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Medical outcome of 8-year-old singleton ICSI children (born ≥32 weeks' gestation) and a spontaneously conceived comparison group

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BACKGROUND: There is little information about the long-term outcome of children born after ICSI. In this study, the eldest cohort of ICSI children worldwide, reaching the age of 8 years, was investigated at the prepubertal stage to monitor subsequent puberty and future fertility. To investigate possible health problems, a thorough medical and neurological examination was performed. METHODS: Medical outcome of 8-year-old singletons (*n* = 150) born through ICSI (≥32 weeks) was compared with that of 147 singletons of the same age born after spontaneous conception (SC). Information about their general health was obtained from the parents by means of a questionnaire. RESULTS: Fifteen of 150 ICSI children experienced a major congenital malformation compared with 5/147 SC children (*P* < 0.05). Pubertal staging was similar in both groups. Neurological examination did not show important differences between ICSI and SC children. ICSI children did not require more remedial therapy or surgery or hospitalization than SC children. CONCLUSION: Physical examination including a thorough neurological examination did not reveal important differences between the two groups. Major congenital malformations were significantly more frequent in the ICSI group. However, most of them were corrected by minor surgery. Further monitoring of these children at an older age is recommended.

Key words: ICSI/outcome/congenital malformation/assisted reproduction techniques/neurological examination

Introduction

ICSI has been in clinical use since 1991 (Palermo *et al.*, 1992) and is now a relatively widespread assisted reproduction technique (ART). It is now possible for azoospermic men to become the genetic fathers of their offspring. In comparison to IVF, ICSI is a more invasive technique and bypasses the process of natural sperm selection. Concerns have been expressed about the outcome for children if poor-quality semen is used (Cummins and Jequier, 1995; Patrizio, 1995; Retzloff and Hornstein, 2003; Tournaye, 2003; Hansen *et al*., 2005).

Initial studies have investigated perinatal outcome with reassuring findings (Wennerholm *et al*., 2000; Bonduelle *et al*., 2002). One of the first studies that addressed developmental outcome at 1 year of age revealed an increased risk of mildly to significantly delayed development (Bayley mental developmental index <85) (Bowen *et al*., 1998). Further neurodevelopmental studies up to 2 years of age failed to find poorer outcome for ICSI children (Bonduelle *et al*., 1998; Sutcliffe *et al*., 2001; Bonduelle *et al*., 2003; Sutcliffe *et al*., 2003). Recently, reassuring findings about 5-year-old ART children were published concerning medical (Bonduelle *et al*., 2004; Bonduelle *et al*., 2005) and developmental outcome (Leslie *et al*., 2003; Place and Englert, 2003; Ponjaert-Kristoffersen *et al*., 2004; Ponjaert-Kristoffersen *et al*., 2005). However, slightly more congenital malformations were found, mainly because of uro-genital malformations in ICSI boys (Bonduelle *et al*., 2005), as described previously by Wennerholm *et al*. in 2000.

To study the possible long-term effects of ICSI, a prospective study was designed in which the psychological and medical outcome of 8-year-old ICSI children was evaluated and compared with the outcome of spontaneously conceived (SC) children. Children were examined at the prepubertal stage to allow a comparison with subsequent monitoring at puberty. A combination of anamnestic data with a detailed clinical and neurological examination at the age of 8 years can reveal important health problems. Moreover, it enables us to stay in contact with the ICSI children with regard to their later fertility.

In this article, medical data are reported. Data on psychological outcome and family functioning are reported elsewhere (Leunens *et al.*, in press).

Material and methods

Study design

The aim of the study was to assess general health by means of written information obtained from the parents and by performing a clinical examination.

The primary outcome measure was neurological testing. Other measures were biometrical data, puberty, congenital anomalies and general health as measured by any hospital admission and chronic disease.

The study was designed to detect trends in morbidity and adverse events in ICSI children at the age of 8 years. Approximately 500 children in each group are required to detect a doubling of rare events such as congenital malformations to reach a power of 80%. Because the included study children are part of the first generation of children born after ICSI, we were not able to reach this required number.

The study was a cross-sectional evaluation of a prospective cohort of ICSI children and a group of SC children. ICSI and SC children ranging from 8 years to 8 years 11 months were included.

Study participants

The study group consisted of 150 children born after ICSI carried out at the Vrije Universiteit Brussel Centre of Reproductive Medicine. They are part of a cohort followed since birth (Bonduelle *et al*., 2002). Of the eligible Dutch-speaking cohort (n = 248) turning 8 years of age between February 2001 and December 2003, ~61% were tested. Of the remaining 39% of the cohort, 16.5% of the families could not be reached (lost to follow-up) and 22.5% refused to participate in the study. Therefore, of all families reached in the cohort (n = 207), 73% responded positively and 27% refused participation.

The comparison group consisted of 147 children, conceived spontaneously. They were recruited from schools in the surrounding area by a letter distributed to the parents during the same study period. All SC children were recruited from 'normal' schools because all but one of the ICSI children attended normal school. The eligible SC comparison group was matched as closely as possible according to age, sex and maternal education level. Mean response rate in schools was ~37.5%. This is an estimate because owing to logistical reasons it was not always possible to measure accurate rates for each school. The reasons for refusal were mainly 'lack of interest or time'.

Inlusion/exclusion criteria

ICSI and SC children were eligible from the established birth cohort if they were singleton and born at least 32 weeks of gestation (to avoid interference with difficulties associated with multiple births and prematurity known to occur more frequently in children born after ART). Children with low birthweight or major malformations were not *per se* excluded from the study. The mother tongue was Dutch and at least one of the parents was European. These restrictions were made to overcome linguistic barriers and differences in sociocultural behaviour, because the psychological outcome of these children was examined in parallel to this study.

Study protocol

Information from both groups was gathered by means of a questionnaire to be filled out by one of the parents. Because recruitment was not based on a national registry as in various studies and participation was voluntary, we did not have access to individual medical records.

Sociodemographic parameters of the parents

Ethnic origins and marital status were enquired. Educational level was initially classified according to a 5-level status and later divided into two groups: parents who achieved levels equivalent to Bachelor or higher university degrees (higher education) and the remaining parents with lower educational levels.

Medical history of the mother and the pregnancy

Information obtained about the mother included age at birth of the child in the study, parity and health during pregnancy. Diseases during pregnancy were classified as pregnancy related or pregnancy unrelated/pre-existent. Minor infections were not recorded. Intake of medication during pregnancy was noted.

Medical history of the child

Information collected about the children consisted of perinatal data such as the child's sex, birthweight, mode of delivery, gestational age, reason and duration of admission to a neonatal care unit.

Information collected concerning childhood medical history included surgical interventions and chronic illnesses. Chronic illness was defined as a disorder of at least 3-month duration in 1 year which had interfered with daily functioning and/or required treatment. Frequency and reason, surgical or other (for instance infections) of hospital admission during childhood was also recorded, as it was recalled by the parents. Mean hospital stay was calculated. Intake of medication was noted. Chronic intake of medication was defined as when taken for more than 3 weeks. Behavioural problems or learning difficulties were recorded. If children needed remedial therapy, the type of therapy was specified (physiotherapy, speech or psychological therapy).

All malformations were classified according to the Brussels system, which has already been used in previous reports (Bonduelle *et al*., 1995, 1999, 2002). A major malformation was defined in the Brussels system as a malformation causing functional impairment and/or requiring surgical correction. The remaining malformations were classified as minor according to an extensive checklist for minor congenital anomalies on the basis of a textbook by Aase (1990). Internal guidelines to code for major or minor were also applied. Our list of malformations was, blinded to conception mode, reclassified by a Western Australian group (Bower *et al*.) according to their system in which the classification system of the British Paediatric Association, based on the International Classification of Diseases, 9th Revision (ICD-9), was used to recode each defect (Hansen *et al*., 2002). All defects were categorized as major or minor according to a method devised by the Centres for Disease Control and Prevention.

An extensive general physical examination was performed by a paediatrician, who was not blinded to conception status because of the recruitment strategy. Although all children were examined in a standardized way, we were sometimes forced to omit some parts of the examination because of restricted time of the children/parents. Medical and psychological examination took place on the same day and lasted more than 2 h for every child. Puberty was evaluated through Tanner scores and genital examination. Special attention was paid to congenital anomalies. Auxological data such as weight, height, head circumference and blood pressure were collected with standard equipment. If a child appeared to have an elevated blood pressure on one measurement, the measurement was repeated later the same day during the examination. Skin abnormalities, including single café-au-lait spots, single angiomata, dry skin or eczema, were noted.

Neurological examination was very detailed and consisted of different subtests (speech, tone, gait, balance, tendonreflexes, plantar reflexes, strength against resistance, resistance against passive movements, coordination, dermographia and stereognostic sense). For all these subtests, Touwen's criteria were used (Touwen and Prechtl, 1977). We selected the worst score of left or right for every item.

Speech was considered abnormal if any of the following occurred: stuttering, lisping or nasal speech.

For the assessment of *gait*, the posture of arms and knees was recorded. Associated movements of the arms were also noted.

Concerning the item *tone*, symmetrical or asymmetrical (idiopathic or post-surgery) posture was noted. Posture was also described by the grade of spooning and deviation from horizontal and median line, while standing with extended arms and eyes closed. This test was repeated with palms in pronation and supination. We also asked the child to walk on tiptoe and associated movements with the arms were recorded. Genua valga (intermalleolar distance of ≥4 cm) were identified as well as flat feet.

To evaluate *balance*, children were asked to stand on one foot for 20 s and to hop on one leg at least 20 times.

Tendonreflexes were checked at the biceps and knee and were classified as normal, non-optimal or poor.

To test the *plantar response*, a scratch along the lateral side of the sole from the toes towards the heel with a thumbnail was made. Movement of the big toe (dorsiflexion or plantar flexion) and the other toes was assessed.

Strength against resistance was scored for the upper and lower limbs and also put in one of the three categories: poor, non-optimal or optimal.

To test *resistance to passive movements* (to assess a spastic component or, on the contrary, hypermobility of the joints), the elbows and knees were moved through their full range.

For the item *coordination*, we used the test of diadochokinesis and fingertip touching. For diadochokinesis, the rate of pronation and supination of the hand and forearm while the elbow is flexed at an angle of 90° was noted. Associated movements (mirror movements in the opposite arm) were also noted. The rate of tremor and/or misplacing of the finger in the fingertip-touching test was recorded.

Graphaesthesia was tested by writing three letters, using the thumbnail of the examiner, on the forearm of the child. To assess *stereognosis*, the child was asked to identify three different objects with the eyes closed.

Visual acuity was tested monocularly at 5-m distance. Binocular vision was checked through the test of Lang. If wearing glasses, the children were tested with their glasses on. Eye movements and pupil light reflexes were also tested.

Hearing was tested by performing a pure tone audiometry; a hearing loss of >30 dB was considered as abnormal. If there was a hearing loss in one ear, the test was regarded as abnormal.

Statistical analysis

The statistical analysis was carried out using SAS software, version 8.2. The testing was performed two-sided at the 5% level of significance. Descriptive statistics are given with median and range or mean.

Differences between groups were analysed using Fisher's exact test for dichotomous variables, the Mann–Whitney test for ordered and the *t*-test for continuous variables.

Ethics

The study was approved by the ethical committees of the university hospital, and written informed consent was obtained from the parents.

Results

Demographics

For the studied groups, two of the ICSI children and five of the SC children had a non-Caucasian mother (difference not statistically significant).

The main language of all children was Dutch.

The mean age of the children at follow-up was 8 years 6 months in the study group and 8 years 5 months in the SC group.

Table I. Socio-demographic parameters of the families of ICSI children and those born after spontaneous conception (SC)

	ICSI	SC	*P*-value
Maternal characteristics Higher educational level	97/141 (68.7%)	68/102 (66.6%)	$P = 0.192$
Paternal characteristics Higher educational level	90/141 (63.8%)	56/97 (57.7%)	$P = 0.266$
Marital status Married/cohabiting Divorced	101/112 (90.2%) 11/112 (9.8%)	125/147 (85.0%) 22/147 (15.0%)	$P = 0.261$

Educational level of the parents was comparable in the two groups. A higher level of education had been obtained by 68.7% (97/141) and 63.8% (90/141) of the ICSI mothers and fathers, compared to 66.6% (68/102) and 57.7% (56/97) of the mothers and fathers from the comparison cohort, respectively (Table I). Family constitution did not differ significantly between the two groups. Of the ICSI children, 101 of 112 (90.2%) lived with their two parents compared to 125 out of 147 (85.0%) SC children (Table I).

The number of mothers taking medication during pregnancy in the ICSI group and SC group was 17/137 and 15/95 respectively (Table II). Medications that were mentioned mostly were thyroid substitution, tocolytica, anti-hypertensive and anti-asthma drugs. Diseases during pregnancy (pregnancy unrelated/pre-existent and pregnancy related) did not occur more often in the ICSI group than the SC group. Seventeen of 137 ICSI mothers experienced pregnancy complications compared to 9 of 94 mothers from SC children (difference not significant). These complications were mainly first-trimester haemorrhages, preterm contractions, gestational diabetes, pregnancy-induced hypertension and/or pre-eclampsia. ICSI mothers were significantly older, with a median age at birth of the study child of 32 versus 30 years in the SC group.

Perinatal characteristics

ICSI offspring were more often first born compared to SC children ($P = 0.000$). A comparable number of children experienced a hospital stay of more than 7 days (Table III). Significantly more ICSI children were hospitalized for ≤7 days. Some ICSI parents (four) did not record the duration of the hospitalization.

Median gestational age did not differ significantly between the two groups. In the ICSI group, four children were born preterm (32–37 weeks) compared to 12 children in the SC group.

Medical history during childhood

Comparable rates of chronic diseases during childhood were reported in the two groups (ICSI 29.5%; SC 30%) (Table IV). ICSI children did not experience more illness morbidity than SC children.

Median hospital stay was 2.5 days for ICSI children and 3 days for SC children.

The number of children who were admitted to a hospital at least once was comparable between the ICSI group (66/142) and the comparison group (47/102). From the 66 ICSI children

Table II. Maternal health during pregnancy

	ICSI	Range	Spontaneous conception	Range	*P*-value
Median age (years) at birth	32	25–43	30	18–42	$P < 0.0001$
Pregnancy-related disease	17/137 (12.4%)		9/94 (9.5%)		$P = 0.534$
Pregnancy-unrelated disease	5/142 (3.4%)		8/97 (8.2%)		$P = 0.147$
Medication intake during pregnancy	17/137 (12.4%)		15/95 (15.8%)		$P = 0.562$

Table III. Neonatal characteristics

	ICSI	Range	Spontaneous conception	Range	*P*-value
Gender					$P = 0.907$
Male	76 (50.7%)		76 (51.7%)		
Median gestational age in weeks, days (*n*)	39.4 (150)	35.3–41.5	40 (144)	33–42	$P = 0.514$
Mode of delivery					$P = 0.271$
Spontaneous vaginal	89/140 (63.6%)		70/97 (72.2%)		
Caesarean section	25/140 (17.9%)		16/97 (16.5%)		
Median birthweight in gram (*n*)	3345 (150)	1950–4970	3250 (98)	2000–4390	$P = 0.374$
Median birthlength in cm (*n*)	50.0 (150)	42.5–58	50.0 (97)	40–58	$P = 0.894$
Median head circumference in cm (*n*)	34.5 (141)	30.5–39	34.5 (59)	29–39	$P = 0.760$
Neonatal admission	30/143 (20.9%)		9/100 (9.0%)		$P = 0.012$
Admission ≤7 days	20		2		
Admission >7 days	6		7		
Days unknown	4		0		

n, number of children included for this item.

Table IV. Medical history during childhood

	ICSI	Range	Spontaneous conception	Range	*P*-value
Any hospital admission	66/142 (46.4%)		47/102 (46.1%)		$P = 1.000$
1 admission	40/66 (60.6%)		30/47 (63.8%)		$P = 0.844$
>1 admission	26/66 (39.4%)		17/47 (36.2%)		
Median hospital stay in days	2.5 ($n = 142$)	1–21	3 ($n = 102$)	1–115	$P = 0.214$
Any surgery	44/142 (30.9%)		31/102 (30.4%)		$P = 1.000$
Genito-urinary surgery	10/142 (7.0%)		5/102 (4.9%)		$P = 0.594$
Medication intake	48/139 (34.5%)		35/103 (34.0%)		$P = 1.000$
Chronic use	28/139 (20.1%)		22/103 (21.3%)		$P = 0.872$
Any chronic illness	39/132 (29.5%)		30/100 (30.0%)		$P = 1.000$
Behavioural problems	22/127 (17.3%)		11/101 (10.8%)		$P = 0.189$
Remedial therapy					
At least one therapy	34/110 (30.9%)		21/96 (21.9%)		$P = 0.157$
Speech/language	23		12		
Physiotherapy	2		3		
Psychological help	5		2		
Combined therapy	4		4		

n, number of children included for this item.

that were ever hospitalized during childhood, 40 (60.6%) were admitted only once. Likewise for SC children, 30 of 47 (63.8%) were only once hospitalized.

A surgical intervention was performed in 44/142 ICSI children compared to 31/102 SC children. Although more ICSI children required genito-urinary surgery compared to the SC children, this difference did not reach statistical significance ($P = 0.594$).

The number of children taking medication (mainly medication for treatment of gastro-oesophageal reflux and for allergy and respiratory diseases) at least once during childhood did not differ significantly between the two groups (ICSI 48/139; SC 35/103). There was no difference in chronic use of medication in the study group (28/139) compared to the comparison group (22/103).

The percentage of children that required remedial therapies was higher in the ICSI group (30.9%) compared to the comparison group (21.9%). More ICSI children (17.3%) had behavioural problems compared to SC children (10.9%), but this difference was not statistically significant.

Physical examination at 8 years of age

After a thorough physical examination, all children studied were found to be well with few abnormalities (Table V).

Because all testing was performed on the same day, we were unfortunately forced to omit some items of the clinical examination because of restricted time of the children/parents, as mentioned previously.

Table V. Biometrical data at 8 years of age

	ICSI[a]	Range	Spontaneous conception[b]	Range	*P*-value
Median weight in kg	28	19–53.5	27.9	18–47.4	$P = 0.812$
Median height in cm	133.2	117.5–151.5	132.2	118.3–155	$P = 0.419$
Median head circumference in cm	53.2	47–57	53	49–56.7	$P = 0.729$
Mean body mass index	16.2		16.3		$P = 0.759$
Mean armwidth in cm	131.5		131.9		$P = 0.658$
Median blood pressure in mmHg	$n = 137$		$n = 143$		
Systolic	100	80–125	95	70–120	$P = 0.0007$
Diastolic	60	45–75	55	35–80	$P < 0.0001$
Abnormality					
Skin	52/143 (36.4%)		38/147 (25.8%)		$P = 0.057$
Heart	2/148 (1.3%)		1/147 (0.7%)		$P = 1.000$
Abdomen	0/148 (0%)		1/146 (0.7%)		$P = 0.496$
External genital anomaly					
Boys	5/74 (6.75%)		5/75 (6.66%)		$P = 1.000$
Girls	0/73 (0%)		0/71 (0%)		
Tanner scores	$n = 143$		$n = 145$		
A	1		≤2		$P = 0.498$
P	≤2		≤2		$P = 1.000$
G/M	1		≤2		$P = 0.498$

[a]*n*, 150 unless otherwise stated.
[b]*n*, 147 unless otherwise stated.

Weight, height and head circumference did not differ significantly in the two groups. Median diastolic and systolic blood pressure was significantly higher in ICSI children ($P < 0.0001$, $P = 0.0007$, respectively). Range upper values for diastolic pressure were measured in one ICSI child (60 mmHg) and four SC children (75 mmHg). Range upper values for systolic pressure were registered in one ICSI child (125 mmHg) and two SC children (120 mmHg). Abnormal heart auscultation was registered in 2/148 ICSI children and in 1/147 SC child. A systolic murmur 1/6 was found in all three children. Abdominal examination showed a variance in 1/146 SC child (distended abdomen with borborygmi without signs of pathology). 52/143 ICSI children presented with a skin abnormality compared to 38/147 SC children.

Pubertal development (Tanner scores) was equal in the two groups. No children presented with premature pubertal onset. Maximum stages that occurred in the ICSI group ($n = 143$) were A1, P2 and G/M1 and in the SC group ($n = 145$) A2, P2 and G/M2.

Genital examination was performed in 74/76 boys and 73/74 girls in the ICSI group and in 75/76 boys and all 71 girls in the SC group. In the ICSI boys, a total of 5/74 with genital abnormalities were found (3 phimosis, 1 cryptorchidism, 1 hypospadias without requirement for surgery). We found 5/75 SC boys with genital anomalies: 4 phimosis and 1 high-scrotal testis. None of the girls in the two groups showed an abnormal genital examination.

Different subtests of the neurological examination were scored in nearly all children.

Speech was recorded as normal in 125/132 children in the ICSI group compared to 130/138 in the SC group ($P = 1.00$).

No difference in *gait* pattern was observed between the two studied groups (Table VI).

Tone was also not significantly different in the two groups. Posture assessed by the "spooning test" did not reveal any significant difference between the study and SC population. Two boys (one ICSI and one SC) presented with idiopathic asymmetry. One ICSI child showed secondary asymmetry (after pes varus correction). Walking on tiptoe was performed with equal results in both groups. Three ICSI children presented with genua valga compared to four SC children (difference not significant). Twenty-two children (13 ICSI children and 9 SC children) showed flat feet, which did not require any therapy.

Concerning the item *balance*, only hopping on one leg was performed equally successfully in both groups. Standing on one leg was performed more successfully by the SC group ($P = 0.0182$).

Table VI. Neurological examination at 8 years of age

	n		
	ICSI	Spontaneous conception	*P*-value
Gait pattern	143	147	$P = 0.1539$
Tone			
Spooning test	107	121	$P = 0.3647$
Tiptoe walking	144	147	$P = 0.9686$
Balance			
Hopping on one leg	145	146	$P = 0.443$
Standing on one leg	144	146	$P = 0.0182$
Tendonreflexes			
Knee jerk	143	146	$P = 0.901$
Biceps reflex	140	131	$P = 0.015$
Plantar response	104	146	$P = 0.387$
Strength against resistance			
Upper limb	111	144	$P = 0.274$
Lower limb	111	144	$P = 0.189$
Resistance to passive movements			
Upper limb	108	108	$P = 0.160$
Lower limb	145	145	$P = 0.160$
Coordination			
Diadochokinesis	137	146	$P < 0.0001$
Fingertip-touching test	143	145	$P = 0.0013$

n, number of children assessed for this item.

Tendonreflexes were checked at the upper and lower limbs. The distribution of normal and subnormal scores of the knee jerk was equal in the study and SC population. Only the intensity of the biceps reflex differed significantly between ICSI children and children from the SC group ($P = 0.0153$).

The performance of the *plantar response* was comparable in the two groups.

Both groups showed sufficient *strength against resistance* in the lower and upper limbs (no statistically significant difference between ICSI and SC children).

Resistance to passive movements was performed equally in ICSI and SC groups.

Diadochokinesis (item *coordination*) was performed better by the SC group ($P < 0.001$). The fingertip touching test, assessing coordination, was performed better by the ICSI children ($P = 0.0013$).

Graphaesthesia was performed with comparable results in the ICSI ($n = 150$) and the SC ($n = 147$) groups ($P = 0.858$) (data not shown).

The number of items that were correctly discriminated with the eyes closed (*stereognostic sense*) was comparable between ICSI children ($n = 145$) and SC children ($n = 147$) ($P = 0.434$).

Major and minor malformations

In our classification system, a total of 15/150 (10%) (Table VII) (seven male/eight female) children with major malformations were found in the ICSI group compared to 5/147 (3.3%) (three male/two female) in the comparison group [$P = 0.035$; relative risk (RR) = 2.94; 95% confidence interval (CI) = 1.09–7.89]. Differences between the ICSI and the SC group were mainly determined by four more children in the ICSI group with an inguinal hernia and two ICSI children with a naevus flammeus on a large bodysurface. After reclassification according to the Western Australian system, 6/150 ICSI children had a major malformation compared to 0/147 SC children. This difference was statistically significant ($P = 0.030$). Reclassification resulted in omission of the following items in the ICSI group: inguinal hernia, short Achilles tendon and surgery for strabism. In the SC group, reclassification resulted in omission of inguinal hernia and surgery for strabism and umbilical hernia.

An increase in major malformation rate from 5.3% in the neonatal period up to the age of 1 year (eight malformations) to 10.6% (16 malformations in 15 children) at the age of 8 years was found in the ICSI group ($n = 150$).

We recorded 35/145 children in the ICSI group with a minor malformation compared to 25/145 children in the comparison group ($P = 0.191$).

The number of children with minor malformations was equal (seven children) in both groups after reclassification based on the Western Australian system (ns). Five ICSI and two SC children were given a routine paediatric examination rather than being examined against the extensive checklist for minor malformations due to restricted time.

Other investigations

A visual acuity test was performed in almost all children. 112/136 (82.35%) ICSI children had a normal vision (≥0.8 in the best eye) compared to 121/146 (82.88%) SC children (ns). Eye movements and pupil light reflexes were performed equally in both groups. The number of items seen binocularly by the test of Lang was comparable between the study and SC population.

Hearing was normal in all tested ICSI children ($n = 145$). Only 5/145 SC children had an abnormal hearing test ($P = 0.060$). Hearing loss was mostly because of cerumen or recent ear infection.

Discussion

Our detailed study of 150 ICSI children, which is the biggest cohort of ART children studied at the age of 8 years, assessing general health through *questionnaires* and *physical examination* showed reassuring findings. Although there were some *methodological limitations*, an increased number of major congenital malformations were found.

Analysis of the *questionnaires* revealed a significant difference in maternal age between SC and ICSI group, which reflects the general tendency of fertility patients to be older, in accordance with other studies. As in previous reports (Bonduelle *et al*., 2004; Bonduelle *et al*., 2005), we also documented more pregnancy complications, but this difference did not reach statistical significance.

We could not report more prematurity or lower birthweight among ICSI children as mentioned in several studies (Helmerhorst *et al*., 2004), probably because children born <32 weeks of gestation were excluded. ICSI children were more often hospitalized in the neonatal period than the SC children, as was mentioned in different other studies on ICSI children (Sutcliffe *et al*., 2003; Bonduelle *et al*., 2004) and on children born after ART in general (Bergh *et al*., 1999; Helmerhorst *et al*., 2004). No more ICSI children were treated in neonatal care units for more than 1 week. On the contrary, significantly more ICSI children born ≥32 weeks of gestation were hospitalized postnatally for a short period, which indicates a good neonatal outcome for these children born after at least 32 weeks of gestation. Those short hospitalizations were mostly for minor reasons (hyperbilirubinaemia or hypoglycaemia) or without any specific medical indication (e.g. ‘observation’), indicating a precautious attitude towards ‘special babies’ regarding their mode of conception. It can also be stated that parents of ICSI children are more accustomed to completing questionnaires and may recall data about the health of their children more easily and accurately than parents of children born after SC (Bonduelle *et al*., 2003, 2005). This fact is reflected in the large amount of missing data in the SC group.

Although the rate of remedial therapy was increased among ICSI children, this increase did not reach statistical significance and was not as large as that reported for 5-year-old ICSI children (Bonduelle *et al*., 2005). Because speech therapy was most frequently applied in the younger study groups, one can assume that the rate of remedial therapy gradually diminishes in the 8-year-old tested population where speech therapy is no longer needed.

Variables measuring general health, such as chronic use of medication and chronic illness, were present at similar rates in both groups. Earlier follow-up until the age of 5 years showed

Table VII. Malformations in ICSI and spontaneous conception (SC) children classified according to the Brussels and the Western Australian classification system

	Brussels system	Western Australian system	Age at diagnosis
Major malformation in ICSI children			
Total major malformations			
Number	15/150	6/150	
Percentage	10	4	
P-value	0.035	0.030	
Dextrocardia	1	1	Birth
Hernia inguinalis[a]	5	Omitted	3, 3.5, 4, 18 months, 5 years
Naevus flammeus (large body surface)[b]	2	2	Birth
Pes equinovarus	1	1	Birth
Short Achilles tendon	1	Omitted	5 years
Strabism surgery	3	Omitted	4 years, 6 years, 7 years
Undescended testes orchidopexy bilateral[b]	1	1	18 months, 5 years
Uretral cyst surgery	1	1	7 years
Uretral web surgery	1	1	6 months
Major malformations in SC children			
Total major malformations			
Number	5/147	0/147	
Percentage	3.3	0	
Hernia inguinalis	1	Omitted	3 years
Strabism surgery	3	Omitted	3 years, 5 years, 6 years
Umbilical hernia surgery	1	Omitted	8 months
Minor malformations in ICSI children			
Total minor malformations			
Number	35/145	7/145	
Percentage	24.1	4.8	
P-value	0.191		
Ear			
Abnormal angulation of ears	1	Omitted	
Absence of earlobe (small part)	1	1	
Indented upper helix ear	1	Omitted	
Prominent ears	2	Omitted	
Small earlobe	1	Omitted	
Face			
Epicanthal folds	4	Omitted	
Facial dysmorphism	1	1	
Hypertelorism	1	Omitted	
Mongoloid eyes	1	Omitted	
Macrognathia	1	Omitted	
Genital			
Hypospadias no surgery	1	1	
Limb			
Fetal pads on fingertops	1	Omitted	
Increased space between toes	10	Omitted	
Irregularity of toe length	1	Omitted	
Overriding toes	1	Omitted	
Single transverse palmar crease	1	Omitted	
Small accessory digit	1	1	
Syndactyly	4	Omitted	
Skin			
Angioma in regression	1	Omitted	
Café-au-lait spot	1	Omitted	
Capillary haemangioma	1	1	
Halo naevus	1	Omitted	
Hypopigmented macula	2	Omitted	
Multiple tuberous angiomata	1	1	
Naevus flammeus (small body surface)	1	1	
Trunk			
Supernumerary nipple	1	Omitted	
Umbilical hernia no surgery	1	Omitted	
Minor malformations in SC children			
Total minor malformations			
Number	25/145	7/145	
Percentage	17.2	4.8	
Ear			
Abnormal angulation of ear	1	1	
Absence of earcartilage	1	1	
Absence of earlobe (small part)	1	1	
Asymmetrical size of ears	2	Omitted	
Face			
Epicanthal fold	1	Omitted	
Facial dysmorphism[c]	1	1	

Table VII. Continued

	Brussels system	Western Australian system	Age at diagnosis
Limb			
Clinodactyly[c]	3	3	
Increased space between toes	7	Omitted	
Irregularity of toe length	2	Omitted	
Overriding toes	1	Omitted	
Syndactyly	3	Omitted	
Skin			
Hypopigmented macula	4	Omitted	
Trunk			
Nipples widely spaced	1	Omitted	
Pectus excavatum[c]	3	3	

[a]If born ≤36 weeks of gestation: hernia inguinalis is considered as minor.
[b]One child had two major malformations.
[c]A child can have more than one minor malformation.

higher illness morbidity in the ICSI group (Bonduelle *et al*., 2005), which we could not confirm in our study (follow-up until the age of 8 years). In contrast to previous reports, we did not find a higher rate of surgical interventions among ICSI children (we excluded probably the most vulnerable group i.e. very prematurely born children). In a recent study of Bonduelle *et al*. (2005), there was an increased hospitalization rate, but no stratification according to aetiology of the admission was made. In our study, with a smaller sample size, no increased hospitalization risk, either for surgical or for non-surgical reasons (e.g. infections), was observed.

Diastolic and systolic blood pressure as part of the *physical examination* showed a statistically significant difference between ICSI children and SC children. Although the mean and median diastolic blood pressure was higher in ICSI children, the maximum diastolic pressure was still below the 97.5th percentile, whereas in the SC group the maximum diastolic pressure is between percentile 97.5 and 97.5 + 10 mmHg when adjusted for height and gender. Maximum systolic blood pressure of 125 mmHg in the ICSI group and 120 mmHg in the SC group was still below the 97.5th percentile when height and gender of those individual children were considered. Owing to the study design, we were not able to visit the children more than once to gather repetitive measurements of the blood pressure. Although the same equipment for measuring blood pressure was used in every child, small differences in measures can be expected in children with relatively higher weight. Speculation of a trend towards higher blood pressure in ART children can be made because an association between low birthweight/prematurity (which are well established findings in ART children) and the subsequent development of diseases such as hypertension later in life is now generally accepted (Barker, 1992; Hofman *et al*., 2004). Owing to our small study group and study design, where we excluded very premature children (born before 32 weeks of gestation), we were not able to add to these data, but this hypothesis should be investigated further.

We did not detect any significant neurological problems in a very detailed examination in our groups of children born after 32 weeks of gestation, recognizing the small sample size (150 ICSI children), which limits the detection of many severe and rare neurological problems, such as cerebral palsy (Stromberg *et al*., 2002). Different subtests of the neurological evaluation showed minor differences between the ICSI and SC children. Although statistically significant differences were found, those differences were of no clinical importance (fingertip-touching test and biceps reflex). Interference of the child's performance by the testing environment cannot be excluded.

The difference in recruitment of ICSI and SC children represents a *methodological weakness* of this study. ICSI parents were contacted by phone and were given oral information about the aim of the study. SC children on the contrary were contacted by letter with written information about the study (i.e. no personal contact and encouragement to participate). Also, whereas ICSI children were examined in the presence of their parents, so creating the possibility of specifying possible health problems, SC children were tested alone in schools without their parents. Owing to the difference in recruitment, the paediatrician was not blinded to the conception mode, and knowledge of the conception status could have influenced the results of the children's examination. However, because children in the two groups were assessed by the same paediatrician using a battery of scaled standardized tests, we think that there was little evidence of consistent bias. ICSI parents were asked to fill in the health questionnaire, while their child was being tested by the psychologist (psycho-emotional and intellectual development was evaluated in parallel). Parents of SC children received the proforma at home and were kindly asked to send them back. This difference in recruitment could lead to differences in obtaining general information about the health of the children, possibly leading to a higher rate of recording problems and thus an overestimation of medical problems in the ICSI group. On the contrary, recruitment of the SC group in 'normal' schools could lead to an underestimation of the risk of major malformations and/or illnesses in this group. However, all but one of the ICSI children also attended normal schools, providing some reassurance that recruitment bias based on ability to attend a normal school has not influenced our study results.

A *limitation* of our study is the rather high rate of non-participation (not reached and refusals) for ICSI children. Although 61% of the eligible cohort was formally assessed,

approximately one-third could not been seen, which could possibly lead to a participation bias and an underestimation of malformations and/or illnesses in this group. However, ICSI children who were not reached were not necessarily dealing with more medical problems/malformations and were probably randomly distributed.

The eligible children in this study were mostly part of a cohort seen at the age of 2 years (Bonduelle *et al*., 1998; Ponjaert-Kristoffersen *et al*., 2004, 2005), which explains a weakening of interest in being tested at the age of 8 years. This finding is inherent to long-term longitudinal follow-up. On the contrary, we could still gather a lot of information from the parents who refused participation (information in up to more than 83% of the initial cohort) because they were asked a standard list of questions about their child in such a way that serious medical problems could be ruled out. Overall hospitalization rate seems to be a little higher in the refusal group (58.9%) compared to the studied ICSI group (46.4%), but this difference was not statistically significant. Surgery was more frequently performed in the refusal group (48.2%) compared to the studied group (30.9%) ($P = 0.031$) due to a higher rate of tympanic drain placements and/or adenotonsillectomy. The group of children that required genito-urinary surgery was comparable in both populations, 10.7 and 7.0%, respectively (ns).

The difference in recruitment strategy as mentioned above probably explains the lower response rate in the SC group (37.5%) where, likewise, a participation bias cannot be ruled out. Parents of SC children with medical problems might have chosen not to expose their children to additional medical testing, which could lead to underestimation of the risk of severe malformations and/or illnesses in this group.

Another *limitation* of this study is the potential for survivor bias because children who had already survived to the age of 8 years were recruited. Although this cross-sectional recruitment strategy could lead to an underestimation of the true risks of malformations and/or serious illnesses, other health outcomes such as general health, medical and psychological development and family functioning, which were the main foci of our parallel studies, can be properly assessed by this strategy.

Our finding of increased RR of major congenital malformation in ICSI children versus SC children at age 8 years is in agreement with the previous reports on children at a younger age of Bonduelle *et al*. in 2004 [major malformation rate: 6.3%; odds ratio (OR) = 2.53; 95% CI = 1.07–5.98], Hansen *et al*. in 2002 (major malformation rate: 8.6%; OR = 2.0; 95% CI = 1.3–3.2) and Wennerholm *et al*. in 2000 (major malformation rate: 5.0%; OR = 1.75; 95% CI = 1.19–2.58). Katalinic *et al* (2004) reported in a prospective study a major malformation rate of 8.7% in ICSI children up to the age of 1 year (RR = 1.44; 95% CI = 1.25–1.65). However, methodological differences, such as small sample size (Sutcliffe *et al*., 2001), different definitions of major/minor malformations (Hansen *et al*., 2002; Katalinic *et al*., 2004) and different inclusion criteria (Hansen *et al*., 2002; Katalinic *et al*., 2004) make it very difficult to compare malformation rates between different studies (Rasmussen and Moore, 2001; Hansen *et al*., 2005). Results of meta-analyses show an overall increased risk of birth defects in children born after ICSI and/or IVF (Hansen *et al*., 2005; Lie *et al*., 2004).

Our finding of a 10% prevalence of major malformations in the ICSI cohort is rather high, but as mentioned previously, malformation rates do depend not only on definitions but also on the age at which children are examined. For example, in a multi-centre study of 5-year-old ICSI children, the prevalence of major malformations was lower at 6% (Bonduelle *et al*., 2005). It is worth restating that our studied groups were small, which may influence statistical outcome. A report from Katalinic *et al*. (2004) suggested that parental background played a role in the apparent increase of congenital malformations in ICSI because after the adjustment for risk factors, such as parental malformations, the RR for major malformations declined. Although parental malformations were explicitly asked for in the questionnaires, we did not gather reliable information concerning this item because parents in the SC group are not so accustomed to specific questions regarding malformations and fertility-related issues as the parents in the ICSI group. Consequently, self-reporting through written questionnaires could lead to an underestimation in the SC group.

As mentioned previously, children in this study were assessed by a paediatrician who was not blinded to conception status. Lack of blinding could bias the reporting of malformations in two different ways: first at the level of detection and second at the level of classification. To minimize the first, children in both groups were assessed by the same paediatrician using a clear definition of major malformation and a checklist for minor malformations. To address the second issue, we asked a Western Australian research team to reclassify our list of malformations without knowledge of conception status. The results of this classification were in line with our findings, although providing different prevalence estimates of malformations.

The same number of children with minor malformations, classified according to the Western Australian system (seven children; 4.8%), was found in ICSI and SC groups. Our rate of minor malformations (using the Brussels system) of 24.1% in the ICSI group and 17.2% in the SC group is rather high (RR = 1.4; 95% CI = 0.88–2.21) compared to different studies, probably because of our thorough examination with special attention to very minor malformations as shown in the comparison between the Western Australian and Brussels classification system.

In conclusion, physical examination including a thorough neurological examination as performed in this study revealed reassuring findings. No clinically important differences between ICSI and SC children were noticed. Major congenital malformations were more frequently found in ICSI children. However, most of them were corrected by minor surgery. The overall general health of 8-year-old singleton ICSI children (born ≥32 weeks of gestation) seems satisfactory, but more children should be examined. To deal with potential risks to health and future fertility, continuing follow-up of these children is indicated. At this moment, the children are being re-assessed at the age of 10.

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