Alcohol during pregnancy (%)	4.7	–	9.9	–	0.076
Parity (% primiparity)	89.3	–	68.8	–	<0.001
Chronic maternal disease[c] (%)	10 (3.3)	–	6 (2.3)	–	0.613
Pregnancy complications[d] (%)	40 (13.3)	–	12 (4.5)	–	<0.001
Fathers					
Age (median) (years)	35.2	23.5–57.9	33.9	20.2–51.7	0.042
Educational level (median)	2.0	1–6	2.5	1–6	0.424
Neonates					
Caesarean section (%)	74 (24.7)	–	38 (14.3)	–	0.021
Gestational age (median) (weeks)	39.3	32.0–42.9	39.4	32.0–43.1	0.754
Preterm birth (<37 week) (%)	33 (11.0)	–	12 (4.5)	–	0.017
Birth weight (median) (g)	3317	960–4830	3410[a]	1560–5530	0.021
LBW (<2500 g) (%)	32 (10.7)	–	10 (3.8)	–	0.006
VLBW (<1500 g) (%)	2 (0.7)	–	0	–	0.241
Apgar score <7 at 5 min (%)	4 (1.3)	–	1 (0.4)	–	0.276
Resuscitation (%)	4 (1.3)	–	3 (1.1)	–	0.701
Ventilation (%)	7 (2.3%)	–	3 (1.1%)	–	0.279
NICU (>7 days) (%)	22 (7.3)	–	7 (2.6)	–	0.008
NICU (>28 days) (%)	5 (1.7)	–	1 (0.4)	–	0.437

[a]Five missing values.
[b]1 = high level = completed university degree (e.g. PhD, lawyer, physician); 2 = completed 2–4 years at university (e.g. BA, teacher, journalist); 3 = completed 1–2 years at university (e.g. nurse, police); 4 = completed upper secondary school education; 5 = 1–2 years at upper secondary school or vocational school; 6 = low level = completed 9 years compulsory school or less.
[c]Chronic hypertension, diabetes, inflammatory bowel disease, chronic renal, endocrine or autoimmune disease.
[d]Gestational hypertension, pre-eclampsia, gestational diabetes, placenta previa, abruptio placentae or pre-term pre-labour rupture of the membranes.

Table 2. Birth weight standard deviation score (BW SDS). CI = confidence interval of the mean.

Centre	n	*ICSI*		*Spontaneous conception*		*Mean difference*[b]		P–*value*
		Mean BW SDS	*95% CI*	*Mean BW SDS*	*95% CI*	*Mean*	*95% CI*	
All	561	–0.38	–0.51 to –0.25	–0.09[a]	–0.22 to 0.04	–0.29	–0.47 to –0.14	0.002
BRU	195	–0.53	–0.76 to –0.31	–0.28	–0.53 to –0.04	–0.25	–0.58 to 0.08	0.14
GOT	209	–0.20	–0.41 to 0.02	0.17	–0.02 to 0.36	–0.37	–0.65 to –0.09	0.011
CNY	157	–0.41	–0.63 to –0.19	–0.28	–0.53 to –0.04	–0.12	–0.47 to 0.23	0.46

[a]Five missing values.
[b]Difference between ICSI group and spontaneous conception group.

Table 3. Frequency of chronic illness at age 5 years. ADHD = attention deficit and hyperactivity disorder.

ICSI (n = *300*)		*Spontaneous conception* (n = *266*)	
Chronic illness	*No. of children*	*Chronic illness*	*No. of children*
Allergy	6	ADHD	2
Allergy food	2	Allergy	1
Asthma	9	Asthma	5
Autistic behaviour	1	Diplegia	1
Constipation	1	Eczema	5
Dyspraxia	1	Gluten intolerance	1
Eczema	3	IgA deficiency	1
Urinary tract infection	1	Neurofibromatosis	1
		Scleroderma	1

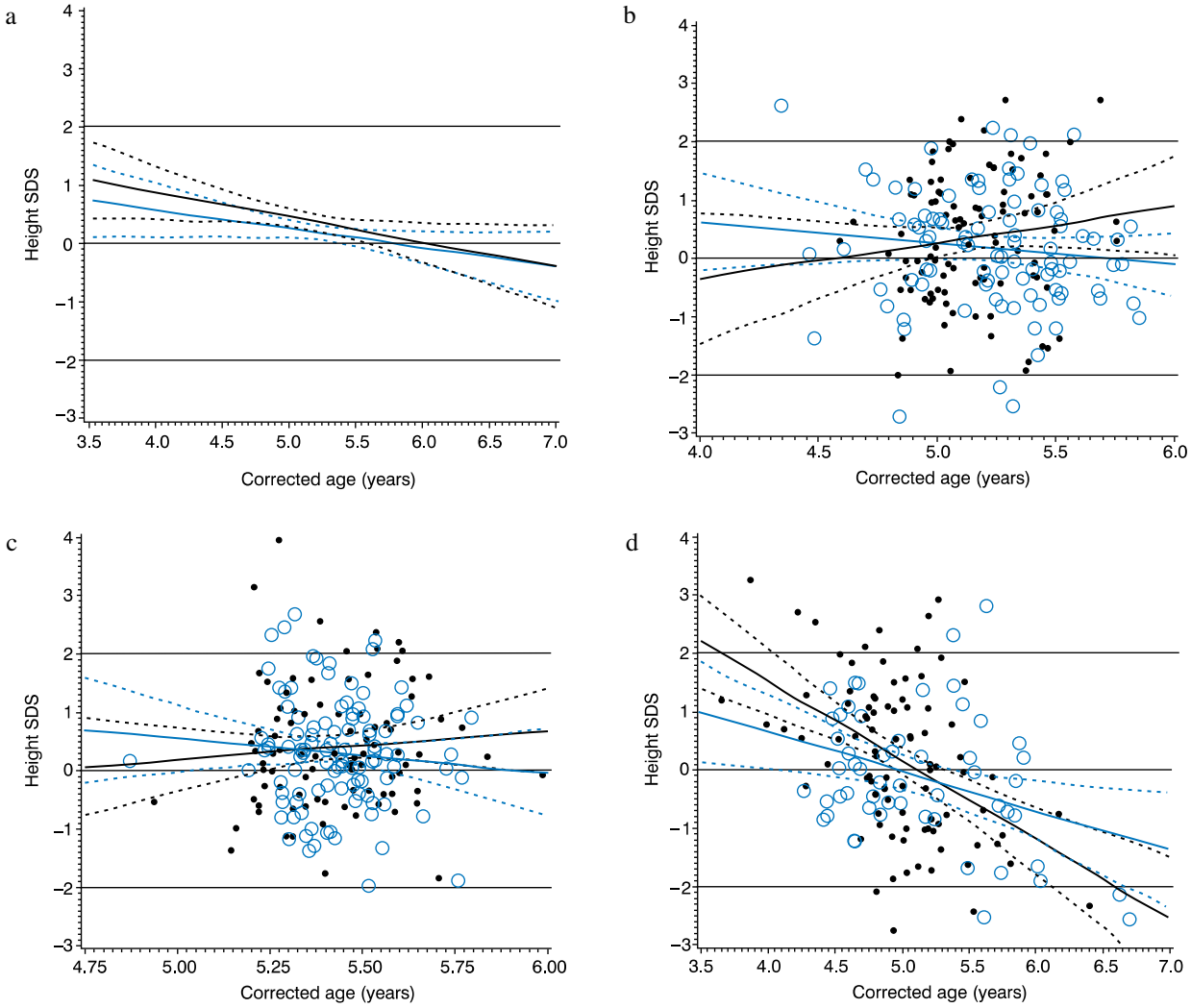


Figure 1. (**a**) Height standard deviation score (Height SDS) versus corrected age at paediatric evaluation for all children given as first order regression lines with 95% confidence intervals. Thin lines indicate cases and heavy lines (in blue colour) controls. (**b**) Height standard deviation score (Height SDS) versus corrected age at paediatric evaluation for all children, subdivided by cases (dots) and controls (open circles) born in Brussels (Belgium). Not adjusted for parental height. (**c**) Height standard deviation score (Height SDS) versus corrected age at paediatric evaluation for all children, subdivided by cases (dots) and controls (open circles) born in Göteborg (Sweden). Note that Swedish children are adjusted for parental height SDS. (**d**) Height standard deviation score (Height SDS) versus corrected age at paediatric evaluation for all children, subdivided by cases (dots) and controls (open circles) born in New York (USA). Not adjusted for parental height.

Table 4. Surgical interventions up to age 5 years[a].

ICSI (n = *300*) *Surgical intervention*	*No. of children*	*Spontaneous conception* (n = *266*) *Surgical intervention*	*No. of children*
Tympanic surgery ± adenoidectomy	39	Tympanic surgery ± adenoidectomy	28
Tonsillectomy	1	Cataract	1
Nasolacrimal duct	2	Strabismus	1
Mandibular surgery	1	Ear tag	2
Strabismus	2	Prominent ear	1
Ear tag	2	Tongue frenulum	1
Prominent ear	1	Neck anomaly	1
Polydactyly	1	Pyloric stenosis	3
Hand and feet surgery	1	Intestinal biopsy	2
Epigastric hernia surgery	1	Appendicitis	1
Inguinal hernia	3	Circumcision	6
Hypospadias	1	Inguinal hernia	2
Orchidopexy	2	Hydrocoele	2
Ovarian cyst	1	Labia minora adhesions	2
Testicular torsion	1		
Circumcision	8		
Vesico-ureteral/urethral reflux	5		
Hip luxation	1		
Cardiac surgery	2		

[a]A child can undergo more than one surgical intervention.

Table 5. Growth parameters at age 5 years.

	ICSI			*Spontaneous conception*			P-*value*
	n	*Median*	*Range*	n	*Median*	*Range*	
Height (cm)	299	112.4	97.0–128.9	261	112.0	98.0–126.0	0.129
Weight (kg)	292	19.5	12.7–51.5	260	19.7	13.7–35.4	0.762
Head circumference (cm)	300	51.5	43.2–56.5	259	51.8	45.2–56.8	0.638

Table 6. Height standard deviation scores at age 5 years. SDS = standard deviation scores, CI = confidence interval.

Centre	n	*ICSI* *Mean height SDS*	*95% CI*	*Spontaneous conception* *Mean height SDS*	*95% CI*	*Mean difference*[a] *Mean*	*95% CI*	P-*value*
All	560	0.37	0.24 to 0.51	0.16	0.04 to 0.29	0.21	0.03 to 0.40	0.023
BRU	194	0.36	0.14 to 0.58	0.18	–0.03 to 0.38	0.19	–0.11 to 0.49	0.28
GOT	209	0.57	0.34 to 0.80	0.28	0.10 to 0.47	0.29	0.00 to 0.57	0.054
GOT[b]	209	0.39	0.19 to 0.60	0.30	0.12 to 0.47	0.09	–0.17 to 0.36	0.48
CNY	157	0.19	–0.06 to 0.44	–0.12	–0.42 to 0.19	0.31	–0.09 to 0.71	0.12

[a]Difference between ICSI group and spontaneous conception group.
[b]Corrected for mean parental height (see materials and methods).

Table 7. Weight standard deviation scores at age 5 years. SDS = standard deviation scores, CI = confidence interval.

Centre	n	*ICSI*		*Spontaneous conception*		*Mean difference*[a]		P–*value*
		weight SDS	*Mean 95% CI*	*weight SDS*	*Mean 95% CI*	*Mean*		*95% CI*
All	552	0.38	0.24 to 0.52	0.33	0.19 to 0.46	0.05	–0.15 to 0.25	0.62
BRU	187	0.19	–0.04 to 0.42	0.30	0.10 to 0.49	0.11	–0.41 to 0.19	0.47
GOT	208	0.56	0.29 to 0.83	0.37	0.16 to 0.58	0.19	–0.15 to 0.51	0.28
GOT[b]	208	0.38	0.10 to 0.66	0.39	0.17 to 0.62	–0.02	–0.37 to 0.34	0.93
CNY	157	0.38	0.15 to 0.61	–0.30	–0.07 to 0.66	0.08	–0.33 to 0.49	0.72

[a]Difference between ICSI group and spontaneous conception group.
[b]Corrected for mean parental height (see materials and methods).

Table 8. General physical examination at age 5 years[a]. SC = spontaneous conception, GOT = Göteborg.

	No. of ICSI children with abnormalities	*No. of examined ICSI children*	*No. of SC children with abnormalities*	*No. of examined SC children*
Cardiac auscultation	3	300	1	266
Systolic blood pressure (median) (only GOT)	103[b]	97	103[b]	110
Diastolic blood pressure (median) (only GOT)	56[b]	97	56[b]	110
Pulmonary auscultation	0	300	0	266
Dentition	2[c]	300	4[d]	266
Mouth	0	300	0	266
Spleen	0	300	0	266
Liver	1	300	1	266
Skin apart from birthmarks	8[e]	300	15[e]	266
Skeleton	2[f]	300	1[g]	266

[a]No. significant differences found between groups.
[b]Values are mmHg, not numbers.
[c]Hypodontia, overlapping incisives.
[d]Enamel dysplasia, distichiasis, dark incisive.
[e]Eczema, vitiligo.
[f]Short thumbs, pectus excavatum.
[g]Tapering fingers.

respectively in the control group. Pulmonary auscultation, mouth and spleen examination were unremarkable in all children in both groups. Two children in the ICSI group and four children in the control group had abnormal dentition, e.g. hypodontia, overlapping incisors, and enamel dysplasia. Enlarged liver was detected in one child in each group. Skin defects, e.g. eczema, vitiligo (birth marks were excluded), were found in eight ICSI children and 15 SC children. Skeletal anomalies were detected in two ICSI children and one SC child.

At neurological examination, two controls (one child with diplegia) were found to have defects in tone and one control child were found to have cranial nerve defects and four ICSI children and one control child had abnormal reflexes. One child in each group had walking disorders, five and six children had running disorders and six and five children had jumping disorders in the ICSI and control groups respectively.

Genital examination was performed on 146 boys and 148 girls in the ICSI group and in 123 boys and 129 girls in the control group. In the ICSI boys a total of 17 boys with genital anomalies were found (hypospadias = 1, undescended testis = 3, long/large penis = 1, hydrocoele = 3, phimosis/circumcision = 9) and in the control boys a total of 11 boys with genital anomalies were found (undescended testis = 2, long/large penis = 1, hydrocoele = 2, phimosis/circumcision = 6) ($P = 0.273$). In the ICSI girls, one ovarian cyst was observed and in the control girls, two labia adhesions were registered.

Major and minor malformations

A total of 19 children (6.3%) with major malformations were identified in the ICSI group compared with eight children (3.0%) in the control group (**Table 9**). These rates were significantly different ($P = 0.031$, OR 2.53; 95% CI

Table 9. Major and minor malformations (Q codes) at age 5 years.

ICSI (n = *300)*		*Spontaneous conception* (n = *266)*	
Major malformations[a]			
Malformation	*No. of children*	*Malformation*	*No. of children*
Aortic stenosis	1	Cataract	1
Cephalosyndactyly	1	Neurofibromatosis	1
Congenital muscle anomaly	1	Non-neoplastic naevus (10 cm)	1
Cystic kidney	1	Pyloric stenosis	3
Dextrocardia	1	Situs inversus	1
Ehlers–Danlos syndrome	1	Undescended testis	1
Hip luxation	1		
Hypospadias	1		
Marcus Gunn syndrome	1		
Pelvic-ureteric obstruction	1		
Polydactyly	1		
Tetralogy of Fallot	1		
Undescended testis	2		
Urethral web	2		
Vesico–ureteral–renal reflux	4		
Minor malformations[b,c]			
47,XYY, asymptomatic	-	Café au lait spots	-
Clinodactyly fingers	-	Clinodactyly fingers	-
Hydronephrosis, transient	-	Congenital ptosis	-
Hypertelorism	-	Hypertelorism	-
Hypopigmented macula	-	Macrocephaly, asymptomatic	-
Klinefelter mosaic, asymptomatic	-	Microcephaly, asymptomatic	-
Macrocephaly, asymptomatic	-	Pectus excavatum	-
Microcephaly, asymptomatic	-	Pes planus	-
Non-neoplastic naevus	-	Preauricular tag -or sinus	-
Pectus excavatum	-	Syndactyly of toes	-
Pes planus	-	Undescended testis, unilateral, no surgery	-
Preauricular tag	-	Unstable hip	-
Prominent ears	-		
Scoliosis, mild	-		
Skin malformation	-		
Syndactyly of toes	-		
Undescended testis, unilateral, no surgery	-		
Unstable hip	-		
Vesico-ureteral reflux	-		
VSD, closed	-		

[a]No child had more than one major malformation.
[b]A child could have more than one minor malformation.
[c]Numbers not available.

1.07–5.98). When centres were analysed individually, significantly more children with major malformations were found in the ICSI group, compared with the control group in BRU (10/100 versus 2/100, *P*=0.037), while the rates in GOT (6/98 versus 6/111) and in CNY (3/102 versus 0/55) were similar. The number of children with minor malformations was 25 in each group and did not differ significantly (**Table 9**). The rate of major malformations in the male-factor infertility group, 14/252 (5.6%), was not significantly higher than in the SC group, 8/266 (3.0%) (*P* = 0.074).

Other investigations

Visual acuity was examined only at the GOT centre. Eighty-six (88.7%) children in the ICSI group and 105 (94.6%) in the SC group (*P* = 0.089) had normal vision (≥0.8 in the best eye). Hearing was investigated at the BRU centre. In the ICSI group, hearing was normal in 73/77 (94.8%) and 72/75 (96.0%) in the left and right ear respectively compared with 96/99 (97.0%) (*P* = 0.701) and 82/89 (96.7%) (*P* = 1.000) respectively in the control group.

Two abnormal karyotypes were found (47,XXY and 47,XXY/46 XY) in 196 tested children in the ICSI group and none of 48 tested children in the control group had abnormal karyotypes.

Discussion

The main findings of this study are that ICSI children grow normally compared with naturally conceived children, assessed as stature at follow-up and corrected for parental height. This is reassuring and may reflect general good health. Since the ICSI children had a lower mean birth weight SDS, this indicates catch-up growth. The finding that most groups, including controls, were taller (mean SDS for height above zero) than a recently updated Swedish reference (Albertsson-Wikland *et al*., 2002) may indicate some sort of selection. Other variables measuring general health such as common diseases and chronic illnesses were also present in similar rates in the two groups. These findings are important, as they have not previously been reported in the literature.

The strength of this report is that the study population represents children from three different countries giving more generality of the results compared to other published one centre studies (Bowen *et al*., 1998; Sutcliffe *et al*., 2001). The reason for choosing growth parameters as the main outcomes was that assessment of growth could be considered to be a robust measurement with limited influence by individual assessors in the three countries. The main weakness of this study is the rather high rate of not reached and refusals for ICSI children in two of the three centres. This is in accordance with other long-term follow-up studies (Bowen *et al*., 1998; Sutcliffe *et al*., 2001). For controls, a participation rate of around 75% could be regarded acceptable for this kind of studies. The participation rate for controls in this study was 77.7% in GOT, 68.5% in BRU and not possible to calculate for CNY. The difference in recruitment of ICSI children and controls represents other methodological weakness of this study. However, in most countries controls are not possible to recruit in other ways. Results for main outcomes have therefore been analysed both totally and per centre. Other methodological differences, e.g. country specific matching of maternal education, have probably had minor influence on the results since the total groups were comparable for these variables.

Matching was done for gender, child age and maternal age within the centres. A significant difference in maternal age was nonetheless found between the ICSI and SC group. This was due to a systematically lower age, within the matching criteria, in the SC group mothers. The slight difference in evaluation age for the two groups of children was probably of no clinical significance and major outcome, e.g. growth parameters were adjusted for age of the child.

Although very pre-term infants were excluded, this did not equalise the neonatal outcome parameters in ICSI and SC children. In ICSI children, there was still a higher rate of preterm birth and low birth weight, and a higher number of children were treated in NICU for more than 1 week. This is in accordance with previous reports on children born after assisted reproduction techniques (Bergh *et al*., 1999).

Significantly more major malformations were found in the ICSI group (6.3%) compared with the SC group (3.0%). This may be due to a recruitment bias at the BRU and CNY centres where SC children were recruited in schools and from advertisement. Parents of children with medical complications in BRU and CNY might have preferred not to expose their children to more medical testing. On the other hand, parents of ICSI children might have been more accustomed to the hospital and more willing to report possible problems. In GOT, recruitment of SC children was performed at birth from the Medical Birth Registry, thereby decreasing recruitment bias. In Sweden, the parents' willingness to participate was high, since all children at the age of 5 years are routinely examined at the Child Health Centres. The individual data from this centre should therefore be emphasized; no significant difference in malformation rate was found between ICSI and SC children in GOT.

It should be stressed, however, that due to the study design, the significant difference in malformation rates between the ICSI and control groups should be interpreted with caution. Both selection and participation bias might well have influenced particularly the composition of the control group.

The finding of 6.3% major malformations at age 5 years in ICSI children is in agreement with the rate of 8.6% found by Hansen and co-workers in 301 children, at the age of 1 year (Hansen *et al*., 2002). These authors conclude that there is a significantly higher malformation rate in ICSI children, compared with naturally conceived children, even after adjustment for maternal age, parity, gender, and sibling correlation of risk of birth defects (OR 2.0; CI 1.3–3.2). A higher crude relative risk, 1.25 (CI 1.11–1.40), was also found by Ludwig and co-workers in 3372 ICSI children (stillborns and terminations included) compared with birth registry data from the general population (Ludwig and Katalinic, 2002). Others (Wennerholm *et al*., 2000), however, found a similar number of major malformations in 1139 ICSI newborns, compared with newborns in the general population (OR 1.19; CI 0.79–1.81), based on data from the medical birth registry and stratified for delivery hospital, year of birth, maternal age and multiplicity. The present study shows that it is

difficult to rule out selection bias even if most of the observation bias was eliminated by applying the same classification system for major malformations, by the same paediatrician examining the children in a standardized manner and by classifying malformation without knowing the mode of conception. It has been argued previously that ICSI children may be subject to detection bias because of more frequent and thorough medical examinations. However, in the present study, major malformations were associated with clinical symptoms in all children.

No increase in rate of hypospadias as previously described by Wennerholm and co-workers (Wennerholm *et al*., 2000), or of any other specific malformation, was found in this study.

Major malformations in relation to male-factor infertility were not more frequent, either compared to the total ICSI group or compared with the SC group. Although the group is too small to detect a minor increase, there is no indication that the ICSI children of men with decreased sperm counts run a higher risk of malformations.

None of the recently reported imprinting disorders such as Angelman disease and Beckwith–Wiedemann syndrome (Cox *et al*., 2002; DeBaun *et al*., 2003) was found in the ICSI or SC group. To clarify the risks of these rare disorders, however, many more children must be examined.

A higher rate of additional therapy was reported in the ICSI group (13.6%) compared with the control group (6.2%). This difference was mainly due to a lower rate of additional therapy in the SC group in BRU, compared with GOT (0/100 and 13/111 respectively). In the ICSI group, the rates were comparable in BRU and GOT (15/100 and 12/98 respectively). A similar selection bias as for the malformations could explain the differences between ICSI and SC groups.

A significantly higher rate of surgical interventions was recorded in ICSI, compared with SC, mainly due to a higher rate of minor ear problems (tympanic drains and adenoidectomy). This could reflect differences in therapeutic attitude as well as a higher incidence of recurrent infections or allergic conditions. However, no higher incidence in ICSI children was reported in the information on recurrent infections.

Neonatal complications in the ICSI group were not reflected by a higher incidence of hearing and vision problems for which a limited group of children were tested. Similar incidences of hearing loss in ICSI and SC probably reflect the incidence of hearing loss due to otitis media effusion, since no children with profound hearing loss were found.

The difficulties of performing this type of follow-up study are shown by the different accessibilities of control children in different countries, leading to selection bias. On the other hand, the multicentric set-up provides opportunities to compare results between groups, although caution must be exercised regarding the conclusions.

The data indicate that even if the ICSI children in this study were in a less favourable position at birth, the general outcome measured as health and growth was comparable to a group of SC children at the age of 5 years. The malformation rate was higher in the ICSI group, probably due to a selection bias. However, a factor related to some step in the assisted reproduction procedure could not be ruled out, which emphasizes the need for further documentation and follow-up of children born after ICSI.

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In the Bertarelli study group, the other contributors were: BRU – Veerle De Ketelaere examined the children, André Van Steirteghem served as scientific adviser and Walter Meul provided technical assistance; GOT – Kerstin Strömland and Lars Hamberger contributed to conception and design of the study, Magnus Borres performed general pediatric examination in some of the children, Margareta Hök-Wikstrand performed the ophthalmological examination, Kerstin Åmark designed the paediatric questionnaire and the physical examination protocols and performed cardiac examinations, Jan Sunnegårdh performed cardiac examinations, and Matts Wikland contributed to recruitment of patients in GOT; CNY – Zev Rosenwaks served as scientific supervisor and contributed to the conception and design of the study, Fred Gilbert for examining the children, Maryanne Williams-Pitman for recruiting patients, and Queenie Neri for coordinating the study and patient recruitment.

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