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Matched follow-up study of 5–8 year old ICSI-singletons: comparison of their neuromotor development to IVF and naturally conceived singletons

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BACKGROUND: Intracytoplasmic sperm injection (ICSI) is an invasive technique of artificial reproduction. We investigated the effect of ICSI on neuromotor development in 5–8 year old singletons. METHODS: We did a follow-up of ICSI-singletons born between 1996 and 1999 after treatment in the Leiden University Medical Center and compared them with matched controls born after *in vitro* fertilization (IVF) and natural conception (NC). Children underwent a thorough neurological examination that focused on minor neurological dysfunction (MND). RESULTS: There were no differences in outcome between ICSI ($n = 81$) and IVF-children ($n = 81$), all born at term: MND prevalence 66.3% versus 61.3%, prevalence ratio (PR) 1.08 [0.83; 1.29]. MND prevalence among all ICSI-children ($n = 87$) was higher than among NC-controls ($n = 85$) (66.3% versus 50.6%, PR 1.31 [1.02; 1.55]). After adjustment for maternal age and parity, the PR remained elevated but was no longer statistically significant (adjusted PR 1.22 [0.86; 1.52]). When comparing only term ICSI and NC-children ($n = 81$; $n = 85$), the PR adjusted for maternal age and parity was 1.20 [0.83; 1.51]. CONCLUSIONS: Neuromotor outcome of 5–8 year old singletons born at term after ICSI or IVF was similar; ICSI-children (both the total group and term children only) deviated slightly from NC-controls. Part of this effect was explained by a difference in parity, but not prematurity.

Keywords: child; development; ICSI; IVF; neurological examination

Introduction

Intracytoplasmic sperm injection (ICSI) is a technique of artificial procreation, in which a single spermatozoon is injected into the oocyte and once fertilized the zygote is transferred to the prestimulated uterus (Palermo *et al.*, 1992). Due to the invasive character of the procedure, e.g. the *in vitro* manipulation of the gametes and the bypassing of natural selection barriers (Sutcliffe, 2000; Schultz and Williams, 2002; Tournaye, 2003; Niemitz and Feinberg, 2004), long-term follow-up of ICSI-children is warranted.

In the present study, we investigated the effect of ICSI on neuromotor development at the age of 5–8. Children born from artificial reproductive techniques are known to be at risk for prematurity and low birthweight (Helmerhorst *et al.*, 2004; Jackson *et al.*, 2004), both risk factors for disturbed neuromotor development (Veen *et al.*, 1991; Marlow *et al.*, 2005). By comparing ICSI-children with carefully matched IVF-controls, we assessed the excess risk of the ICSI-procedure on neuromotor development, given the common characteristics of underlying infertility, hormonal stimulation of the mother, *in vitro* manipulation of the gametes and an increased risk of prematurity and low birthweight. In a comparison with naturally conceived (NC) control children, we studied both the overall effect of ICSI on neuromotor outcome, including the increased risk due to prematurity, and the net effect of ICSI in children born at term.

Previous studies on neurological and psychomotor development of ICSI-singletons painted a reassuring general picture (Sutcliffe *et al.*, 1999; Sutcliffe *et al.*, 2001; Pinborg *et al.*, 2004; Ponjaert-Kristoffersen *et al.*, 2004; Ponjaert-Kristoffersen *et al.*, 2005; Leunens *et al.*, 2006; Belva *et al.*, 2007), but only one study has reached beyond the child age

of 5 (Leunens *et al.*, 2006; Belva *et al.*, 2007). The test-instruments used in former studies were (except for Belva *et al.*, 2007) limited to gross and fine motor assessment or diagnosed neurological sequelae. In the present study, neuromotor development was recorded with well-defined outcome measures based on the assessment of posture, muscle tone, reflexes, gross and fine motor function, associated and involuntary movements, sensory deficits and cranial nerve dysfunctions.

Patients and Methods

All ICSI singleton children born between June 1996 and December 1999 after fertility treatment in the Leiden University Medical Center were invited. Exclusion criteria were: oocyte or sperm donation, cryo-preservation of the embryo and selective embryo reduction with medical indication. Similar inclusion criteria were applied in the selection of IVF-children, who were matched person-to-person to ICSI-participants for gender, socio-economic status (SES), gestational age [preterm/term], maternal age at the time of pregnancy [$\pm$3 years] and birth date [closest]. SES-level low, medium or high was ascribed according to the zip code/SES indicator of Statistics Netherlands (Van Duijn and Keij, 2002), based on home price and income. If no match was available within the maternal age range of $\pm$3 years, larger deviations were permitted.

Regular pre-schools and primary schools (i.e. schools not providing special education) with zip codes that indicated social class distributions similar to the ICSI-cohort were approached for the sampling of naturally conceived singletons. Teachers distributed letters among singletons within the defined age range (5–8 years old or born between June 1996 and December 1999) without further selection. In this way, we applied group matching for SES, birth date and, additionally, gender.

Paternal educational level was indexed according to the SOI-register of Statistics Netherlands (1998). Demographic information on ICSI and IVF non-participants was obtained from the Leiden University Medical Center database to evaluate selection bias.

The study design was approved by the Ethics Committee of the Leiden University Medical Center and written informed consent was obtained from at least one parent of each child.

Examination and outcome measures

All children underwent a standardized neurological examination developed by Touwen (1979), which focuses on minor neurological dysfunction (MND) and is applicable between 4 and 18 years of age. Outcome measures consist of total neuromotor outcome and clusters of dysfunction separately (Table 1): posture and tone, reflexes, involuntary movements, gross motor development, fine motor development, associated movements, sensory deficits and cranial nerve dysfunctions (Hadders-Algra, 2003).

Simple MND (one or two clusters of dysfunction) reflects the presence of a normal but non-optimally functioning brain and forms the lower tail of the distribution of the quality of brain function, which is seen as non-pathological (Hadders-Algra, 2002). Complex MND (more than two clusters of dysfunction) can be considered as a distinct form of perinatally acquired brain dysfunction that is likely to be associated with a structural deficit of the brain (Hadders-Algra, 2002). Children with dysfunctional patterns in two or more clusters (complex MND) who meet the criteria of cerebral palsy (CP) are classified as CP. CP is defined as movement and posture deviations due to a defect or lesion of the immature brain that manifest early in life and are permanent and non-progressive (Bax *et al.*, 2005). Clinically, a child with coordination problems, fine motor dysfunction and excessive associated movements would be reported as complex MND; CP would be diagnosed in the case of e.g. hemiplegia.

One trained investigator (MK) who was blinded for the mode of conception did all neurological examinations. Blinding was achieved by scheduling and assessing the children in order of birth date. No information on mode of conception was available in this procedure. During the examination, we instructed the parents not to reveal the family name or the conception mode of the child. The assessments were videotaped and a sample of 32 children was reviewed by a specialist in neurological developmental assessment (SV), who was also blinded for mode of conception. The sample included 10 children haphazardly chosen with score 'normal' (10%), 15 children with 'simple MND' (10%), all six children with 'complex MND' and the one child with CP (excluded from main analysis due to prematurity).

General characteristics and additional information on the study groups were obtained through questionnaires.

Statistical analysis

We performed statistical analysis using the SPSS 11.0 for Windows package (SPSS Inc., Chicago, IL, USA). The original power calculation was based on an intelligence test that was carried out in parallel (RAKIT, mean 100, SD 15; minimal detectable difference 7.5, power 0.80, $n \geq 63$). Additionally, a *post-hoc* power calculation on MND showed that a sample size larger than 59 was required to detect an increase in MND prevalence from a baseline of 25% in the NC-group [anticipated from Hadders-Algra *et al.* (Hadders-Algra, 2002)] to 50% in the ICSI-group, with a power of 0.80. Cross tabulations and logistic regression analyses provided odds ratios (OR) and the corresponding 95% confidence intervals (95%CI). As the prevalence of outcome values exceeded 10%, the OR did not sufficiently approximate the relative risk any longer and, therefore, all OR and 95%CI were translated to prevalence ratios (PR) (relative risks) using the method of Zhang (Zhang and Yu, 1998): $PR = OR/((1- Po) + (Po \times OR))$, with Po = the prevalence of the outcome of interest in the non-exposed group.

We used the Pearson chi-square test to assess the distribution of outcome values between groups if outcome consisted of more than two categories. Multiple logistic regression analysis was done to adjust for possible confounders.

We performed both ICSI–IVF and ICSI–NC analyses in an unpaired design. ICSI–IVF analyses were suitable for paired testing, as we had matched the children person-to-person. The advantage of unpaired testing was the possibility of presenting the results as crude data instead of differences only. A possible disadvantage was the slight widening of the 95%CI.

The ICSI–NC comparison was carried out in two ways. First, we assessed the overall difference in neuromotor development between ICSI and NC-children; the clinical question that parents are interested in. For this purpose, the data were analysed without controlling for intermediate factors such as prematurity, which are associated with both ART and neuromotor outcome. Secondly, we assessed the net difference between ICSI and NC-children. For this purpose, preterm born children were excluded from the analyses.

Results

Selection

The overall response in the ICSI-group was 97/110 (88%), of which 87 children enrolled (90% of responders, 79% of

Table 1. Clusters of minor neurological dysfunction (MND) based on the neurological examination of Touwen (1979) and Hadders-Algra (2003)

Cluster of dysfunction	Based on	Criteria for dysfunctional cluster
Dysfunctional muscle tone regulation	Muscle tone Posture during sitting, crawling, standing and walking	One or more of the following: -Consistent mild deviations in muscle tonus -Consistent mild deviations in posture
Reflex abnormalities	Abnormal intensity and/or threshold or asymmetry in: -Biceps reflex -Knee jerk -Ankle jerk Foot sole response: uni- or bilateral Babinski sign	Presence of at least two signs
Choreiform dyskinesia	Spontaneous motor behaviour Test with extended arms Movements of face, eyes and tongue	Presence of at least one of the following: -Marked choreiform movements of distal and facial muscles -Slight or marked choreiform movements of proximal muscles, eyes or tongue
Coordination problems	Finger-nose test Fingertip-touching test Diadochokinesis Kicking Knee-heel test Reaction to push (sitting, standing) Romberg Tandem gait Standing on one leg	Presence of age-inadequate performance of at least two tests
Fine manipulative disability	Finger-opposition test: -Smoothness -Transition Follow-a-finger test Circle test	Presence of age-inadequate performance of at least two tests
Rarely occurring miscellaneous disorders	Motor behaviour of face, eyes, pharynx, tongue Associated movements during diadochokinesis, finger-opposition test, walking on toes or heels	Evidence of at least one of the following: -Mild cranial nerve palsy -Excessive amount of associated movements for age
Age specific criteria for simple and complex MND (Hadders-Algra, 2003)		
Age	Simple MND	Complex MND
Four year to onset of puberty	1–2 MND clusters of dysfunction	>2 MND clusters of dysfunction

all children invited) and 10 refused for various reasons. Participating and non-participating children were comparable for gender, SES, maternal age and gestational age (data not shown). Higher participation rates were seen in the higher SES groups (participation percentage: high SES: 91%, medium SES: 71% and low SES: 59%).

In the IVF group, 257 children met the inclusion criteria. To find a match for each ICSI-child, 126 IVF-children were invited. The overall response was 100/126 (79%), of whom 92 participated (92% of responders, 73% of all invited) and 8 refused. Because no matches within the range of [−3, +3 years] for maternal age were available, larger deviations were permitted in 11 cases. Reasons for refusal were similar as for ICSI-families. The 92 participants differed from the 34 non-participants in gender-distribution (male gender in participants 49% versus 71% in non-participants), but were comparable for maternal age, gestational age and birthweight (data not shown). The participation rates according to SES approximated those of the ICSI-group (high: 81%, medium: 73% and low: 50%). In five cases, two IVF-matches were available for one ICSI-child. By selecting the best match we restricted $n = 92$ to $n = 87$.

Of the 87 ICSI-children, six were born preterm. For 4/6 cases we failed to include an IVF-match. As two children and their matches could not represent the preterm born children in the ICSI and IVF cohorts, we decided to exclude them from further analyses in the ICSI/IVF-comparison ($n = 81$). This decision was in line with our aim to investigate an effect of ICSI as compared to IVF that was not mediated by low birth weight or prematurity.

From 16 schools, 87 children enrolled, of which two were excluded for being a twin and being conceived with intrauterine insemination ($n = 85$). Forty-three children refused for various reasons. The response rate for all children invited and selection were hard to estimate in the NC-group, as we did not know the exact size of the target group or the characteristics of non-responders. However, of those who responded, 67% participated. Within the schools, the response was higher among NC-children of higher SES.

The ICSI–NC comparison was initially not restricted to term children because we aimed to assess the overall effect of ICSI on the outcome measures (ICSI $n = 87$; NC $n = 85$). However, in parallel we assessed the net effect of ICSI on neuromotor development by excluding preterms from the analysis (ICSI $n = 81$; NC $n = 85$).

Characteristics

Parental and child characteristics are listed in Table 2. The ICSI and IVF-groups were comparable except for diagnosed infertility factors, incidence of pregnancy complications, paternal smoking behaviour and paternal educational level.

Despite the matching, the ICSI and NC-groups varied in age at the time of examination, parity, parental age, diagnosed infertility factors, paternal smoking behaviour, SES and maternal level of education. Mean birthweight was lower after ICSI, and a higher frequency of prematurity, low birthweight, small-for-gestational-age (Niklasson *et al.*, 1991) and caesarean sections was found for ICSI-children compared with NC-controls.

Of the participating ICSI-children, all but one attended regular preschools and primary schools. This had justified the retrieval of NC-controls via regular education.

Table 2. Demographic characteristics of parents and children: ICSI versus IVF and ICSI versus NC

	ICSI $n = 81$	IVF $n = 81$	ICSI $n = 87$	NC $n = 85$
Gender: male, *n* (%)	40 (49)	40 (49)	44 (51)	47 (55)
Age at examination, mean (range)	6.1 (5.3–7.7)	6.2 (5.3–8.3)‡	**6.1 (5.3–7.7)**	**6.3 (5.1–8.0)**
Parity: first-born, *n* (%)	61 (75)	59 (73)	**65 (75)**	**31 (36)**
Birth parameters				
Gestational age, mean (range)	40.1 (37–43)	39.8 (37–42)	39.9 (35–43)	39.8 (37–43)
Birth weight, mean (range)	3447 (2300–4750)	3379 (1835–4730)	**3370 (1485–4750)**	**3555 (2300–4800)**
Prematurity (gest. age <37 weeks)	0 (0)	0 (0)	**6 (7)**	**0 (0)**
Birth weight <2500 g, *n* (%)	3 (4)	3 (4)	**7 (8)**	**1 (1)**
Small-for-gestational-age †, *n* (%)	4 (5)	2 (2)	6 (7)	1 (1)
If Apgar score available, *n* (%)	57 (70)	58 (72)	60 (69)	62 (73)
Apgar 1 min <5 or 5 min <7, *n* (%)	2 (4)	2 (3)	2 (3)	1 (2)
Caesarian section, *n* (%)	11 (14)	9 (11)	12 (14)	6 (7)
Vanishing twin	6 (7)	7 (9)	9 (10)	
timing unknown	1	4	3	
<9 weeks	4	1	4	
9–21 weeks	0	2	1	
>21 weeks	1	0	1	
Parental age at pregnancy, mean (range)				
Mother	32.8 (22–41)	33.4 (24–42)	**32.8 (22–41)**	**30.6 (20–41)**
Father	36.9 (23–65)	37.3 (27–60)	**36.9 (23–65)**	**32.6 (20–49)**
Diagnosed infertility factor, *n* (%)				
Mother	**13 (16)**	**37 (46)**	**15 (17)**	**0 (0)**
Father	**64 (79)**	**11 (14)**	**70 (80)**	**0 (0)**
Pregnancy complications, *n* (%)	17 (21)	27 (33)	23 (26)	17 (20)
Medication during pregnancy, *n* (%)	10 (13)*	8 (10)	10 (12)*	14 (17)§
Smoking during pregnancy, *n* (%)				
Mother	*		*	
No	70 (88)	70 (86)	76 (88)	75 (88)
Yes, <10 per day	9 (11)	10 (12)	9 (10)	8 (9)
Yes, >10 per day	1 (1)	1 (1)	1 (1)	2 (2)
Father		‡		*
No	**57 (70)**	**61 (77)**	**61 (70)**	**62 (74)**
Yes, <10 per day	**7 (9)**	**11 (14)**	**9 (10)**	**15 (18)**
Yes, >10 per day	**17 (21)**	**7 (9)**	**17 (20)**	**7 (8)**
Ethnicity, *n* (%) ‖				
Mother: non-Caucasian	7 (9)	9 (11)	9 (10)	8 (9)
Father: non-Caucasian	8 (10)	8 (10)	10 (11)	11 (13)
Socio-economic status, *n* (%)				
Low	8 (10)	8 (10)	10 (11)	7 (8)
Medium	26 (32)	26 (32)	27 (31)	18 (21)
High	47 (58)	47 (58)	50 (57)	60 (71)
Level of education, *n* (%)				
Mother		*		
No education	0 (0)	1 (1)	**0 (0)**	**0 (0)**
Low	25 (31)	25 (31)	**27 (31)**	**11 (13)**
Medium	28 (35)	27 (34)	**29 (33)**	**37 (44)**
High	28 (35)	27 (34)	**31 (36)**	**37 (44)**
Father	*		*	
No education	0 (0)	2 (2)	0 (0)	1 (1)
Low	28 (35)	26 (32)	31 (36)	22 (26)
Medium	26 (33)	16 (20)	26 (30)	26 (31)
High	26 (33)	37 (46)	29 (34)	36 (42)
Child, special education	1 (1)	2 (2)	1 (1)	0 (0)

*One missing value.
†Birth weight for gestational age < -2SDS (Niklasson *et al.*, 1991).
‡Two missing values.
§Three missing values.
‖Turkey classified under non-Caucasian.
Bold $P < 0.05$.

Neuromotor development

The principal investigator (MK) and the reviewing specialist (SV) agreed in 30 out of 32 cases that were reassessed (rate of agreement 0.94). The two cases with disagreement were analysed according to the score of the principal investigator. One ICSI-boy did not complete the examination and questionnaires were incompletely returned in three ICSI-cases. Two children in the IVF-group did not undergo the physical examination because of (i) severe developmental delay of the child (estimated total score of complex MND was assigned, based on parents' interview) and (ii) many previous hospital visits due to a congenital malformation (no score assigned).

Total neuromotor outcome in the ICSI and IVF-groups was similar (Table 3). The outcomes of simple and complex MND were combined to outcome MND, shown in the second part of Table 3. The crude PR of ICSI versus IVF considering neuromotor development normal versus MND was 1.08 (95%CI [0.83; 1.29]). To further investigate the effect of ICSI, we performed logistic regression analysis with the following covariates: maternal age, parity and low birthweight. After adjustment, the ICSI-procedure was still not a predictor for neuromotor development (Adjusted PR = 1.09, 95%CI [0.83; 1.30]). Adjustment for differences in patient characteristics between the two groups (pregnancy complications, paternal smoking and paternal education) did not result in a material change of this PR.

Neither the occurrence of the specific clusters of dysfunction nor movement-quantity and quality was different between the ICSI and IVF groups (Table 4). We found a doubled frequency of children who had ever required physical therapy in the IVF-group and a third fewer IVF-children received speech therapy as compared to ICSI-children. The increase in physical therapy after IVF was mainly due to a higher frequency of gross or fine motor delay (ICSI $n = 3$, IVF $n = 8$). The decrease in speech therapy after IVF disappeared if the comparison was limited to speech therapy due to articulation problems and deviating mouth behaviour, the most relevant causes in this study on neuromotor development.

Comparing ICSI versus NC showed that ICSI-children were more often classified as simple MND than NC-controls (63% versus 49%) (Table 3). The crude PR of ICSI versus NC, considering neuromotor development normal versus MND, was 1.31 95%CI [1.02; 1.55]. Logistic regression analysis, adjusting for maternal age and parity, showed an increased risk of 22%, which was not statistically significant (Adjusted PR = 1.22, 95%CI [0.86; 1.52]) (Table 3). Parity seemed to account for a part of the crude ICSI-effect, as first-born children performed worse than children born with higher parity, and ICSI-children were more often first born (PR parity = 1.33, 95%CI [0.96; 1.64]). Furthermore, ICSI and NC-groups varied in age at the time of examination, and in factors such as paternal age, paternal smoking behaviour, SES and maternal educational level, but none of these factors influenced the adjusted PR.

The occurrence of specific clusters of dysfunction, the frequencies of supporting physical and speech therapy, and

Table 3. Crude and adjusted outcomes of neuromotor development: ICSI versus IVF and ICSI versus NC

Score	ICSI $n = 81$* n (%)	IVF $n = 81$* n (%)		
Normal	27 (34)	31 (39)		$P = 0.802$
Simple MND†	50 (63)	46 (58)		
Complex MND	3 (4)	3 (4)		
Cerebral palsy	0 (0)	0 (0)		
	ICSI $n = 87$* n (%)	NC $n = 85$ n (%)		
Normal	29 (34)	42 (49)		$P = 0.087$
Simple MND	54 (63)	42 (49)		
Complex MND	3 (3)	1 (1)		
Cerebral palsy	0 (0)	0 (0)		
ICSI versus IVF	ICSI $n = 81$* n (%)	IVF $n = 81$* n (%)	PR [95%CI] §	Adjusted PR [95%CI] ‖
Normal	27 (34)	31 (39)	1.08 [0.83; 1.29]	1.09 [0.83; 1.30]
MND‡	53 (66)	49 (61)		
ICSI versus NC, total groups	ICSI $n = 87$* n (%)	NC $n = 85$ n (%)	PR [95%CI]	Adjusted PR [95%CI]**
Normal	29 (34)	42 (49)	1.31 [1.02; 1.55]	1.22 [0.86; 1.52]
MND	57 (66)	43 (51)		
ICSI versus NC, children born at term	ICSI $n = 81$* n (%)	NC $n = 85$ n (%)	PR [95%CI]	Adjusted PR [95%CI]**
Normal	27 (34)	42 (49)	1.31 [1.01; 1.55]	1.20 [0.83; 1.51]
MND	53 (66)	43 (51)		

*One missing value.
†MND, minor neurological dysfunction.
‡Simple MND and complex MND combined.
§Prevalence ratio with 95% confidence interval.
‖Adjustment for maternal age, parity and low birthweight.
**Adjustment for maternal age and parity.

Table 4. Clusters of dysfunction ICSI versus IVF and ICSI versus NC

	ICSI $n = 81$ n (%)	IVF $n = 81$ n (%)	PR*	[95%CI PR] or P-value
Posture and tonus†	9 (11)	4 (5)§	2.19	[0.70; 5.86]
Reflexes	20 (25)	21 (27)§	0.93	[0.52; 1.50]
Involuntary movements	5 (6)	3 (4)§	1.63	[0.39; 5.86]
Coordination	43 (54)‡	35 (44)§	1.21	[0.87; 1.55]
Fine manipulative disability	1 (1)	3 (4)§	0.33	[0.03; 2.88]
Associated movements	0 (0)	0 (0)§		
Sensory deficits	0 (0)	0 (0)§		
Cranial nerve dysfunction	0 (0)	1 (1)§		$P = 0.310$
Quantity of movement	0 (0)	0 (0)§		
Quality of movement				
Normal, fluent	76 (94)	72 (91)§		$P = 0.747$
Moderate	2 (2)	2 (3)		
Abnormal	3 (4)	5 (6)		
Physical therapy	8 (10)§	16 (20)	0.51	[0.22; 1.10]
Speech therapy	16 (20)‡	10 (12)	1.62	[0.78; 3.01]
	ICSI $n = 87$ n (%)	NC $n = 85$ n (%)	PR*	[95%CI PR] or P-value
Posture and tonus‖	9 (10)	3 (4)	2.93	[0.83; 8.69]
Reflexes	21 (24)	14 (16)	1.46	[0.79; 2.45]
Involuntary movements	5 (6)	1 (1)	4.89	[0.59; 29.6]
Coordination	47 (55)‡	34 (40)	1.37	[0.99; 1.72]
Fine manipulative disability	1 (1)	2 (2)	0.49	[0.04; 4.91]
Associated movements	0 (0)	2 (2)		$P = 0.150$
Sensory deficits	0 (0)	0 (0)		
Cranial nerve dysfunction	0 (0)	0 (0)		
Quantity of movement**	1 (1)	3 (4)	0.33	[0.03; 2.90]
Quality of movement				
Normal, fluent	81 (93)	79 (93)		$P = 0.800$
Moderate	2 (2)	1 (1)		
Abnormal	4 (5)	5 (6)		
Physical therapy	8 (9)§	10 (12)	0.79	[0.31; 1.83]
Speech therapy	18 (21)‡	17 (20)	1.05	[0.56; 1.79]

*PR, prevalence ratio.
†ICSI: one hypertonic, eight hypotonic; IVF: one hypertonic, one hypotonic, two changing hypo-/hypertonic.
‡One missing value.
§Two missing values.
‖All hypotonic and hyperlax, except for one ICSI-child.
**All hyperkinetic.

the occurrence of abnormalities in movement-quantity and quality (Table 4) were not significantly different between ICSI-children and NC-controls. However, a dysfunction on cluster coordination (gross motor skills) occurred in 55% of ICSI-children versus 40% of NC-controls (PR = 1.37, 95%CI [0.99; 1.72]). Stratification for parity revealed that this difference was only present in first-born children. When reasons for physical therapy were compared, gross and/ or fine motor dysfunction was equally frequent in the ICSI and NC-group (ICSI $n = 3$ and NC $n = 4$). Frequencies of speech therapy due to articulation problems or deviating mouth behaviour were comparable (articulation ICSI $n = 9$ (10.3%) versus NC $n = 10$ (11.8%); mouth behaviour ICSI $n = 2$ (2.3%) versus NC $n = 5$ (5.9%)).

When considering only term ICSI and NC-children ($n = 81$; $n = 85$, respectively), the crude and adjusted PRs for MND ICSI versus NC were 1.31 [1.01; 1.55] and 1.20 [0.83; 1.51] (Table 3). A non-significant elevation of 34% was found on the coordination cluster (ICSI versus NC PR = 1.34 [0.96; 1.71], data not shown); as in the comparison of the total ICSI and NC-groups, this elevation too was limited to first-born children.

Discussion

From this detailed neurological investigation of 5–8 year old singleton children conceived by ICSI, in comparison with children conceived by IVF and naturally conceived children two conclusions can be drawn. First, there was no effect of the ICSI-procedure itself on neuromotor development in comparison to the more common IVF-procedure, neither on total MND score nor on subscores. Second, in the comparison of ICSI-children with the NC control children, the crude data showed a higher prevalence of simple and overall MND among ICSI-children in both the total group and term children only. This difference largely disappeared upon controlling for parity among mothers.

The comparison between ICSI and IVF was limited to children born at term. The number of premature ICSI-children was small, and we had difficulty in finding matched prematurely born IVF-children. Prematurity is more frequent after ART (Helmerhorst *et al.*, 2004; Jackson *et al.*, 2004) and is associated with neuromotor delay (Veen *et al.*, 1991; Marlow *et al.*, 2005). A comparison of term singletons from ICSI to those from IVF allowed us to assess if there was any extra

effect of ICSI over IVF in addition to the risks following prematurity.

In the comparison between ICSI and NC children, at first the complete groups were compared. Premature ICSI-children were left in, as we wanted to assess what the future parents of an ICSI child might expect on neuromotor development in comparison to that of an NC child (assuming similar parental characteristics up to the time of conception). The same argument was considered in the adjustment for confounding factors. Secondly, we compared ICSI and NC-children who were born at term to assess the net effect of ICSI.

We found that the overall difference in MND between ICSI and NC-children largely disappeared when controlling for parity. In our study, first-born children performed worse on neuromotor development than children of higher birth order. As ICSI-children were more often first-born, this influenced our crude results. The way in which parity influences neuromotor development is unclear. Although adjustment for parity lowered the PR to non-significance, it did not completely explain the findings between the ICSI and NC groups. As we found similar outcomes for ICSI and IVF-children, a shared factor of ICSI and IVF that was not present in NC-children may explain the difference. In a *post-hoc* analysis, we found that low birthweight and being small-for-gestational-age showed only minimal effects in our data. Residual factors that may play a role are infertility status, hormonal stimulation of the mother and *in vitro* manipulation of the oocyte.

The clinical significance of the difference in MND-outcome between ICSI and NC after correction for parity is moderate. However, a slight shift of ICSI-outcomes to poorer neuromotor development is (i) a topic of interest within the scope of science and (ii) may not only result in children shifting from normal to simple MND, but also from simple MND to complex MND.

The increase in rate of physical therapy and decrease in rate of speech therapy in IVF-children seem contradictory. The doubled rate of physical therapy for IVF versus ICSI remained when we limited the children to those who ever needed therapy for fine or gross motor movement. Regarding speech therapy, the most relevant categories in the scope of neuromotor development are articulation and mouth behaviour. The one-third decrease in speech therapy for IVF versus ICSI-children was not present if only these categories were considered. A hypothetical reason why the doubled rate of physical therapy was not reflected in the MND outcomes may be that IVF-children had poorer neuromotor development than ICSI-children, which was diagnosed at a young age and was compensated for with physical therapy up to age 5–8, the age at which we examined the children.

Strengths and weaknesses of the study

What our study adds to those previously carried out is the assessment at a higher age and with a more specific test-instrument with well-defined outcome measures. As children should reach more milestones with ageing, assessment at a higher age allows for a more precise distinction in neuromotor development. Further, the strength of our study lies in the matched controlled design, the blinded examination of each individual child by one trained investigator in a single centre and the blinded review of video-recordings.

A limitation of the study is that in the ICSI–IVF comparison we can only draw conclusions on term children. At the same time, this allowed us to focus on the potential effect of the procedure itself, irrespective of any difference in prematurity rate.

Composing the NC-group of children from regular preschools and primary schools had the disadvantage that control children were inherently neurologically developed to a degree that they could attend regular education. As only one participating ICSI-child relied on special education, we were confident that the ICSI and NC-children had similar educational backgrounds.

By the matching process, we intended to increase the validity of the comparisons. The benefit of this matching procedure was that we needed less control for confounding in the analyses, but the downside was that our sample sizes decreased. Although we reached the number of children to achieve a power of 80%, the difference in MND-prevalence that was found between the two groups was smaller than the difference used for the power calculation. This led to broader confidence intervals than aimed for, but the results remain interpretable.

With response rates of 79% and 73%, respectively, we assumed to have retrieved representative samples of ICSI and IVF-children. Part of the non-responders may have never been reached due to expired home addresses and of the 97 ICSI-responders (88% of those invited) 87 participated (90%). Of the 126 invited IVF-children, 100 had responded (79%), of which 92 (92%) enrolled. The increased participation rate in higher SES families compared with lower SES families will not have influenced our outcomes as we matched for SES and the rates were comparable between ICSI and IVF. The higher rate of male gender in IVF non-participants as compared with participants was unexpected. Hypothetically, the non-participating boys may have had more neuromotor problems and their parents may have been less keen to volunteer them for the study. In that case, the prevalence of neuromotor problems in IVF-children would be an underestimation and finding no differences between ICSI and IVF would reflect a higher than average neuromotor development in ICSI-children.

The NC-group should represent that part of the general population that matches the ICSI-group, but the prevalence of MND in the NC-group was higher than in the general population [51% versus 21% for Dutch children aged 9 (Hadders-Algra *et al.*, 1988; Hadders-Algra, 2002)]. Reassuringly, this increase involved mainly simple MND, which represents non-optimality. However, combined with the apparently high prevalences of physical therapy and speech therapy among NC-children, the impression may a rise that the controls were ‘too pathological’: NC-parents may have been keener to volunteer when they worried about their child’s health or development. If such selection happened, this would change our results and conclusions: the true difference in MND-prevalence between ICSI and NC-children might be larger, and might remain after adjustment for confounding factors.

The high rates of physical and speech therapy do not necessarily point to such selection. Statistics Netherlands reports that 4.6% of the children aged 0–11 visited the physical therapist at least once in the year 2005 (Statistics Netherlands (CBS), 2005). Our data covered the complete history of the children. When excluding children with indications that could not have occurred in 2005 (e.g. hyperextension at infant age), we found that 7.1% of the NC-controls had visited a physical therapist. As this percentage covered several years, we consider it comparable to the 4.6% of the Dutch population that relates to a single year. Speech therapy (including language therapy) is also common in the Netherlands. This is mainly the result of screening at age 5 in schools. In a sample of 20.000–30.000 children covered by about 15 health services (GGD-NL), 7–33% of the children were referred to a speech therapist (Sluijmers and Ter Horst, 2005).

Selection bias based on the child's health or development was also not seen in outcomes that were measured in parallel to the current study. The mean IQ of NC-children was 110; 35% of the NC-children did not visit a general practitioner once in the past year; NC-parents considered their children healthier than other children in 31%, equally healthy in 67% and less healthy in 2% (data not shown). These findings are not in line with the potential selection of children from worried parents. Another argument against selection bias is that a large part of the children that scored simple MND would have never been recognized by the parents as such, as the deviations are minor. Finally, parents had been asked for their reasons to volunteer; in the NC-group answers were mainly 'to support medical sciences/ help other people' and 'being keen to follow my child's development'. 'Worries' were mentioned in four cases, of which only one involved neuromotor development.

Alternative explanations for the increased MND rate in our findings as compared to the reference population could be: (i) a stricter method of examination, (ii) a hypothetical increase in MND in children over the past decades (norm population was born in the '70s) and (iii) other differences between the NC-control group and the norm population (e.g. parity).

We did not take in consideration the possibility of hereditary MND. Diagnosing MND among the parents would have required their full assessment. An alternative would have been to collect information on their need for physical and speech therapy due to motor delay in childhood.

The death of a co-twin *in utero* (vanishing twin) may cause neurological sequelae in the surviving 'singleton' (Melnick, 1977; Pharoah and Adi, 2000; Pinborg *et al.*, 2005). In our study, the number of vanishing twins was comparable for ICSI and IVF. No information was available on the incidence after natural conception, but this was probably lower than after ICSI or IVF, as the incidence of twinning is also lower after natural conception. However, as being the survivor of a vanishing co-twin did not influence our adjusted PR in regression analysis, bias due to vanishing twins was excluded.

Related studies

Our findings of no significant differences in neuromotor development between ICSI and IVF and neither between ICSI and NC, at least after adjustment for parity, are in line with the literature (Sutcliffe *et al.*, 1999; Sutcliffe *et al.*, 2001; Pinborg *et al.*, 2004; Ponjaert-Kristoffersen *et al.*, 2004; Ponjaert-Kristoffersen *et al.*, 2005; Leunens *et al.*, 2006; Belva *et al.*, 2007). Ponjaert-Kristoffersen *et al.* (2004) noted significantly lower scores on the Peabody Developmental Motor Scales for Gross Motor and Fine Motor abilities comparing ICSI-children to NC-controls at age 5. The authors explained that after stratification for site, the difference in Gross Motor Quotient was only present in one (New York) of the two centres (New York and Brussels) studied. This finding could be in line with the effect of adjustment for parity in our study: in Brussels only primiparous women had been included, whereas in New York a higher number of primiparous women in the ICSI-group compared with the NC-group might have lead to worse motor outcomes for the ICSI-group.

From the present study, in which ICSI-children underwent a complete and detailed neuromotor examination at the more advanced age of 5–8, we can conclude that neuromotor outcome of 5–8 years old singleton children born at term after an ICSI or IVF-procedure was similar, but ICSI-children (both the total group and term children only) deviated slightly from NC-controls. Part of the latter effect might be explained by a difference in the mother's parity. Thus, the overall conclusion of this study on neuromotor development is in line with the literature, and is reassuring for future parents of ICSI-children.

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