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ORIGINAL ARTICLE ***Reproductive epidemiology***

Two-year development of children conceived by IVM: a prospective controlled single-blinded study

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STUDY QUESTION: Is there a difference in mental development of children conceived by IVM in comparison to IVF or ICSI, independently, at the age of 2 years?

SUMMARY ANSWER: No differences could be found in mental development of IVM children compared to IVF and IVM children compared to ICSI as well.

WHAT IS KNOWN ALREADY: Only few retrospective or non-controlled studies addressed the health of IVM children and did not show a negative impact of the IVM procedure.

STUDY DESIGN, SIZE, DURATION: Prospective controlled single-blinded study including 63 pregnancies (21 per IVM, IVF and ICSI groups) with 70 children expected. Examinations of 62 embryos at first trimester screening, of 57 fetuses at 21st week of pregnancy, of 60 children at birth and of 37 children at their second birthday were performed during the study period from January 2009 until October 2016. Bayley score at the age of 2 was the primary outcome parameter. Data of 40 children after spontaneous conception from a previous prospective unrelated study were further used as control at 2 years examination and compared to the pooled ART group (IVM, IVF and ICSI).

PARTICIPANTS/MATERIALS, SETTING, METHODS: Twenty-one IVM pregnancies achieved in the study period were included. For each of them, the following IVF- and ICSI pregnancies were recruited as controls. Ultrasound examinations during pregnancy, examinations of newborns and of children around their second birthday were done by blinded prenatal specialists, pediatricians and neuropediatricians, respectively.

MAIN RESULTS AND THE ROLE OF CHANCE: Children conceived after IVM did not show differences during embryonic development, at birth nor in their neuropediatric development at the age of 2 compared to their counterparts after IVF and after ICSI (Bayley score 91.3 ± 21.0 for IVM, 96.8 ± 13.2 for IVF and 103.9 ± 13.1 for ICSI) and of the pooled ART group compared to children after spontaneous conception (96.6 ± 16.4 ART and 103.2 ± 9.4 spontaneous conception). When analyzing singleton pregnancies only, again no differences during pregnancy, at birth and at their 2-year evaluation were detected between IVM versus IVF and IVM versus ICSI.

LIMITATIONS, REASONS FOR CAUTION: Due to the small sample size data must be interpreted with caution. To allow a confirmative answer that there are no health risks for children conceived by IVM, large multicenter cohort or registry-based studies are urgently needed.

WIDER IMPLICATIONS OF THE FINDINGS: The study adds further information to previous uncontrolled or retrospective studies, which were unable to detect risks for the health of IVM children.

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TRIAL REGISTRATION NUMBER: Not applicable.

Key words: IVM / health of children / IVF / ICSI / mental development / Bayley score

Introduction

After the first successful IVM in an animal model by Pincus and Enzmann (1935), the method was transferred to human oocytes by Edwards in 1965 and the first human live birth after IVM was obtained in 1991 (Cha *et al.*, 1991). Today, it is estimated that more than 5000 babies were born worldwide after IVM up to now (Sauerbrun-Cutler *et al.*, 2015). There is constant concern that IVM of oocytes might lead to epigenetic disorders eventually increasing the risk for diseases based on imprinting defects such as Beckwith–Wiedeman or Angelman syndrome (Chen *et al.*, 2013). However, Kuhtz *et al.* (2014) could not detect major epigenetic alterations in IVM oocytes. Furthermore, we were unable to detect an unusually high rate of imprinting defects in umbilical cord blood samples and placental tissues of children conceived by IVM in a previous study (Pliushch *et al.*, 2015). Up to now, no prospective controlled study has been performed to examine the long-term health and the development of children conceived by IVM. Some retrospective or uncontrolled smaller studies did not find any risks for the offspring (Mikkelsen 2005; Söderström-Anttila *et al.*, 2006; Cha *et al.*, 2005). In a recent retrospective study, Fadini *et al.* (2012) have analyzed obstetric and perinatal outcome of 79 children definitely derived from IVM oocytes compared to 104 children conceived from mature oocytes and further 194 children after conventional ICSI. They found comparable obstetric and perinatal outcomes including minor and major abnormalities. Buckett *et al.* (2007) also described no differences in birth weight at term between IVM, IVF and ICSI children in a retrospective analysis. Hence, we decided to set up a prospective controlled single-blinded study to evaluate the mental development of children conceived by IVM at the age of 2 as our primary aim. IVM children were compared to simultaneously prospectively recruited children after IVF or ICSI. To our knowledge, this is the first prospective controlled single-blinded study on this topic so far up to the age of 2 years.

Participants and Methods

The study was designed as a prospective controlled single-blinded study.

Study population

Between January 2009 and October 2015, each woman getting pregnant after IVM at the Department of Gynecological Endocrinology and Fertility Disorders, University of Heidelberg, Germany, and who presented with positive fetal heartbeats was invited to participate in the study. IVM pregnancies resulting from cryopreserved embryos were also included following the same protocol. For each IVM pregnancy, the next following intact pregnancies after conventional IVF and ICSI were prospectively recruited and matched as controls. To search for possible differences in children conceived after ART at all regardless of the techniques used and natural conception, data from IVM/IVF/ICSI pregnancies were pooled and compared with data of a group of 40 children after spontaneous conception (control group). These children from a previous unrelated study were matched for maternal profession and age. This study included 69 children born between June 2008 and October 2009 with prospective recruitment. All children were delivered at term, risk pregnancies, multiples and children born after ART had been excluded. Socio-economic status was controlled with regard to professions of parents in all groups.

All but one woman, pregnant after IVM, who have been approached, agreed to participate. One woman refused participation due to the long distance from her place of residence. Ten patients with an IVM pregnancy had an early miscarriage at week 6 or earlier before they could be contacted.

Study design

Potential risk conditions (age of mother and father, smoking habits and BMI of the mother) were recorded. Between 12+2 and 13+6 weeks of pregnancy, ultrasound examinations of embryonic parameters (crown-rump-length, biparietal diameter (BIP), femur length (FL), nuchal translucency (NT) and nasal bone) and biochemical analysis of free human chorionic gonadotropin (hCG) as well as placental associated protein-A were performed by a blinded prenatal specialist.

Between 19+0 and 22+0 weeks of gestation, a second ultrasound of the fetal development (BIP, head and abdominal circumference, FL, weight (Headlock)) was also performed in a blinded manner.

After birth, fetal umbilical cord blood and placental tissue were collected for genetic analysis. Genetic analyses were published elsewhere (Pliushch *et al.*, 2015). The newborn was examined by a neonatologist, and neonatal parameters were assessed (sex, birth modus, week of pregnancy at birth, weight, length, head circumference and pH).

At the age of 2 years, children were examined by a specially trained blinded neuropediatrician. Again weight, length and head circumference were measured. Global cognitive performance was evaluated using the mental scale of the German version of *Bayley Scales of Infant Development* Second Edition Bayley-II (Reuner *et al.*, 2008). Raw scores were transformed into standardized scores with a mean of 100, standard deviation (SD) 15 (Mental Developmental Index, MDI) using the US normative data (Bayley, 1993). Development was rated as normal when MDI was above 85, lower MDI were classified as delayed development.

Ethical approval

The local ethics committee of the medical faculty, university of Heidelberg approved the protocol. From each patient, written informed consent was obtained. Also the local ethics committee had

approved the previous unrelated study from which the control group was extracted.

Statistical analysis

Statistical analysis was performed with SAS 9.4WIN (SAS Institute, Cary, NC, USA). The empirical distributions of continuous data were expressed with mean and SD, by categorical data with absolute and relative frequencies. Possible differences between ART groups (IVM versus IVF and IVM versus ICSI) were verified with Student's *t*-test for independent data. Birth weight was analyzed by covariance analysis (ANCOVA) using linear adjustment for gestational duration and maternal age, and the fitted estimates and their standard errors were shown. Since there was no difference between the groups regarding neither the cognitive performance (Bayley-II MDI) nor the biometric parameters (Tables IV–VIII) at birth and at the age of 2 years, we pooled the assisted reproduction patients (IVM/IVF/ICSI) in one group (ART group) for comparison to the control group of children after spontaneous conception with analysis of covariance and linear adjustment for gestational age at birth. Chi-square test was used for categorical data. *P*-values <0.05 were regarded as statistically significant. Primary endpoint was the mental and motoric development at 24 months evaluated by Bayley score.

Results

During the study time frame, 21 pregnant patients in each group after IVM (19 fresh transfers, 2 frozen-thawed transfers), IVF and ICSI were recruited, representing a total of 70 embryo implantations (1 twin pregnancy after IVM, 4 twin pregnancies after IVF, 2 twin pregnancies after ICSI, Fig. 1). Indications for ART were one case of cycle disorders, 20 cases of andrological factor in the ICSI group, 4 patients with endometriosis Grades 1 and 2, one case of endometriosis Grade 3–4, 12 cases with tubal pathology, 19 cases with PCOS. In six patients, a single indication was missing. No other severe maternal diseases were reported. In seven IVM patients, fertilization was achieved by IVF and in 14 patients by ICSI.

Sixty-two out of 70 embryos could be examined in first trimester screening. Five pregnancies ended as early miscarriages after recording of fetal positive heartbeats (four IVM, one ICSI), one withdrawal from the study (ICSI), two patients (one IVM, one ICSI) refusing this ultrasound and three examinations were excluded because patients presented outside the defined screening window (one IVM, one IVF, one ICSI). The second ultrasound examination could be done in 60 fetuses, 2 late miscarriages took place before (both twin pregnancies after IVF). Three of 60 second trimester ultrasound examinations were not performed within the defined time frame (two IVM, one ICSI) and therefore excluded from this part of the analysis. By the end of October 2015, 60 children were born. For analysis at birth, full data sets of 60 babies were available. At the age of 2 years, 37 children were examined up to now, 30 of them underwent a complete follow-up including assessment with Bayley-II (10 IVM, 13 IVF, 7 ICSI, see Fig. 1). Dropouts with incomplete 2 years testing were due to lack of cooperation (four children) and Moebius syndrome (one child).

When analyzing socio-demographic data of parents ($n = 63$ mothers and $n = 63$ fathers), we found a lower maternal age in the IVM group compared with the IVF and ICSI groups ($P = 0.025$, $P = 0.031$, respectively). Mean maternal age of the total group of IVM/IVF/ICSI (ART group) did not differ statistically compared to controls with spontaneous pregnancies (data not shown). All other parameters also did not differ significantly (Table I).

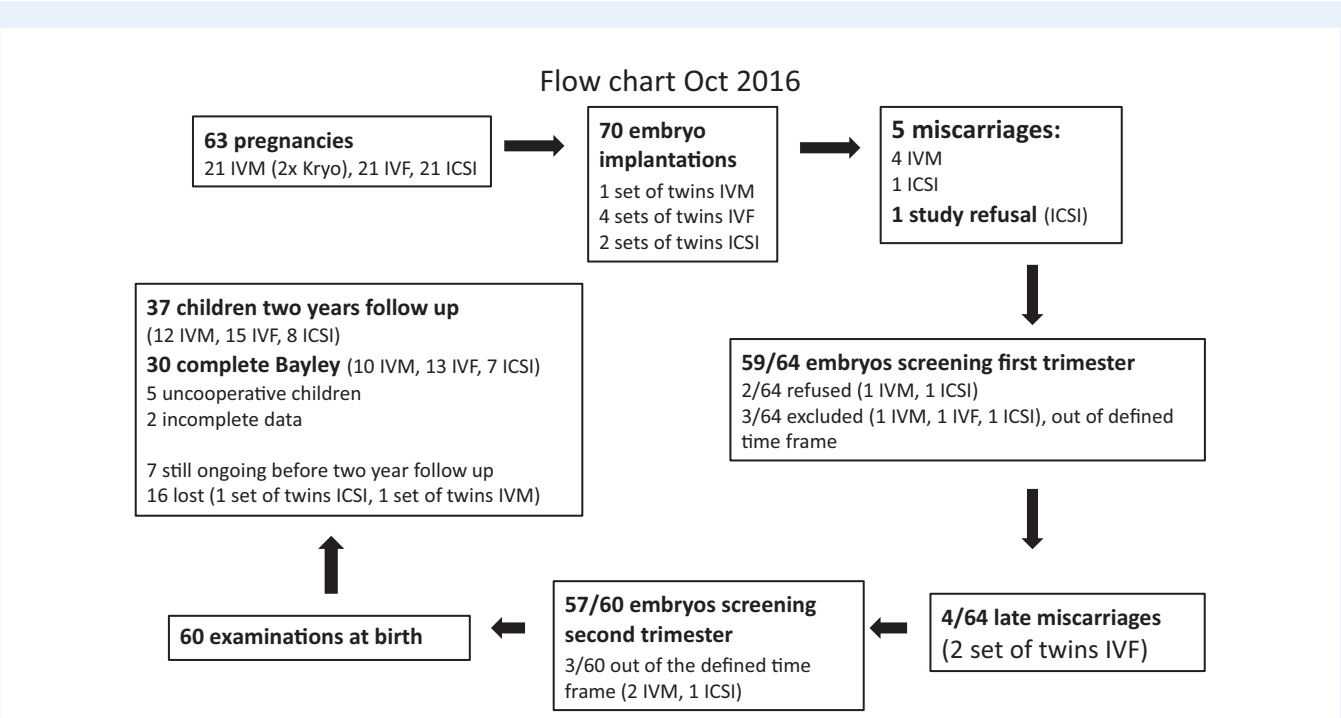

Figure 1 Flow chart of patients enrolled.

Table I Descriptive data of parents.

	IVM	IVF	IVM versus IVF, P^+	ICSI	IVM versus ICSI, P^+	Controls
n	21	21		21		40
Age mother (*y*)	31.6 ± 3.4	34.2 ± 3.9	0.025	34.5 ± 4.8	0.031	33.9 ± 4.6
BMI mother (kg/m²)	26.0 ± 6.0	23.4 ± 4.1	0.104	23.0 ± 4.3	0.067	n.a.
Mother smoking (*n*)	6	6		6		0
Age father (*y*)	35.3 ± 4.6	35.7 ± 6.6	0.829	37.7 ± 5.1	0.119	37.3 ± 7.0
Father smoking (*n*)	3	5		4		n.a.

Values are given as mean ± SD or number (*n*).
y, years; n.a., not assessed.
$^+$Student's *t*-test.

Table II Biometric parameters at first ultrasound (12+2 to 13+6th weeks of pregnancy).

Parameters	IVM	IVF	IVM versus IVF, P^+	ICSI	IVM versus ICSI, P^+
Total (*n* = 59)	16	24		19	
CRL (mm)	68.3 ± 4.5	67.6 ± 5.6	0.634	68.9 ± 6.4	0.757
BIP (mm)	22.3 ± 1.5	22.8 ± 1.9	0.350	22.2 ± 2.1	0.942
FL (mm, *n* = 58)	9.0 ± 1.3	9.5 ± 1.6	0.309	9.5 ± 1.9	0.362
NT (mm)	1.6 ± 0.2	1.6 ± 0.4	0.852	1.6 ± 0.3	0.899
Nasal bone (mm, *n* = 48)	2.6 ± 0.6	2.5 ± 0.5	0.862	2.6 ± 0.4	0.975
PAPP-A (U/l, *n* = 51)	6.2 ± 6.7	9.1 ± 17.3	0.497	6.2 ± 4.4	0.996
hCG (IU/l, *n* = 52)	45.8 ± 42.7	49.4 ± 47.6	0.816	60.6 ± 46.7	0.367
Singletons (*n* = 49)	14	20		15	
CRL (mm)	69.4 ± 3.7	67.7 ± 4.9	0.282	69.8 ± 6.9	0.836
BIP (mm)	22.3 ± 1.6	23.0 ± 1.8	0.235	22.5 ± 2.2	0.726
FL (mm, *n* = 48)	9.1 ± 1.4	9.7 ± 1.7	0.287	9.9 ± 1.8	0.174
NT (mm)	1.6 ± 0.2	1.6 ± 0.4	0.749	1.7 ± 0.3	0.687
Nasal bone (mm, *n* = 38)	2.6 ± 0.7	2.5 ± 0.4	0.607	2.7 ± 0.3	0.794
PAPP-A (IU/l, *n* = 46)	6.4 ± 6.9	9.2 ± 18.3	0.561	5.7 ± 4.5	0.760
hCG (IU/l, *n* = 46)	40.5 ± 39.3	35.1 ± 15.4	0.649	47.3 ± 22.6	0.587

Values are given as mean ± SD.
CRL, crown-rump-length; BIP, biparietal diameter; FL, femur length; NT, nuchal translucency; PAPP-A, placental associated protein-A; hCG, human choriogonadic gonadotropin.
$^+$Student's *t*-test.

Development during pregnancy

At first ultrasound examination between 12+2 and 13+6 weeks of pregnancy, no differences were found in parameters of embryonic development (Table II). During the second ultrasound examination between 19+0 and 22+0 weeks of pregnancy (Table III) again, we found no differences between groups (Tables II and III).

Children's outcome at birth

Biometric parameters at birth showed no significant differences between IVM versus IVF and IVM versus ICSI. Because range of birth weight was 1080–4000 g in IVM, 980–3900 g in IVF and 760–4080 g in ICSI due to three preterm deliveries birth weight was adjusted for maternal age and gestational age at birth to include the three preterm births without showing a significant difference (Tables IV and V). If singletons were analyzed separately, again no differences could be detected (Tables IV and V). In the IVM group, two children were observed with abnormalities at birth: one child with a Moebius syndrome and one with spasticity. Five abnormalities were found in the IVF group (ventricular septum defect, appendix of the skin, intrauterine growth retardation (IUGR), renal congestion, hyperbilirubinaemia) and two abnormalities after ICSI (disturbance of breathing, IUGR).

Two-year follow-up examination

At the 2-year follow-up examination, no differences in anthropometric parameters were found between IVM versus IVF and IVM versus ICSI, also if singletons were analyzed separately (Table VI). Two children of

Table III Biometric parameters at second ultrasound (19+0 to 22+0th weeks of pregnancy).

Parameters	IVM	IVF	IVM versus IVF, P^+	ICSI	IVM versus ICSI, P^+
Total ($n = 57$)	16	21		20	
BIP (mm)	49.8 ± 1.8	51.1 ± 2.7	0.073	50.0 ± 2.8	0.717
Head circumference (mm)	182.8 ± 6.8	184.5 ± 12.4	0.598	183.8 ± 9.8	0.725
Abdominal circumference (mm)	157.9 ± 8.5	158.6 ± 11.1	0.838	154.9 ± 12.8	0.398
FL (mm)	33.5 ± 2.0	34.7 ± 2.6	0.107	34.0 ± 2.6	0.486
Weight headlock (g, $n = 54$)	374.2 ± 39.8	394.5 ± 60.9	0.236	387.1 ± 58.4	0.458
Singletons ($n = 47$)	14	17		16	
BIP (mm)	49.9 ± 1.7	50.7 ± 2.7	0.298	50.4 ± 2.8	0.575
Head circumference (mm)	183.5 ± 6.9	182.6 ± 12.7	0.816	183.7 ± 9.7	0.938
Abdominal circumference (mm)	158.5 ± 8.9	156.3 ± 10.7	0.555	156.7 ± 12.6	0.654
FL (mm)	33.8 ± 1.9	34.7 ± 2.9	0.304	34.1 ± 2.7	0.747
Weight headlock (g, $n = 44$)	379.2 ± 40.4	386.5 ± 63.7	0.704	396.5 ± 55.0	0.357

Values are given as mean ± SD.
$^+$Student's *t*-test.

the IVM group were suspicious for an auditory deficit. In the IVF group, three abnormalities were found: two children suspicious for an auditory deficit and one with microcephalus. No abnormalities were seen in the ICSI group.

With regard to cognitive development at 2 years of age, all groups (IVM, IVF and ICSI) had mean MDIs in the normal range. On a descriptive level, the IVM group had the lowest mean performance. On a statistical level, the IVM group did not differ significantly from the IVF group or the ICSI group (Tables VII and VIII). We found also no differences if singletons were analyzed separately (Tables VII and VIII). Absolute values are shown in Tables VII and VIII. Although children born after assisted reproduction had a higher proportion of abnormal MDI below 85 when compared to controls, no statistical differences could be detected, neither in the raw nor in the adjusted data (Table IX). When analyzing singletons after ART compared to the control group, a significant difference could be found in the adjusted data for MDI (95.9 ± 2.6 versus 103.2 ± 2.1, $P = 0.0307$, Table IX).

Discussion

To the best of our knowledge, this is the first prospective controlled single-blinded study to observe the mental development of children born after IVM including a 2-year follow-up period. Twenty-one pregnancies after IVM as well as 21 pregnancies per IVF and ICSI were included, starting with 70 embryos and leading to the birth of 60 children. Furthermore, data of 40 children after spontaneous conception from a previous unrelated study served as controls for perinatal outcome and cognitive development at the age of 2 years. The prospective controlled design including IVF and ICSI is a definite strength of our study.

Neither the embryonic development during pregnancies nor the examination at birth nor at 2-year follow-up revealed statistically significant differences between IVM compared to IVF or ICSI.

We observed four miscarriages in the IVM group, one in the ICSI group and none in the IVF group. In a large retrospective analysis of a total of 1 581 positive pregnancy tests (120 IVM, 849 IVF and 612 ICSI), Buckett *et al.* described a clinical miscarriage rate after IVM of 25.3%, which was statistically significantly different from 15.7% after IVF and 12.6% after ICSI. If they analyzed cycles of PCOS patients, only the clinical miscarriage rates were similar (24.5% after IVM and 22.2% after IVF, Buckett *et al.*, 2008). This is in accordance with Walls *et al.* (2015), who also found no significant difference in miscarriage rates after IVM and conventional IVF in a retrospective analysis of 80 IVM cycles compared to 98 IVF cycles in patients with PCOS. A small prospective study by Choi *et al.* (2012) also failed to show a higher miscarriage rate after IVM compared to two conventional IVF protocols. Our sample size is rather small, so we cannot comment sufficiently on a possibly higher miscarriage rate due to the IVM technique. However, a higher miscarriage rate after IVM is rather unlikely from the previous literature at least in PCOS patients.

Fetal development and ultrasound assessments during pregnancies have not been addressed by other studies so far. There are convincing data published that children conceived by ART in general have perinatal outcomes different from naturally conceived ones regardless of the method used (Pinborg *et al.*, 2013). Perinatal data on IVM have been published in some not prospectively controlled studies and are confirmed by our results (Cha *et al.*, 2005; Shu-Chi *et al.*, 2006; Buckett *et al.*, 2007; Fadini *et al.*, 2012). In our study, three children were born before 32 weeks of pregnancy. Therefore, we have used an analysis adjusted to gestational age. The birth weight of children did not show any significant differences between groups. These results confirm the findings of a retrospective study by Buckett *et al.* (2007), who also found similar birth weights at term between IVM, IVF and ICSI children in their study group of 432 children conceived by ART. On the other hand, Fadini *et al.* (2012) reported that children delivered at term of the IVM group had a higher birth weight than ICSI children. However, 13 babies of their IVM group were delivered from oocytes of both types, *in vitro* and *in vivo* matured. When they excluded pregnancies potentially derived from both, mature or immature, oocytes and analyzed pregnancies from *in vitro* matured oocytes only, no differences in birth weight of singletons were detected. This rather excludes a negative impact of IVM on children's weight at

Table IV Biometric parameters at birth IVM versus IVF.

Parameters	IVM	IVF	*P*	Difference [95% CI]
Total (*n*)	18	21		
Gestational age (weeks)	39.0 (3.0)	38.9 (2.5)	0.873[+]	0.14 [−1.62; 1.91]
Birth weight (g)				
Raw	3036.4 (704.0)	2990.5 (737.7)	0.844[+]	45.9 [−434.3; 516.1]
Adjusted	2981.4 (83.6)	2894.2 (79.8)	0.469[++]	42.4 [−206.3; 291.2]
Length (cm, *n* = 57)	50.4 (3.9)	49.7 (4.3)	0.613[+]	0.67 [−2.02; 3.37]
Head circumference (cm, *n* = 55)	34.1 (2.9)	33.7 (2.7)	0.701[+]	0.36 [−1.52; 2.24]
pH (*n* = 57)	7.26 (0.08)	7.29 (0.05)	0.206[+]	−0.028 [−0.070; 0.014]
Female/male (*n*)	9/9	7/14		
Way of delivery (*n*)				
Spontaneous	8	9		
CS	8	11		
VE	2	1		
Forceps	0	0		
Singletons (*n*)	16	17		
Gestational age (weeks)	39.0 (3.2)	39.4 (2.3)	0.675[+]	−0.42 [−2.37; 1.55]
Birth weight (g)				
Raw	3050.3 (747.6)	3166.8 (704.8)	0.649[+]	−116.5 [−632.1; 400.4]
Adjusted	3051.9 (89.9)	3035.9 (87.4)	0.902[++]	−14.1 [−287.1; 258.8]
Length (cm, *n* = 47)	50.3 (4.2)	50.6 (4.3)	0.853[+]	−0.28 [−3.29; 2.74]
Head circumference (cm, *n* = 45)	34.1 (3.1)	34.1 (2.8)	0.987[+]	0.02 [−2.17; 2.21]
pH (*n* = 47)	7.25 (0.08)	7.28 (0.05)	0.273[+]	−0.027 [−0.077; 0.022]
Female/male (*n*)	7/9	6/11		
Way of delivery (*n*)				
Spontaneous	8	9		
CS	6	7		
VE	2	1		
Forceps	0	0		

Values are given as mean ± SD, adjusted values as fitted estimates ± SE.
CS, Cesarean section; VE, vacuum extraction.
[+]Student's *t*-test.
[++]ANCOVA adjusted for maternal age and gestational age at birth.

delivery. In the study of Foix-L'Hélias *et al.* (2014), significant differences in weight, height and head circumference at birth could be found in girls derived from IVM cycles compared with ICSI girls. At the age of 1 year, IVM girls were still heavier and taller than ICSI girls. No differences were found in male children. This gender-specific difference might be rather the result of underlying maternal characteristics like PCOS, which might have more influence on the intrauterine development of girls than of the IVM technique itself. Due to our small sample size, we were unable to comment on any gender differences between the three groups.

With regard to cognitive development at 2 years of age, mean MDI for IVM, IVF and ICSI was within the normal range, i.e. within 1 SD of the standardized MDI score. Mean MDI after IVM was ~5 points above the score of 85, which was defined as lower limit. In our IVM group, both techniques for fertilization, IVF and ICSI, were represented. Due to the sample size, we were unable to look at these small subgroups separately.

Our study has some potential limitations. We saw remarkable differences of means, for example of more than 5% for singletons between IVM and IVF, and of more than 14% between IVM and ICSI. If we look at lower confidence limits, we observed an even more pronounced effect with differences of means between −20 and −30 points, leading to an estimated mean of even below 80. If this effect is confirmed by larger data sets, we cannot exclude a potential clinical relevance of our findings on mental development of IVM versus IVF or ICSI children. Beside our small study size, another reason for this finding might depend on the magnitude of absolute MDI values below 85. The two IVM children with MDI <85 had an MDI of 62 and below 50, whereas minimal MDIs after IVF (70 and 76) and after ICSI (80) were closer to the lower MDI threshold. Therefore, a finding of 'no difference' in means of MDI must be interpreted with caution. Our mean MDIs in the normal range are comparable to results of the few available studies on IVM children to this point (Shu-Chi *et al.*, 2006; Söderström-Anttila *et al.*, 2006). Comparison is somehow difficult, since Söderström-

Table V Biometric parameters at birth IVM versus ICSI.

Parameters	IVM	ICSI	*P*	Difference [95% CI]
Total (*n*)	18	21		
Gestational age (weeks)	39.0 (3.0)	38.3 (4.1)	0.560[+]	0.67 [−1.68; 3.02]
Birth weight (g)				
Raw	3036.4 (704.0)	2984.8 (903.6)	0.842[+]	51.6 [−480.7; 584.0]
Adjusted	2981.4 (83.6)	3072.8 (74.8)	0.428[++]	−72.7 [−315.0; 169.7]
Length (cm, *n* = 57)	50.4 (3.9)	50.2 (2.8)	0.885[+]	0.17 [−2.16; 2.49]
Head circumference (cm, *n* = 55)	34.1 (2.9)	34.7 (1.9)	0.487[+]	−0.60 [−2.26; 1.07]
pH (*n* = 57)	7.26 (0.08)	7.22 (0.12)	0.209[+]	0.042 [−0.027; 0.110]
Female/male (*n*)	9/9	12/9		
Way of delivery (*n*)				
Spontaneous	8	4		
CS	8	13		
VE	2	3		
Forceps	0	1		
Singletons (*n*)	16	17		
Gestational age (weeks)	39.0 (3.2)	38.9 (4.2)	0.929[+]	0.12 [−2.56; 2.79]
Birth weight (g)				
Raw	3050.3 (747.6)	3135.9 (936.4)	0.773[+]	−85.6 [−689.6; 518.5]
Adjusted	3051.9 (89.9)	3179.6 (82.9)	0.312[++]	−98.0 [−369.0; 172.9]
Length (cm, *n* = 47)	50.3 (4.2)	51.0 (2.5)	0.586[+]	−0.69 [−3.32; 1.95]
Head circumference (cm, *n* = 45)	34.1 (3.1)	35.4 (1.4)	0.192[+]	−1.22 [−3.03; 0.59]
pH (*n* = 47)	7.25 (0.08)	7.19 (0.12)	0.078[+]	0.065 [−0.008; 0.139]
Female/male (*n*)	7/9	8/9		
Way of delivery (*n*)				
Spontaneous	8	4		
CS	6	9		
VE	2	3		
Forceps	0	1		

Values are given as mean ± SD, adjusted values as fitted estimates ± SE.
[+]Student's *t*-test.
[++]ANCOVA adjusted for maternal age and gestational age at birth.

Table VI Biometric parameters at the age of 2 years.

	IVM	IVF	IVM versus IVF, *P*[+]	ICSI	IVM versus ICSI, *P*[+]
Total (*n* = 35)	12	15		8	
Weight (g)	11.8 ± 1.4	12.1 ± 1.5	0.656	12.8 ± 1.8	0.228
Length (cm)	88.0 ± 3.6	87.4 ± 3.3	0.688	88.7 ± 3.6	0.665
Head circumference (cm)	48.7 ± 1.8	48.1 ± 1.6	0.446	48.9 ± 1.8	0.747
Singletons (*n* = 31)	12	11		8	
Weight (g)	11.8 ± 1.4	11.9 ± 1.7	0.941	12.8 ± 1.8	0.228
Length (cm)	88.0 ± 3.6	87.0 ± 3.5	0.536	88.7 ± 3.6	0.665
Head circumference (cm)	48.7 ± 1.8	48.0 ± 1.8	0.405	48.9 ± 1.8	0.747

Values are given as mean ± SD or number (*n*).
[+]Student's *t*-test.

Table VII Cognitive performance at the age of 2 years IVM versus IVF (Bayley-II mental development index (MDI), primary endpoint).

Endpoint	IVM	IVF	*P*	Difference [95% CI]
Total (*n*)	10	13		
Age at assessment (months)	24.8 (1.2)	24.1 (0.7)	0.122[+]	0.69 [−0.13; 1.50]
Bayley-II MDI				
Raw	91.3 (21.0)	96.8 (13.2)	0.483[+]	−5.50 [−20.35; 9.42]
Adjusted	90.8 (5.1)	96.8 (4.5)	0.387[++]	−6.02 [−20.06; 8.03]
MDI <85				
n (%)	2 (20.0)	2 (15.4)	0.772[+++]	0.05 [−0.35; 0.44]
Absolute value	49, 62	70, 76		
Singletons (*n*)	10	9		
Age at assessment (months)	24.8 (1.2)	24.4 (0.7)	0.357[+]	0.41 [−0.53; 1.35]
Bayley-II MDI				
Raw	91.3 (21.0)	94.7 (15.6)	0.695[+]	−3.37 [−21.21; 14.47]
Adjusted	90.8 (5.1)	93.5 (5.7)	0.778[++]	−2.24 [−18.51; 14.04]
MDI <85				
n (%)	2 (20)	2 (22.2)	0.906[+++]	−0.02 [−0.43; 0.43]
Absolute value	49, 62	70, 76		

Values are given as mean ± SD, adjusted values as fitted estimates ± SE.
Bayley MDI <50 was scored as 49.
[+]Student's *t*-test.
[++]ANCOVA adjusted for gestational age at birth.
[+++]Chi-square.

Table VIII Cognitive performance at the age of 2 years IVM versus ICSI (Bayley-II mental development index (MDI), primary endpoint).

Endpoint	IVM	ICSI	*P*	Difference [95% CI]
Total = singletons (*n*)	10	7		
Age at assessment (months)	24.8 (1.2)	24.7 (0.8)	0.865[+]	0.08 [−1.00; 1.16]
Bayley-II MDI				
Raw	91.3 (21.0)	103.9 (13.1)	0.151[+]	−12.56 [−30.26; 5.15]
Adjusted	90.8 (5.1)	104.4 (6.1)	0.103[++]	−13.61 [−23.23; 8.05]
MDI <85				
n (%)	2 (20.0)	1 (14.3)	0.761[+++]	0.06 [−0.42; 0.50]
Absolute value	49, 62	80		

Values are given as mean ± SD, adjusted values as fitted estimates ± SE.
Bayley MDI <50 was scored as 49.
[+]Student's *t*-test.
[++]ANCOVA adjusted for gestational age at birth.
[+++]Chi-square.

Attila *et al.* did not use a control group beside standards of healthy Finnish children. Shu-Chi *et al.* also did not find a developmental delay after IVM. However, they tested children between the age of 6 months and 2 years matched to controls and had no final 2-year examination performed for the entire group. In our study, we cannot provide real evidence that there are definitely no differences between techniques due to the rather small sample size. Therefore, any conclusions on safety of IVM must be drawn with caution.

Mean MDI after assisted reproduction was also within normal range. When we compared our pooled ART data to a matched control group of children born after spontaneous conception, we saw a lower MDI after ART with no statistical significance in the overall comparison but a slight difference in regard to singletons only. However, mean differences between ART and controls are remarkable and were above 7 points with the upper confidence limit exceeding 13. So again we cannot exclude clinically meaningful differences, although mean MDIs

Table IX Cognitive performance at the age of 2 years ART group versus controls (Bayley-II mental development index (MDI), primary endpoint).

Endpoint	ART	Control	*P*	Difference [95% CI]
Total (*n*)	30	40		
Age at assessment (months)	24.5 (0.9)	23.9 (0.9)	0.005[+]	0.62 [−1.04; −0.19]
Bayley-II MDI				
Raw	96.6 (16.4)	103.2 (9.4)	0.054[+]	−6.63 [−0.11; 13.36]
Adjusted	96.8 (2.4)	103.1 (2.0)	0.0496[++]	−6.25 [0.01; 12.48]
MDI <85				
n (%)	5 (16.7)	2 (5.0)	0.107[+++]	0.12 [−0.12; 0.35]
Absolute value	49, 62, 70, 76, 80	84, 84		
Singletons (*n*)	26	40		
Age at assessment (months)	24.6 (0.9)	23.9 (0.9)	0.0008[+]	0.77 [−1.21; −0.33]
Bayley-II MDI				
Raw	95.8 (17.5)	103.2 (9.4)	0.0563[+]	−7.38 [−0.21; 14.97]
Adjusted	95.9 (2.6)	103.1 (2.0)	0.0307[++]	−7.28 [0.70; 13.86]
MDI <85				
n (%)	5 (16.7)	2 (5.0)	0.067[+++]	0.14 [−0.10; 0.38]
Absolute value	49, 62, 70, 76, 80	84, 84		

Values are given as mean ± SD, adjusted values as fitted estimates ± SE.
Bayley MDI <50 was scored as 49.
[+]Student's *t*-test.
[++]ANCOVA adjusted for gestational age at birth.
[+++]Chi-square.

were in normal range. If this finding is confirmed, it might have an impact on the assessment of ART. Only two children of the control group had minimally lower MDIs below 85 (both 84) in contrast to five children with more pronounced low MDI values in the ART group (between 80 and below 50). This underlines further the difficulty of interpretation of our findings. So we cannot exclude a possible overall impact of ART on mental development based on our small data set. On the opposite, the long-time outcome of children with a lower MDI is hard to predict. The predictive validity of the Mental Development Index MDI by Bayley-II in the age of 2 years is rather poor making conclusions from our findings even more difficult (Potharst *et al.*, 2012). Smoking was surprisingly common in women with ART. In contrast no female smokers were reported in the control group of natural conception. So smoking might be one possible confounder and we cannot exclude a possible influence of smoking during pregnancy in the ART groups.

In addition, we could not detect epigenetic alterations in blood samples from umbilical cord blood and placental tissue from IVM children compared to ICSI and IVF. These results have been published previously by our group (Pliushch *et al.*, 2015).

Two abnormalities were found in IVM children, five in IVF children and two in ICSI children. Except of one case of Moebius syndrome, all other abnormalities were unlikely associated with a genetic defect and were of intermittent character (renal congestion, hyperbilirubinaemia and disturbance of breathing). Parents of the child with Moebius syndrome were consanguineous partners (cousins), what might be the underlying cause rather than the IVM technique. The child presenting with spasticity at birth could not be examined at second birthday because of lost to follow-up. Buckett *et al.* (2007) detected a higher risk for congenital abnormalities in all their ART cycles without any difference between IVM, IVF and ICSI. Our small simple size did not allow a calculation of possible differences between the three groups.

As a result of our findings, we strongly recommend that the impact of IVM on children's development and behavior must be analyzed in proper larger scale studies to allow a conclusive answer, which seems very much warranted from the evidence presented in our study and in the other studies mentioned above. Due to our sample size, our observed effect sizes and the tremendous variations of the corresponding confidence intervals, our results of mean MDIs within the normal range do not allow a conclusive answer about the impact of IVM on children's mental development. Although reassuring and confirming our clinical practice, the study size is too small for a final conclusion that IVM is a safe method in ART comparable to IVF or ICSI at the current time.

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Authors' roles

S.R. participated in the collection, analysis and interpretation of the data and in drafting the manuscript, M.v.W. contributed to the design of the study and collection of the data, M.E. performed the ultrasound examinations, K.R. participated in the data collection, G.R. contributed

to the collection, analysis and interpretation of the data, to writing the manuscript and to the critical revision of the manuscript, J.P. performed the examination of the children at second birthday, T.B. performed the extensive statistical evaluation and T.S. has designed the study, supervised the evaluation and contributed to writing the manuscript, revising it critically for important intellectual content.

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Conflict of interest

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