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Full length article

The effects of intrauterine insemination and single embryo transfer or modified natural cycle *in vitro* fertilization on offspring's health—Follow-up of a randomized clinical trial

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ABSTRACT

Objective: Does ovarian hyperstimulation and/or the *in vitro* procedure of assisted reproduction affect neurodevelopmental and physical health of the offspring?

Study design: Infertile couples were randomly allocated to intrauterine insemination with controlled ovarian hyperstimulation (IUI-COH), modified natural cycle in vitro fertilization (IVF-MNC) or single embryo transfer IVF (IVF-SET). We compared neurodevelopmental and physical health in childhood (4–7 years). We used age-appropriate questionnaires to assess behavioral problems (Child Behavior Check List (CBCL)) and executive functioning (Behavior Rating Inventory of Executive Function (BRIEF)). We measured body mass index Z-score, waist- and hip-circumference, body fat percentage, blood pressure Z-scores, pulse wave velocity, glucose, insulin, insulin resistance, total cholesterol, high- and low-density lipoprotein cholesterol, triglycerides, and high sensitivity c-reactive protein. We compared groups by analysis of variance.

Results: We examined 191 (57%) of the 333 children born in the study at a mean age of 5.5 years (range 4.0–7.6 years). We found no statistically significant differences between randomization groups in children's neurodevelopmental or physical health indices (all p-values > 0.05). Comparing the outcomes between actual method of conception, including a naturally conceived group, also did not show statistically significant differences.

Conclusions: Although this follow-up study was not powered on childhood outcomes and limited power due to attrition may have hampered detection of subtle effects, we found no indications of differences in neurodevelopmental and physical health between ovarian hyperstimulation and/or the *in vitro* procedure of assisted reproduction. Future trials should be powered on child outcomes, and aim to optimize follow-up rates to provide answers that are more definitive.

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Introduction

Since in 1978 Louise Brown was born as the first baby conceived through *in vitro* fertilization (IVF) [1], millions of children have been born after assisted reproductive technology [2]. Early environmental alterations have been suggested to have lasting consequences for the offspring conceived through these techniques [3]. Compared to naturally conceived children, those conceived through assisted reproduction were shown to have increased risks of prematurity and low birth weight [4,5]. While postnatal growth and neurodevelopment seems mostly unaffected [6–9], there is some evidence for increased adiposity, blood pressure and altered metabolic profiles among assisted reproduction children compared to spontaneously conceived children [7,10–13].

The information on neurodevelopmental and physical health of assisted reproduction children comes from observational studies, and might therefore be hampered by confounding by indication. For example, parental infertility itself is associated with higher blood pressure and glucose levels in offspring [14], and maternal stress related to infertility may also affect offspring's neurodevelopmental and physical health [15]. On the other hand, animal studies have shown causal effects of ART on offspring physical health. In these experiments, offspring conceived by assisted reproduction had lower birth weight, displayed catch-up growth, altered fat distribution, vessel dysfunction, hypertension and impaired glucose tolerance compared to naturally conceived offspring [16,17]. However, results of animal studies of the effects of assisted reproduction on neurodevelopment have been conflicting [18].

Assisted reproduction involves different techniques, including controlled ovarian (hyper)stimulation (COH), intrauterine insemination (IUI), IVF and intracytoplasmic sperm injection (ICSI) [19]. Each of these techniques may influence embryo development in different ways that affect future neurodevelopmental and physical health. For instance, exposure to a very high estrogen level in ovarian hyperstimulation has been linked to impaired cardiovascular function in offspring [20]. Conventional ovarian hyperstimulation has been associated with increased rates of oocyte aneuploidy [21]. Also, hyperstimulation may affect blood pressure of IVF children, as children conceived through IVF with hyperstimulation had higher blood pressure than those conceived through modified natural cycle IVF (IVF-MNC) [22]. Besides the stimulation regime, the *in vitro* embryo culture conditions have been shown to affect offspring's health. The composition of *in vitro* culture medium led to differences in birth weight and anthropometry at 9 years of age [23].

Currently, there are no data in humans from randomized clinical trials on the effect of the different aspects of assisted reproduction on neurodevelopmental and physical health of the offspring. In this follow-up of a randomized trial we examined children of parents/couples with a diagnosis of unexplained or mild male factor infertility who were randomized to IUI-COH, IVF-MNC, or IVF with single embryo transfer (IVF-SET) [24]. We aimed to disentangle whether ovarian hyperstimulation and/or the *in vitro* procedure of assisted reproduction might affect offspring's neurodevelopmental and physical health at age 4–7 years.

Materials and methods

This follow-up study is based on the INeS trial (NTR939), of which details have previously been published [24]. This multicenter, open label, three arm, parallel group, randomized controlled trial took place in 17 fertility clinics in the Netherlands between January 2009 and February 2013. Couples were eligible if they were seeking infertility treatment after at least 12 months of unprotected intercourse, where the female partner was between 18–38 years, had an unfavorable prognosis of natural conception (12-month natural conception chance <30%), and had a diagnosis of unexplained or mild male infertility. Women with anovulation, bilateral tubal disease, severe endometriosis, premature ovarian failure, or known endocrine disorders were not included.

Both the initial trial and this follow-up study were approved by the medical Ethics Committee of the Academic Medical Centre, the Netherlands (NL56267.018.16). Both parents gave written informed consent for follow-up of their child, if legally able to.

INeS randomization

Randomization and intervention details of the INeS trial have been previously described [24]. Briefly, couples were randomly allocated in a 1:1:1 ratio to either one of the following three strategies: IUI-COH (6 cycles of COH achieved by clomiphene citrate or follicle stimulating hormone (FSH)), IVF-MNC (6 cycles) or IVF-SET (3 cycles). In both IVF groups, embryo transfer took place on day three. If applicable, surplus embryos were cryopreserved and transferred after thawing. Primary endpoint of the original trial was conception within 12 months after randomization from a singleton pregnancy leading to the birth of a healthy child.

Follow-up assessment

Eligible for follow-up were all singletons and firstborns of multiple pregnancies, to ensure independence of the measurements, who were known to be alive and who had been conceived within 12 months after randomization (N = 333). Parents were asked to participate with their child in this follow-up study, when the child was 4–7 years of age.

Maternal and pregnancy related characteristics were collected during the INeS trial. Child's birth weight was calculated as a gestational age and gender adjusted Z-score based on Dutch reference curves [25], with the LMS (lambda-mu-sigma) methodology. During follow-up parents completed questionnaires concerning demographics, neurodevelopmental and physical health of their child. We used a socioeconomic status (SES) score based on average household income by postal codes at time of randomization, and SES scores were provided by the Netherlands Institute for Social Research (Sociaal Cultureel Planbureau). We visited participants near/at their homes with a mobile research vehicle, providing a standardized environment for the measurements. Two assessors from a pool of six trained assessors performed measurements with one parent present. Parents were asked to not disclose any information about the conception of their child to the assessors to enable the assessors to remain blinded to the group the mother was allocated to.

Neurodevelopmental and physical health questionnaires

To measure child's behavioral problems, one of the parents completed the Dutch version of the Child Behavior Checklist (CBCL) 1,5-5 [26], when their child was 4 or 5 years old, and the CBCL 6–18 [27] when their child was 6 or 7 years old. Good reliability and validity have been reported for both the CBCL 1.5–5 and CBCL 6–18 [26,27]. We used the total CBCL scale as a measure of total behavior problems. The total CBCL scale was dichotomized into a normal score and a clinically deviant score using the clinical cut-off based on age-specific reference norm scores appropriate for the Dutch population [27,28].

To measure child's executive functioning, one of the parents filled out the Dutch translation of the Behavior Rating Inventory of Executive Function-Preschool (BRIEF-P) [29] questionnaire when their child was 4 or 5 years, and the Behavior Rating Inventory of Executive Function (BRIEF) [30] when the child was 6 or 7 years.

Good reliability and sufficient validity have been reported for both the BRIEF-P and the BRIEF [29,30]. We used the total BRIEF(-P) scale as a measure of global executive functioning. The total BRIEF (-P) score was dichotomized into a normal score and a clinically deviant score using the clinical cut-off based on age- and sex-specific reference norm scores of the Dutch population [29,30].

Physical health was assessed by posing the following question to parents. "Could you indicate whether your child currently or chronically had one or more of the following health problems: allergies; ear, nose, throat; lung; heart; liver; kidney; urinary tract; gastrointestinal; metabolic; neurologic; dermatologic; hematologic; skeletal; cancer; psychosocial; other." Parents were asked to specify the problem, if any was applicable to their child. In case their child had no problems, parents answered none.

Physical health measurements

Anthropometry. We measured height to the nearest 0.1 cm by a SECA® 206 wall attached measuring tape. Weight was measured by a SECA® 877 digital scale to the nearest 0.1 kg. We calculated BMI as weight in kg divided by height in meters squared. We calculated an age and sex adjusted Z-score based on WHO reference values, with the LMS methodology. We measured waist- and hip-circumference to the nearest 0.1 cm with a SECA® 201 measuring tape. We assessed body composition by bioelectrical impedance analysis (BIA) using the Bodystat 1500mdd® (Douglas, UK). Children were asked to refrain from drinking at least 90 min and to empty their bladder prior BIA measurements. We used a validated, age-appropriate equation of De Beer et al. to calculate body-fat percentage [31]. We took measurements in duplicate, in case there was too much discrepancy between measurements, e.g. more than 0.5 kg for weight, we took a third measurement. We averaged all measurements.

Cardiovascular health. After 5 min of rest, we measured blood pressure seated in triplicate on the non-dominant arm with a validated oscillometric device with an age-appropriate cuff (Omron HBP-1300®, Kyoto, Japan). We calculated the mean of these three systolic blood pressure and diastolic blood pressure (DBP) measurements, respectively, followed by calculation of age and sex adjusted Z-scores based on NIH (National Institute of Health) reference values [32]. We assessed arterial stiffness by ALAM Medical Complior® (St. Quentin Fallavier, France) to measure pulse wave velocity.

Metabolic health. Parents provided separate consent to take a blood sample of their child. If they consented, a separate appointment was made during which a research nurse took a morning fasting venous blood sample. We measured the following: insulin, glucose, total cholesterol, high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol, triglycerides, and high sensitivity c-reactive protein (hsCRP). We calculated the homeostatic model assessment of insulin resistance (HOMA-IR) as insulin (μIU/mL) times glucose (mmol/L) divided by 22.5.

Statistical analyses. The sample size of the initial INeS trial was already set at 600 women based on the primary outcome [24]. Based on findings from observational studies between IVF and naturally conceived children we determined a difference of 5 mmHg in systolic blood pressure to be clinically relevant [11]. A post-hoc sample size calculation that was performed preceding the start of follow-up indicated that 3 groups of 73 children (70% participation rate) would result in 87% power to detect a 5 mmHg difference in systolic blood pressure between groups (significance level alpha is 0.05). With the actual number of children participating, the achieved power to detect a 5 mmHg difference in systolic blood pressure between groups was 75%.

We examined differences between maternal and pregnancy related characteristics of participating and non-participating eligible children. Similarly, of those that participated we compared characteristics based on maternal randomization group (IUI-COH; IVF-MNC; and IVF-SET). We compared outcome measures (deviant CBCL and BRIEF(-P) scores, parent reported health problems, BMI Z-score, body-fat percentage, blood pressure Z-score, pulse wave velocity, serum lipids, glucose, insulin, HOMA-IR, and hsCRP concentrations) between children based on maternal randomization group by intention to treat principles. In secondary analyses, we stratified groups for actual conception method instead of randomization group, which resulted in four groups: IUI-COH; IVF-MNC; stimulated IVF or ICSI; and natural conception.

We present values as means and standard deviations (±SD) for continuous data and as frequency distributions for categorical data. We examined differences in characteristics between participants and non-participants by student's *t*-test or Fisher's exact test, as appropriate. We used ANOVA or Fisher's exact test to compare differences in characteristics between randomization groups. We assessed differences in BMI Z-score and blood pressure Z-scores by ANOVA between randomization groups and based on actual method of conception. For all other outcomes, we used ANCOVA, correcting for offspring's age and gender as no Z-scores could be calculated. However, we assessed differences in the dichotomized neurodevelopmental scores between the randomization groups and actual method of conception with Fisher's exact tests, without correcting for offspring's age and gender since we used appropriate reference norms.

We performed sensitivity analyses adjusting for baseline factors that were no longer similar in those participating in the follow-up. We repeated our analyses now additionally adjusting for household SES score, to assess whether this would change the outcomes. Furthermore, we tested whether censoring the first born of a twin and only including singletons in the analyses would affect outcomes. A p-value <0.05 was considered to indicate statistical significance.

Results

Fig. 1 depicts the flow of participants. There were 333 eligible children (among which 19 were the first born of a twin) from 602 randomized couples. Of these 333 children, there were 21 of whom we could not acquire any contact information (6%), while 121 parents (36%) declined participation. Among the 191 parents who participated with their child (57%); 65 children (3 twins) were born from the IUI-COH group, 55 (1 twin) born from the IVF-MNC group, and 71 (3 twins) born from the IVF-SET group. Of those, 159 children (83%) took part in the physical measurements, and 74 (39%) provided blood samples.

Maternal and pregnancy related characteristics did not differ between participating and non-participating eligible children (Supplementary Table 1). Overall, 36% of children were conceived by another method than the ART treatment their mother was randomly allocated to, which was statistically significantly different between participants and non-participants (26% vs 49%, $p < 0.05$). In mothers of participating children, baseline characteristics based on randomization group were not different, except that the IVF-MNC group had the lowest SES scores (Table 1).

Table 2 provides details of participating children by maternal group allocation. Mean age of participating children was 5.5 years (range 4.0–7.6 years). There were 93 boys (49%). Child's characteristics were similar between randomization groups, except that birth weight Z-scores were lower in the hormone stimulated groups compared to the IVF-MNC group. Additionally, fewer children were conceived by the ART method their mother was randomized to in the IVF-MNC group compared to the other groups. Treatment allocation was not

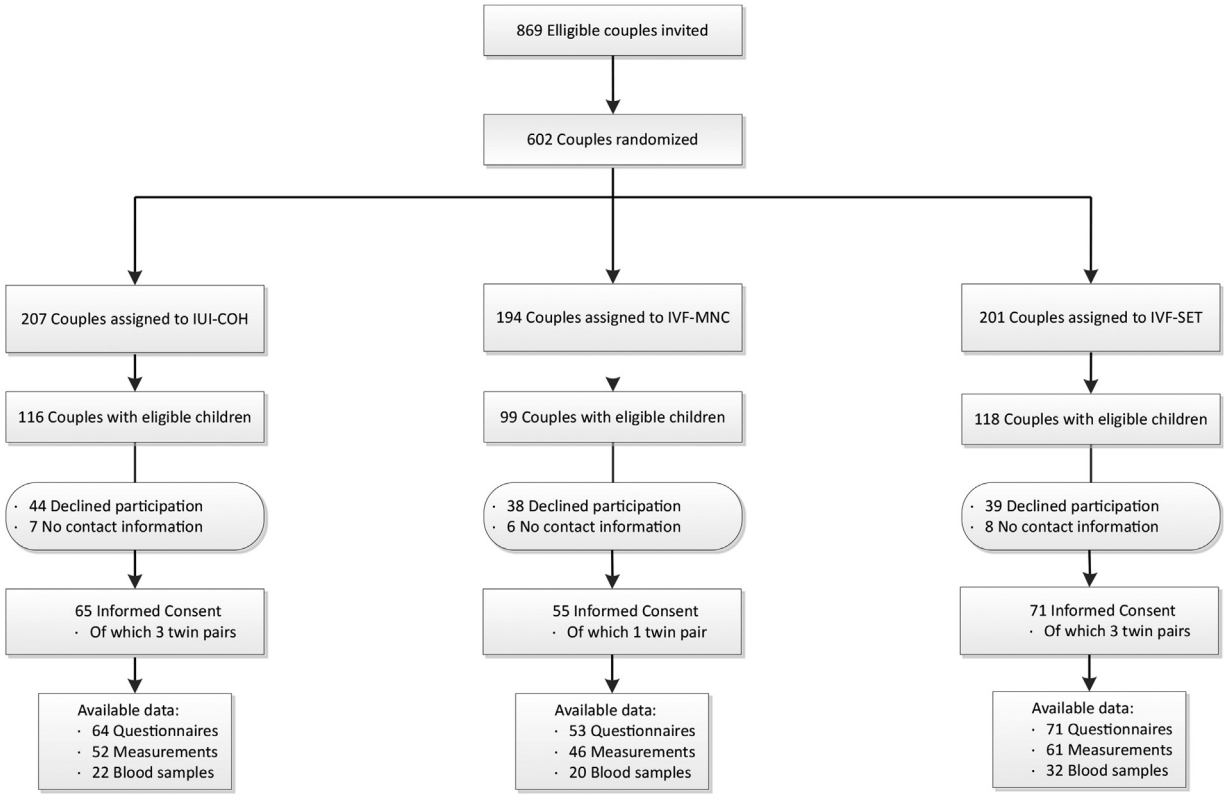

Fig. 1. Flowchart of included patients.

associated with any differences in terms of parental report of child's health problems. Over 55% of parents reported no health problems in their child, and of those that did, most reported allergies, ear, nose and throat or skin problems. When child's characteristics were compared between groups based on actual conception method, mean (±SD) birth weight Z-scores were −0.26 (0.95) for IUI-COH, 0.39 (0.93) for IVF-MNC, −0.29 (0.93) for stimulated IVF and −0.10 (0.78) for natural conceptions (Supplementary Table 2). Mean (±SD) birth weight Z-scores of all children born in the initial INeS trial (N = 333) were −0.31 (1.09) for IUI-COH, 0.07 (1.07) for IVF-MNC, −0.19 (1.02) for stimulated IVF and −0.01 (0.85) for natural conceptions.

Intention to treat analyses

Table 3 shows offspring's neurodevelopmental, anthropometric, cardiovascular, and metabolic health indices based on maternal randomization group. There were no statistically significant differences between groups for these health indices of the offspring. Overall, neurodevelopmental and physical health outcomes were normal, the percentage of children with a deviant neurodevelopmental score was similar to the percentage in the general, normative population and also, the BMI and systolic blood pressure Z-scores were close to zero. However, diastolic blood pressure was slightly elevated (0.70 ± 0.46) without statistically significant differences between groups. Sensitivity analyses adjusting for SES score (not conducted for neurodevelopmental outcomes due to the limited number of children scoring clinically deviant), or censoring twins did not alter effect estimates.

Secondary analyses

Table 4 shows childhood neurodevelopmental, anthropometric, cardiovascular, and metabolic health indices based on the actual method of conception. There were no statistically significant differences in childhood outcomes. Again, sensitivity analyses adjusting for SES score (not conducted for neurodevelopmental outcomes due to the limited number of children scoring clinically deviant), or censoring twins did not alter effect estimates.

Table 1
Baseline characteristic of women who participated with their child by maternal randomization group.

	N	IUI-COH	*N*	IVF-MNC	*N*	IVF-SET
Maternal baseline characteristics						
Age at randomization (years) – mean (SD)	65	33.7 (3.2)	55	33.3 (2.9)	71	33.0 (2.8)
Caucasian – no. (%)	65	61 (94)	53	50 (94)	71	68 (96)
SES score (AU) – mean (SD)*	65	0.2 (1.2)*	54	−0.2 (1.4)*	71	0.4 (1.0)*
Smoker – no. (%)	64	8 (13)	55	7 (13)	71	13 (18)
BMI (kg/m^2) – mean (SD)	64	24.3 (3.6)	51	23.5 (3.5)	67	23.6 (3.6)
Primary infertility – no. (%)	65	49 (75)	55	41 (75)	71	56 (79)
Duration of infertility (years) – mean (SD)	65	2.7 (1.4)	55	2.4 (0.9)	70	2.4 (1.2)
Total motile sperm count (Log transformed) – mean (SD)	59	1.7 (0.5)	52	1.8 (0.6)	67	1.7 (0.6)

Differences between groups assessed by ANOVA. *$p < 0.05$. IUI-COH = intra-uterine insemination with controlled ovarian hyperstimulation, IVF-MNC = in vitro fertilization in a modified natural cycle, IVF-SET = in vitro fertilization with single embryo transfer, SES = socioeconomic status, AU = arbitrary units, BMI = body mass index.

Table 2
Characteristics of participating children by maternal randomization group.

	N	IUI-COH	N	IVF-MNC	N	IVF-SET	p-value
General							
Age (years) – mean (SD)	64	5.5 (0.74)	54	5.5 (0.94)	71	5.4 (0.80)	0.68
Boys – no. (%)	65	26 (40)	55	25 (46)	71	42 (59)	0.07
Only child in household – no. (%)	63	11 (18)	53	14 (26)	71	19 (27)	0.38
Went to daycare – no. (%)	62	50 (81)	53	45 (85)	71	59 (83)	0.86
Pregnancy related characteristics							
Maternal age at time of pregnancy (years) – mean (SD)	65	34.1 (3.3)	55	33.6 (3.0)	71	33.5 (2.9)	0.49
Conception mode – no. (%)	65		55		71		<0.01
Natural		11 (17)		12 (22)		8 (11)	
IUI-COH		48 (74)		3 (6)		1 (1)	
IVF-MNC		0 (0)		32 (58)		0 (0)	
Stimulated IVF		4 (6)		3 (6)		61 (86)	
ICSI		2 (3)		5 (9)		1 (1)	
Twins – no. (%)	65	3 (5)	55	1 (2)	71	3 (4)	0.80
Gestational age at birth (weeks) – mean (SD)	65	39.4 (2)	55	39.7 (2)	71	39.4 (2)	0.71
Birth weight (grams) – means (SD)	65	3329 (511.9)	55	3528 (492.3)	71	3269 (527.1)	0.02
Birth weight (Z-score) – mean (SD)	65	−0.19 (0.91)	55	0.18 (0.99)	71	−0.34 (0.86)	<0.01
Any breastfeeding – no. (%)	64	46 (72)	53	39 (74)	71	52 (73)	1.0
Health problems by parental report							
No problems – no. (%)	64	36 (56)	53	32 (60)	71	41 (58)	0.90
Allergies – no. (%)	64	8 (13)	53	6 (11)	71	7 (10)	0.96
Ear, nose, throat – no. (%)	64	9 (14)	53	8 (15)	71	9 (12)	0.96
Lung – no. (%)	64	7 (11)	53	4 (8)	71	6 (9)	0.85
Heart – no. (%)	64	2 (3)	53	0 (0)	71	1 (1)	0.63
Urinary tract – no. (%)	64	1 (2)	53	1 (2)	71	2 (3)	1.0
Gastroenterological – no. (%)	64	6 (9)	53	3 (6)	71	3 (4)	0.49
Neurological – no. (%)	64	0 (0)	53	0 (0)	71	1 (1)	1.0
Dermatological – no. (%)	64	9 (14)	53	6 (11)	71	9 (13)	0.96
Hematological/immunological – no. (%)	64	2 (3)	53	1 (2)	71	0 (0)	0.38
Skeletal – no. (%)	64	1 (2)	53	1 (2)	71	1 (1)	1.0
Psychosocial – no. (%)	64	1 (1)	53	3 (6)	71	2 (3)	0.51

Differences between groups assessed by ANOVA or Fisher's Exact Test. IUI-COH = intra-uterine insemination with controlled ovarian hyperstimulation, IVF-MNC = in vitro fertilization in a modified natural cycle, IVF-SET = in vitro fertilization with single embryo transfer, IVF = in vitro fertilization, ICSI = Intracytoplasmic sperm injection, CRYO = cryopreservation, Stimulated-IVF refers to hormone stimulated IVF including single embryo transfer and multiple embryo transfer.

Table 3
Childhood neurodevelopmental, anthropometric, cardiovascular, and metabolic health indices of participating children by maternal randomization group.

	N	IUI-COH	N	IVF-MNC	N	IVF-SET	p-value
Neurodevelopmental							
Behavioral problems – no. (%)^	63	3 (5)	53	2 (4)	70	1 (1)	0.59
Executive functioning – no. (%)^	62	5 (8)	53	3 (6)	70	5 (7)	0.94
Anthropometric							
BMI (Z-score) – mean (SD)#	52	0.18 (0.95)	46	0.07 (0.98)	61	0.10 (0.78)	0.80
Waist circumference (cm) – mean (SD)	51	53.36 (3.73)	46	53.04 (4.00)	61	52.68 (3.17)	0.60
Hip circumference (cm) – mean (SD)	51	59.61 (5.40)	46	59.59 (4.88)	61	59.00 (3.98)	0.95
Body fat (%) – mean (SD)	51	19.97 (6.76)	46	20.00 (5.83)	61	18.79 (5.09)	0.66
Cardiovascular							
SBP (mmHg) – mean (SD)	52	96.4 (7.0)	46	97.4 (7.1)	61	97.8 (6.9)	0.55
SBP (Z-score) – mean (SD)#	52	0.06 (0.64)	46	0.08 (0.67)	61	0.15 (0.62)	0.71
DBP (mmHg) – mean (SD)	52	63.3 (4.6)	46	64.0 (4.6)	61	63.1 (5.9)	0.68
DBP (Z-score) – mean (SD)#	52	0.69 (0.40)	46	0.72 (0.47)	61	0.69 (0.50)	0.88
PWV (m/sec) – mean (SD)	49	4.79 (0.78)	40	4.63 (0.89)	56	4.38 (0.95)	0.08
Metabolic							
Glucose (mmol/L) – mean (SD)	22	4.71 (0.39)	19	4.75 (0.23)	31	4.80 (0.29)	0.65
Insulin (pmol/L) – mean (SD)	22	24.95 (9.39)	18	22.83 (9.69)	30	24.07 (11.49)	0.89
HOMA-IR – mean (SD)	22	0.89 (0.38)	19	1.19 (1.69)	30	0.87 (0.42)	0.89
Total cholesterol (mmol/L) – mean (SD)	22	4.32 (0.62)	20	4.03 (0.41)	30	3.92 (0.61)	0.07
HDL cholesterol (mmol/L) – mean (SD)	22	1.52 (0.35)	20	1.40 (0.30)	31	1.48 (0.28)	0.60
LDL cholesterol (mmol/L) – mean (SD)	22	2.55 (0.53)	20	2.38 (0.44)	30	2.21 (0.60)	0.11
Triglycerides (mmol/L) – mean (SD)	22	0.58 (0.24)	20	0.55 (0.27)	30	0.52 (0.26)	0.92
hsCRP mg/L – mean (SD)	21	0.58 (0.67)	19	0.84 (2.01)	31	0.55 (0.35)	0.66

Differences between groups assessed by ANCOVA, corrected for age and sex, or ANOVA for outcomes marked by # or Fisher's Exact Test marked by ^. IUI-COH = intra-uterine insemination with controlled ovarian hyperstimulation, IVF-MNC = in vitro fertilization in a modified natural cycle, IVF-SET = in vitro fertilization with single embryo transfer, BMI = Body mass index, SBP = systolic blood pressure, DBP = diastolic blood pressure, PWV = pulse wave velocity, HOMA-IR = homeostatic model assessment of insulin resistance, HDL = high density lipoprotein, LDL = low density lipoprotein, hsCRP = high sensitivity c-reactive protein.

Discussion

We evaluated whether ovarian hyperstimulation and/or the *in vitro* procedure of assisted reproduction affect offspring's neurodevelopmental and physical health at age 4–7 years. We found no statistically significant differences in children's neurodevelopmental and physical health between IVF with single embryo transfer, IVF in a modified natural cycle, and intrauterine

Table 4
Childhood neurodevelopmental, anthropometric, cardiovascular, and metabolic health indices of participating children by actual conception method.

	N	IUI-COH	*N*	IVF-MNC	*N*	Stimulated-IVF	*N*	Natural	*p*-value
Neurodevelopmental									
Behavioral problems – no. (%)^	51	3 (6)	32	0 (0)	75	2 (3)	28	1 (4)	.55
Executive functioning – no. (%)^	50	4 (8)	32	1 (3)	75	5 (7)	28	3 (11)	.70
Anthropometric									
BMI (Z-score) – mean (SD)#	41	0.14 (0.97)	29	0.11 (0.83)	64	0.03 (0.73)	25	0.32 (1.18)	0.58
Waist circumference (cm) – mean (SD)	40	53.4 (4.0)	29	53.0 (2.5)	64	52.3 (2.9)	25	54.2 (5.1)	0.24
Hip circumference (cm) – mean (SD)	40	59.6 (5.2)	29	59.7 (3.1)	64	58.6 (4.0)	25	60.7 (6.7)	0.64
Body fat (%) – mean (SD)	40	19.6 (6.4)	29	20.4 (4.5)	64	18.5 (5.1)	25	21.1 (7.8)	0.39
Cardiovascular									
SBP (mmHg) – mean (SD)	41	95.6 (6.5)	29	98.0 (6.2)	64	97.3 (6.9)	25	98.9 (8.3)	0.36
SBP (Z-score) – mean (SD)#	41	−0.06 (0.56)	29	0.15 (0.64)	64	0.14 (0.62)	25	0.22 (0.78)	0.30
DBP (mmHg) – mean (SD)	41	63.1 (4.7)	29	64.3 (4.8)	64	63.3 (5.7)	25	63.3 (4.9)	0.88
DBP (Z-score) – mean (SD)#	41	0.67 (0.40)	29	0.75 (0.51)	64	0.72 (0.47)	25	0.64 (0.45)	0.81
PWV (m/sec) – mean (SD)	39	4.9 (0.8)	28	4.6 (0.8)	58	4.4 (0.9)	20	4.4 (1.0)	0.09
Metabolic									
Glucose (mmol/L) – mean (SD)	20	4.71 (0.40)	13	4.81 (0.24)	29	4.78 (0.28)	10	4.74 (0.28)	0.77
Insulin (pmol/l) – mean (SD)	20	24.40 (9.38)	12	24.50 (10.12)	28	22.54 (11.34)	10	26.90 (9.99)	0.74
HOMA-IR – mean (SD)	20	0.87 (0.39)	12	1.43 (2.01)	28	0.81 (0.41)	10	0.96 (0.38)	0.21
Total cholesterol (mmol/L) – mean (SD)	20	4.29 (0.71)	14	4.05 (0.35)	28	3.90 (0.54)	10	4.17 (0.64)	0.19
HDL cholesterol (mmol/L) – mean (SD)	20	1.53 (0.35)	14	1.43 (0.35)	29	1.46 (0.30)	10	1.45 (0.17)	0.84
LDL cholesterol (mmol/L) – mean (SD)	20	2.50 (0.60)	14	2.35 (0.38)	28	2.21 (0.54)	10	2.52 (0.63)	0.25
Triglycerides (mmol/L) – mean (SD)	20	0.58 (0.24)	14	0.60 (0.27)	28	0.53 (0.28)	10	0.44 (0.14)	0.43
hsCRP mg/L – mean (SD)	19	0.57 (0.72)	13	1.09 (2.42)	29	0.52 (0.35)	10	0.50 (0.28)	0.59

Differences between groups assessed by ANCOVA, corrected for age and sex, or ANOVA for outcomes marked by # or Fisher's Exact Test marked by ^. IUI-COH = intra-uterine insemination with controlled ovarian hyperstimulation, IVF-MNC = in vitro fertilization in a modified natural cycle, Stimulated-IVF refers to hormone stimulated IVF including single embryo transfer, multiple embryo transfer and intracytoplasmic sperm injection, BMI = Body mass index, SBP = systolic blood pressure, DBP = diastolic blood pressure, PWV = pulse wave velocity, HOMA-IR = homeostatic model assessment of insulin resistance, HDL = high density lipoprotein, LDL = low density lipoprotein, hsCRP = high sensitivity c-reactive protein.

insemination. Our data suggest that neither ovarian hyperstimulation nor the IVF procedure have major effects on neurodevelopmental and physical health of the offspring, but we cannot exclude potential subtle effects.

This is the first follow-up of an randomized controlled trial comparing three commonly used assisted reproduction technology. The study adhered to standardized protocols in concurrence with local protocols, and thus realistically reflects daily clinical practice. Follow-up assessment took place in a specially designed research vehicle to provide a standardized environment and to eliminate travel time for participants, in order to optimize participation rates. Before and during data collection assessors remained blinded to the group allocation of participants, to ensure objective assessment of children.

This follow-up study had some limitations. First, there was loss to follow-up, which reduced statistical power. Post-hoc sample size calculations suggested our sample could have detected a relatively large effect (i.e. > 5 mmHg in SBP), if it were present, however our results only showed very small differences between groups. Second, selective participation led to differences between groups in SES, not present in the initial trial (data not shown) [24]. However, adjustment for this potential confounder did not alter our results. Third, this study only included couples with unexplained or mild male factor infertility, and thus our findings apply to this population and cannot be directly translated to all couples seeking infertility treatment. Finally, the study was not designed to assess the effects of assisted reproduction versus natural conception, and as such, our secondary analyses must be considered explorative.

Our study found no differences in childhood neurodevelopmental and physical health based on ovarian hyperstimulation or IVF procedures. Our data are in line with the results of a prospective cohort study that found no differences in anthropometry or blood pressure at 9 years of age in the offspring of couples that underwent IVF with or without ovarian hyperstimulation [33]. In our study BMI and systolic blood pressure were normal and not different between the groups, thus our study does not suggest programming effects of assisted reproduction treatment on cardiometabolic health. This contrasts most observational studies linking assisted reproduction to poorer cardiometabolic health in offspring [7,10,12,13]. In these studies, assisted reproduction offspring are often compared to naturally conceived children from fertile parents, and as such, the poorer cardiometabolic health in assisted reproduction children may be due to parental infertility [14]. The slightly elevated diastolic blood pressure Z-scores in all groups compared to population reference values in our study may suggest that parental infertility indeed affects offspring's cardiovascular function to some extent.

Our follow-up was in early childhood. It is possible that differences in neurodevelopmental and physical health may only become apparent at a later age. Indeed, studies of young assisted reproduction children do not show differences in blood pressure while those of young adults suggest that blood pressures and glucose levels were higher than among spontaneously conceived controls [10,16,22]. This fits with other studies suggesting that early life programming effects increase with age [34]. Perhaps, we were unable to detect the subtle differences in cardiovascular function in assisted reproduction children reported in other studies [35]. These vascular alterations in assisted reproduction offspring have been shown to persist and evolve into elevated blood pressure in apparently healthy young adults [36,37]. Similarly, neurodevelopmental problems may need more time to become apparent, as few observational studies have suggested that IVF could be associated with an increase of depression in adolescence [9]. Hence, while in our study there were no indications of assisted reproduction technology affecting childhood neurodevelopmental and physical health, following up these children until later age remains warranted as effects may change over time.

Perhaps, we were unable to detect effects of assisted reproduction on childhood neurodevelopment and health because of innovation in assisted reproduction technologies [38], or shifts in the indications for assisted reproduction, as an increasing number of couples without a clear reason for being unable to

conceive are treated with assisted reproduction nowadays. Indeed, less harmful effects of assisted reproduction over the last decades was shown in subgroup analyses of a meta-analysis; assisted reproduction conceived children had higher blood pressure than naturally conceived children if they were born between 1990 and 1999, but there was no difference in those born between 2000–2009 [10]. The current assisted reproduction treatments may be less invasive and may more closely resemble *in vivo* circumstances than those used previously [38,39]. The COH protocol with strict cancellation criteria as used in our study, is more commonly used nowadays, and has been linked to reduced risks of adverse health in offspring compared to more aggressive protocols used in the past [40]. Furthermore, single embryo transfer has reduced the rates of multiple gestations, which itself is related to adverse cardiovascular health [41], and neurodevelopmental outcomes due to prematurity, but may also more closely resemble natural conception and therefore reduce the impact on offspring's health [42]. Moreover, the initial INeS trial and other recent studies showed low rates of neonatal complications, including prematurity and congenital abnormalities, which was only partly explained by reduced rates of multiple pregnancies [24]. Although modern assisted reproduction technology seem to minimally affect offspring's health, each new medical intervention should be carefully examined before it is implemented. Since early life influences can program future health, it may be especially crucial in fertility care to not only focus on a healthy live birth but also include long-term health outcomes [43].

Conclusions

We did not detect differences in neurodevelopmental and physical health at age 4–7 years of children whose mother was randomly allocated to IUI-COH, IVF-MNC or IVF-SET. We found no indications for effects of mode of conception, including natural conception, on child outcomes. Suggesting that assisted reproduction treatment itself does not have major effects on offspring's health, although this follow-up was not powered on childhood outcomes and more subtle effects may have gone undetected due to attrition. Even though there is evidence of lower birth weight following hormonal stimulation, our findings seem reassuring, as the effects of assisted reproduction on children's health at age 4–7 years are, if present, likely to be small. Future trials should be powered on child outcomes, include repeat follow-up measurements, and aim to optimize follow-up rates to provide answers that are more definitive.

Author's roles

Madelon van Wely is the principal investigator (PI) of the INeS trial, and Tessa Roseboom is the PI of the follow-up. Stijn Mintjens, Malou Menting, Reinoud Gemke, Mireille van Poppel, Madelon van Wely, Alexandra Bensdorp, Raïssa Tjon Kon Fat, Ben Willem Mol, Rebecca Painter, Cornelieke van de Beek and Tessa Roseboom planned the study procedures. Stijn Mintjens and Malou Menting collected and analyzed the data. Stijn Mintjens, Malou Menting, Cornelieke van de Beek and Tessa Roseboom interpreted the data. Stijn Mintjens wrote the manuscript and Malou Menting, Reinoud Gemke, Mireille van Poppel, Madelon van Wely, Alexandra Bensdorp, Raïssa Tjon Kon Fat, Ben Willem Mol, Rebecca Painter, Cornelieke van de Beek and Tessa Roseboom revised the manuscript. All authors approved the final submitted manuscript.

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Stijn Mintjens, Malou Menting, Reinoud Gemke, Mireille van Poppel, Madelon van Wely, Alexandra Bensdorp, Raïssa Tjon Kon Fat, Ben Willem Mol, Rebecca Painter, Cornelieke van de Beek and Tessa Roseboom declare no conflicts of interest relevant to this study. Outside the work submitted, Ben Willem Mol is supported by a NHMRC practitioner Fellowship and reports consultancy for ObsEva, Merck, Merck KGaA and Guerbet. Rebecca Painter reports board membership for the Dutch Gezondheidsraad and NVVH guideline committees, and travel expenses reimbursement for ESHRE meeting Barcelona 2018 (June).

Declaration of Competing Interest

Stijn Mintjens, Malou Menting, Reinoud Gemke, Mireille van Poppel, Madelon van Wely, Alexandra Bensdorp, Raïssa Tjon Kon Fat, Ben Willem Mol, Rebecca Painter, Cornelieke van de Beek and Tessa Roseboom declare no conflicts of interest relevant to this study. Outside the work submitted, Ben Willem Mol is supported by a NHMRC practitioner Fellowship and reports consultancy for ObsEva, Merck, Merck KGaA and Guerbet. Rebecca Painter reports board membership for the Dutch Gezondheidsraad and NVVH guideline committees, and travel expenses reimbursement for ESHRE meeting Barcelona 2018 (June).

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ejogrb.2019.09.026.

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