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IVF procedures are not, but subfertility is associated with neurological condition of 9-year-old offspring

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ABSTRACT

In vitro fertilization (IVF) is not associated with neurological abnormalities in offspring's early childhood. Yet, it is unclear whether this is also true for school age. Neither do we know the role of parental subfertility in offspring's neurological development. The present study examined neurological condition at 9 years of 57 singletons born after controlled ovarian hyperstimulation IVF (COH-IVF), 46 singletons born after modified natural cycle IVF (MNC-IVF) and 66 singletons born to subfertile parents after natural conception (Sub-NC). To assess the effect of subfertility, the groups were pooled to form a subfertile group, and compared with a prospectively followed fertile reference group (n = 282). The sensitive Minor Neurological Dysfunction (MND) examination was used, resulting in the detailed Neurological Optimality Score (NOS) and the prevalence of the clinically relevant complex MND. Neurological condition of the three subfertile groups did not differ significantly: median NOS was 53 in each subfertile group and the prevalence of complex MND in the three subfertile groups was 30%, 37% and 36%, respectively. However, the NOS was lower and the prevalence of complex MND higher in children born to subfertile couples than in children of fertile couples (adjusted mean difference [95% CI]: −4.48 [−5.53 to −3.42]) and adjusted OR [95% CI]: 5.13 [2.60–10.16], respectively). We conclude that ovarian hyperstimulation, in vitro procedures, and the combination of both were not associated with a less favourable neurological outcome of 9-year-old singletons. However, the presence of parental subfertility was associated with less favourable neurological outcome of offspring at 9 years follow up.

1. Introduction

In vitro fertilization (IVF) is associated with adverse perinatal outcomes such as preterm birth and low birthweight [1]. It has been suggested that these less optimal perinatal outcomes in children born after IVF do not result from the in vitro culture procedures itself but from the underlying parental subfertility [2,3]. Parental subfertility may also be associated with less optimal neurodevelopmental outcome at preschool age. Previous studies showed that in vitro culture procedures itself are not associated with short-term effects on neurological outcome during the first postnatal years [4]. However, it is unclear if there is an association between the IVF procedures, parental subfertility, perinatal adversities and an adverse neurological outcome at school age. As often, neurological dysfunctions may emerge after the significant neurodevelopmental transition that takes place between 7 and 9 years [5].

To unravel this multiple factor question, the Groningen ART cohort study was designed. Its design allows for disentangling the effects of ovarian hyperstimulation (used to induce timed multiple ovulation aiming to increase the chance of pregnancy) and the effects of the in vitro laboratory procedures on offspring's neurodevelopmental outcome. The study revealed that ART components did not affect neurodevelopmental outcome up to and including the age of 4 years [6–9]. However – as indicated above – the presence of subfertility was associated with less favourable neurological condition at 3 months and the severity of subfertility with less optimal neurological condition at 2 and 4 years [6,8,10].

Notwithstanding these reassuring results of studies on the effect of

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ART and its specific components on neurodevelopmental outcome in infancy and at preschool age, it is possible that components of ART are associated with less favourable neurological outcome at 9 years, as dysfunctions in more complex neurological functions, including coordination and fine manipulative ability, only emerge over time [5,11].

Therefore, the primary aim of the current study was to evaluate whether ovarian hyperstimulation and/or the in vitro procedure have an effect on neurological condition of the offspring at the age of 9 years. The secondary aims were to study the effects of the presence and severity (in terms of time to pregnancy [TTP]) of subfertility on neurological outcome at 9 years. We hypothesized that the ART procedures and the presence and severity of subfertility have unfavourable effects on neurological condition of 9-year-old offspring.

2. Methods

2.1. Study population

This study is part of the Groningen ART cohort study, a prospective, assessor-blinded, longitudinal follow-up study. The general methods and characteristics have been described previously [8]. Pregnant subfertile couples with a term date between March 2005 and December 2006 were asked to participate. The couples had been referred for subfertility evaluation and/or treatment to the Department of Reproductive Medicine of the University Medical Center Groningen (UMCG). In the present study only singleton offspring were included. The cohort consists of singletons born after IVF or intracytoplasmic sperm injection (ICSI), as well as naturally conceived singletons born to subfertile parents (Sub-NC). The singletons born after IVF or ICSI were subdivided into two groups: a group of children born after either IVF or ICSI with controlled ovarian hyperstimulation (COH-IVF) and a group of children born after IVF or ICSI with a modified natural cycle (MNC-IVF), in which medication use is minimal [12]. The third group (Sub-NC) consists of naturally conceived children born to couples who had tried to conceive for at least one year and were either evaluated for the cause of subfertility or on the waiting list for fertility treatment.

Information on prenatal, perinatal, and neonatal periods, parental and socioeconomic characteristics was collected on standardized charts during the first follow-up assessment at 2 weeks after term age [8]. TTP was defined as the number of months between the start of unprotected intercourse or the end of a previous pregnancy and conception, converted into decimal years for analysis.

The reference group consisted of the 9-year-old participants of the Groningen LCPUFA cohort study born to fertile parents [13]. The Groningen LCPUFA cohort study is a prospective, double-blind, randomized controlled study in healthy term singletons on the effect of LCPUFA supplementation of infant formula on neurodevelopment. Mothers were recruited during pregnancy check-up visits in the two hospitals and the midwife clinics in the city of Groningen from February 1997 until October 1999; it resulted in the inclusion of 472 healthy term infants [14]. At the follow-up at 9 years, 341 (72%) children of the cohort were assessed. Obstetrical, neonatal and social background information was available, including some information on subfertility and subfertility treatment. For the current reference group we excluded a priori the 59 children born to the parents who had indicated any type of subfertility problem, as details on type and severity of subfertility and on subfertility treatment were lacking. The 282 children born to fertile parents and assessed at 9 formed the fertile reference group of the present study. In these children data on TTP were missing (see Supplementary Fig. 1).

The ethics committee of the UMCG approved the follow-up of both cohorts. Written informed consent was obtained from all participants and their parents.

2.2. Neurological assessment

The children of both cohorts were assessed with the age-specific and standardized MND-assessment [11]. The MND-assessment evaluates neurological signs in eight functional domains: posture and muscle tone, reflexes, dyskinesia, coordination, fine manipulative ability, associated movements, sensory deficits, and cranial nerve functioning. Each domain can either be scored as typical or dysfunctional according to the criteria of the assessment manual [11]. The classification 'neurologically normal' implies the absence of dysfunctional domains or the isolated presence of dysfunctional reflexes; simple MND means the presence of one or two dysfunctional domains; whereas complex MND denotes the presence of more than two dysfunctional domains. The classification 'neurologically abnormal' implies the presence of a distinct neurological syndrome, such as cerebral palsy. Complex MND is associated with prenatal, perinatal and neonatal morbidity and is regarded as the clinically relevant form of MND, since it is strongly related to learning and behavioural disorders [5].

The MND-assessment may also be summarized by means of the quantitative NOS. The NOS at 9 years consists of 64 items for which optimality criteria are defined [11]. The summation of the number of items fulfilling the optimality criteria yields the NOS, implying that the maximum score is 64. Note that neurological optimality is more narrowly defined than neurological normality. This turns the NOS into a sensitive tool to assess the child's neurological condition and therefore is our primary outcome parameter.

The assessment at 9 years was performed by trained assessors under the supervision of a neurodevelopmental expert (MHA). Assessors and supervisor were blinded to prenatal and perinatal history, including the mode of conception of the three subfertile groups.

2.3. Statistical analyses

To test the differences between the groups' background characteristics Student's *t*-tests, Fisher's exact tests and Mann-Whitney *U* tests were used where appropriate. Linear regression analysis was used to detect associations between the various groups and the NOS. Logistic regression was used to evaluate associations between group status and complex MND and dysfunction in specific neurological domains.

First, we analysed the differences between the three groups of the ART-cohort. We adjusted in the multivariable regression analyses for potential confounders which were identified based on differences in background characteristics between the groups and on a priori basis: sex, maternal education, maternal age, TTP, vanishing twins, gestational age, and birth weight. Second, the three ART-cohort groups were pooled to form the subfertile group. In this subfertile group the associations between TTP and neurological outcome were assessed using linear and logistic regression analyses, adjusting for the same variables as in the previous analyses, except for TTP. Third, in order to assess the effect of the presence of subfertility, neurological outcome of the pooled subfertile group was compared with that of the fertile reference group. In the linear and logistic regression analyses we adjusted for sex, maternal education, maternal age, maternal smoking and alcohol use during pregnancy, caesarean section, gestational age, birth weight, firstborn child, breastfed > 6 weeks, age at follow-up.

A post hoc power analysis showed that our study samples allowed for a detection of 0.61–0.73 points in NOS scores between the three groups (power 80%, $\alpha = 0.05$), which underscores the sensitive nature of the NOS to detect differences between groups.

Throughout the analyses *P*-values < 0.05 were considered statistically significant. In the pairwise comparisons of the three ART study groups and the underlying causes of subfertility, Bonferroni corrections were applied, implying that cut-off for statistical significance was $P < 0.017$ for the ART study groups and $P < 0.013$ for the underlying causes of subfertility. The analyses were performed using SPSS Statistics version 23.0.

3. Results

3.1. The Groningen ART cohort

At 9 years 57 of the 68 neonatally recruited COH-IVF children were assessed (84%), 46 of the 57 MNC-IVF children (81%) and 66 of the 90 Sub-NC children (73%) (see Supplementary Fig. 1). Reasons for non-participation were parental withdrawal of consent due to overloaded family agendas, inability to contact families and emigration. The background characteristics of the children assessed at 9 years and children lost to follow-up were similar (data not provided). The background characteristics of the children assessed are shown in Table I. Most background characteristics of the three ART groups were similar, except for the following: both COH-IVF and MNC-IVF couples had a longer TTP than Sub-NC couples (medians 4.0 and 3.8 vs. 2.0 years; $P < 0.001$ and $P = 0.003$, respectively); COH-IVF mothers had more often experienced vanishing twins than Sub-NC mothers (11% vs. 0%; $P = 0.009$); COH-IVF children had a shorter gestational age and a lower birth weight than Sub-NC children (39.4 vs. 40.1 weeks; $P = 0.036$, and 3340 vs. 3594 g; $P = 0.049$, respectively); and finally, COH-IVF fathers were older than MNC-IVF fathers (36.4 vs. 33.7 years; $P = 0.020$).

3.2. ART-components and neurology at 9

At 9 years, 17 (30%) COH-IVF children, 17 (37%) MNC-IVF children and 24 (36%) Sub-NC children were classified as having complex MND, the clinically relevant form of MND (Table II). None of the children were classified as neurologically abnormal. Statistical analyses showed that the prevalence of complex MND in the three ART groups did not differ, also when confounders were taken into account (Tables II and III). Also no statistically significant group differences were present between the three groups in terms of our primary outcome parameter, the NOS (Tables II and III).

3.3. Severity of subfertility and neurology at 9

The three ART-cohort groups were combined to form the subfertile group. In this group, a longer TTP was not associated with complex MND (adjusted odds ratio [OR] [95% CI]: 1.09 [0.93–1.26]) or the NOS (adjusted mean difference [95% CI]: 0.00 [−0.32–0.33]; Table III). In addition, we did not find an increased risk of a less optimal neurological condition when dichotomising TTP into a group with a TTP of > 3 years and a group with a lower TTP (complex MND: adjusted OR [95% CI]:

Table I
Characteristics of participating children and parents of the Groningen ART cohort study groups.

Characteristics	COH-IVF	MNC-IVF	Sub-NC
	n = 57	n = 46	n = 66
Child characteristics			
Male gender, n (%)	32 (56)	22 (48)	33 (50)
First born, n (%)	38 (67)	33 (72)	39 (59)
Age at examination in months, median (range)	110.4 (108.5–126.5)	110.3 (108.2–131.8)	109.9 (100.7–119.9)
Fertility parameters			
TTP in years[a], median (range)	4.0 (0.1–13.3)***	3.8 (0.1–7.5)**	2.0 (0.1–11.3)***/**
ICSI, n (%)	37 (65)	21 (46)	n.a.
Gestational characteristics			
Smoking during pregnancy, n (%)	6 (11)	5 (11)	6 (9)
Alcohol use during pregnancy, n (%)	3 (5)	0 (0)	2 (3)
Use of folic acid during pregnancy[b], n (%)	50 (93)	46 (100)*	57 (86)*
Vanishing twins, n (%)	6 (11)**	1 (2)	0 (0)**
Birth characteristics			
Gestational age in weeks, median (range)	39.4 (33.4–42.3)*	39.9 (34.6–42.6)	40.1 (30.1–42.6)*
Preterm birth (< 37 weeks), n (%)	6 (11)	6 (13)	4 (6)
Birth weight in grams, mean (σ)	3340 (563)*	3382 (604)	3594 (517)*
Low birth weight, n (%)	3 (5)	4 (9)	2 (3)
Small-for-gestational age[c], n (%)	0 (0)	3 (7)	1 (2)
Caesarean section, n (%)	15 (26)	8 (17)	19 (29)
Neonatal characteristics			
NICU admission, n (%)	1 (2)	2 (4)	4 (6)
Apgar score at 5 min < 7[b], n (%)	0 (0)	0 (0)	0 (0)
Breastfed for > 6 weeks[b], n (%)	29 (52)	21 (46)	33 (51)
Signs of foetal distress[d], n (%)	19 (33)	12 (26)	27 (41)
Parental characteristics			
Maternal age at conception, median (range)	33.2 (27.0–40.9)	32.8 (26.2–37.5)	33.7 (23.1–40.3)
Paternal age at conception[b], median (range)	36.4 (27.5–56.1)*	33.7 (28.3–47.8)*	35.4 (25.5–48.7)
Education level mother high[e], n (%)	20 (35)	20 (43)	31 (47)
Education level father high[b,e], n (%)	26 (48)	15 (33)	25 (38)

Student's *t*-tests, Fisher's exact tests and Mann-Whitney *U* tests were performed to investigate differences between the groups.
ART: assisted reproductive techniques, COH-IVF: children born following controlled ovarian hyperstimulation IVF or ICSI, MNC-IVF: children born following modified natural cycle IVF or ICSI, Sub-NC: naturally conceived children born to subfertile parents, TTP: time to pregnancy, ICSI: intracytoplasmic sperm injection, NICU: neonatal intensive care unit.

[a] Time to pregnancy of the three ART groups was recorded in years and months and finally converted into decimal years. In case of a miscarriage, the onset of TTP was reset, therefore TTP may be shorter than one year.

[b] Missing data in three groups: Use of folic acid — COH-IVF: n = 3; Apgar score at 5 min < 7 — MNC-IVF: n = 1, COH-IVF: n = 1; breastfed > 6 weeks — COH-IVF: n = 1, Sub-NC: n = 1; paternal age at conception — COH-IVF: n = 2, MNC-IVF: n = 1; education level father — COH-IVF: n = 3, MNC-IVF: n = 1; ovulatory cycle – COH-IVF: n = 1.

[c] Birthweight for gestational age is below 2 standard deviations compared with a Dutch reference population.

[d] Signs of foetal distress defined by meconium stained amniotic fluid and/or cardiotocographic signs and/or acidosis.

[e] University education or vocational colleges.

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

Table II
Neurological outcome in the Groningen ART cohort study groups, the pooled subfertile group and the fertile group.

Outcome	COH-IVF	MNC-IVF	Sub-NC	Subfertile group[a]	Fertile group
	n = 57	n = 46	n = 66	n = 169	n = 282
Neurological optimality score, mean [95% CI]	51.9 [50.5; 53.3]	53.1 [51.9; 54.3]	52.3 [51.2; 53.4]	52.4 [51.7; 53.1]	56.7 [56.3; 57.2]
Neurological classification					
Neurologically normal, n (%)	8 (14)	9 (20)	7 (11)	24 (14)	137 (49)
Simple MND, n (%)	32 (56)	20 (44)	35 (53)	87 (51)	109 (39)
Complex MND, n (%)	17 (30)	17 (37)	24 (36)	58 (34)	36 (13)
Neurologically abnormal, n (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Domains of neurological dysfunction					
Posture and muscle tone dysfunction, n (%)	22 (39)	16 (35)	27 (41)	65 (38)	46 (16)
Dysfunctional reflexes[b], n (%)	39 (68)	33 (72)	42 (64)	114 (67)	130 (46)
Dyskinesia, n (%)	7 (12)	2 (4)	6 (9)	15 (9)	9 (3)
Dysfunctional coordination, n (%)	39 (68)	34 (74)	52 (79)	125 (74)	80 (28)
Fine manipulative disability, n (%)	13 (23)	12 (26)	21 (32)	46 (27)	76 (27)
Excessive associated movements, n (%)	1 (2)	0 (0)	2 (3)	3 (2)	4 (1)
Sensory deficits, n (%)	1 (2)	0 (0)	0 (0)	1 (1)	1 (0)
Cranial nerve dysfunction, n (%)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0)

ART: assisted reproductive techniques, COH-IVF: children born following controlled ovarian hyperstimulation IVF or ICSI, MNC-IVF: children born following modified natural cycle IVF or ICSI, Sub-NC: naturally conceived children born to subfertile parents, MND: minor neurological dysfunction.
[a] Subfertile group: pooled COH-IVF, MNC-IVF and Sub-NC groups.
[b] The high prevalence of minor deviations in reflex function, e.g. tendon reflexes with high intensity and low thresholds, underscores the notion that minor reflex impairments occurring as an isolated phenomenon (i.e., not co-occurring with other signs of MND) have no clinical significance.

Table III
Associations between subfertility treatment, presence and severity of subfertility and neurological outcome.

Outcome measure: NOS	Unadjusted		Adjusted	
	Mean difference [95% CI]	*P*-value	Mean difference [95%]	*P*-value
COH-IVF versus MNC-IVF[a]	−1.19 [−3.10–0.71]	0.217	−0.86 [−2.87–1.15]	0.398
MNC-IVF versus Sub-NC[a]	0.80 [−0.86–2.46]	0.342	0.37 [−1.37–2.11]	0.674
COH-IVF versus Sub-NC[a]	−0.39 [−2.17–1.39]	0.663	−0.12 [−2.02–1.78]	0.898
Time to pregnancy[b]	−0.52 [−0.38–0.28]	0.756	0.00 [−0.32–0.33]	0.980
Pooled subfertile[c] versus fertile group[d]	−4.36 [−5.18 to −3.55]	< 0.001	−4.48 [−5.53 to −3.42]	< 0.001

Outcome measure: complex MND	Unadjusted		Adjusted	
	Odds ratio [95%CI]	*P*-value	Odds ratio [95%CI]	*P*-value
COH-IVF versus MNC-IVF[a]	0.73 [0.32–1.65]	0.445	0.81 [0.33–1.96]	0.636
MNC-IVF versus Sub-NC[a]	1.03 [0.47–2.24]	0.949	1.02 [0.43–2.40]	0.964
COH-IVF versus Sub-NC[a]	0.74 [0.35–1.59]	0.444	0.59 [0.24–1.42]	0.240
Time to pregnancy[b]	1.10 [0.95–1.27]	0.202	1.09 [0.93–1.26]	0.283
Pooled subfertile[c] versus fertile group[d]	3.57 [2.23–5.73]	< 0.001	5.13 [2.60–10.16]	< 0.001

NOS = neurological optimality score. COH-IVF: children born following controlled ovarian hyperstimulation IVF or ICSI, MNC-IVF: children born following modified natural cycle IVF or ICSI, and Sub-NC: naturally conceived children born to subfertile parents. MND = minor neurological dysfunction.
In general $P < 0.05$ was considered to be significant. Bonferroni corrections were applied in the pairwise comparison analyses of the three Groningen ART cohort groups: $P < 0.05/3 = 0.017$ was considered to be significant. In the analysis on the cause of underlying subfertility $P < 0.05/4 = 0.013$ was considered significant.
[a] Adjusted for sex, gestational age, birthweight, vanishing twins, time to pregnancy, maternal age and maternal educational level.
[b] Adjusted for sex, gestational age, birthweight, vanishing twins, maternal age and maternal educational level.
[c] Subfertile group: pooled COH-IVF, MNC-IVF and Sub-NC.
[d] Adjusted for sex, gestational age, birthweight, caesarean section, breastfed > 6 weeks, firstborn, age at examination, smoking during pregnancy, alcohol use during pregnancy, maternal age and maternal educational level.

1.69 [0.84–4.00]); NOS: adjusted mean difference [95% CI]: -0.64 [−2.15–0.86]).

3.4. Neurology at 9 in the pooled subfertile group and the fertile reference group

The background characteristics of the pooled subfertile group and fertile group are shown in Table IV. The children in the subfertile group were more often firstborn (65% vs. 44%; $P < 0.001$), were slightly older at the time of assessment at 9 years (110.2 vs. 107.9 months; $P < 0.001$), were more often born preterm (9% vs. 0%; $P < 0.001$), had a lower birth weight (5% vs. 1%: $P = 0.021$), were more often born through caesarean section (25% vs. 10%; $P < 0.001$), and were more often breastfed for longer than 6 weeks (50% vs. 30%; $P < 0.001$). Moreover, the subfertile mothers smoked less often during pregnancy (10% vs, 29%; $P < 0.001$), consumed less often alcohol during pregnancy (3% vs. 15%; $P < 0.001$), were older at the age of conception (33.2% vs. 29.5%; $P < 0.001$) and were more often highly educated (42% vs. 26%; $P < 0.001$).

At 9 years none of the children of both groups was classified as neurologically abnormal. In the pooled subfertile group 58 (34%) children were classified as having complex MND compared to 36 (13%) children of the fertile group (adjusted OR [95% CI]: 5.13 [2.60–10.16]). The increased prevalence of complex MND was brought about by higher prevalences in the domains of posture and muscle tone (adjusted OR [95% CI]: 2.84 [1.36–5.90]), reflexes (adjusted OR [95%

Table IV
Characteristics of children and parents of the pooled subfertile group and the fertile group.

Characteristics	Subfertile group	Fertile group	*P*-value
	n = 169	n = 282	
Child characteristics			
Male gender, n(%)	87 (51)	151 (54)	0.697
Firstborn, n (%)	110 (65)	123 (44)	< 0.001
Age at examination in months, median (range)	110.2 (100.7–131.8)	107.9 (102.1–115.2)	< 0.001
Gestational characteristics			
Smoking during pregnancy, n (%)	17 (10)	82 (29)	< 0.001
Alcohol use during pregnancy, n (%)	5 (3)	43 (15)	< 0.001
Birth characteristics			
Gestational age in weeks, median (range)	39.9 (30.1–42.6)	40.0 (37.0–42.0)	0.285
Preterm birth (< 37 weeks), n (%)	16 (9)	0 (0)	< 0.001
Birthweight in grams, mean (σ)	3471 (563)	3549 (474)	0.134
Low birthweight, n (%)	9 (5)	4 (1)	0.021
Small-for-gestational age[a], n (%)	4 (2)	5 (2)	0.733
Caesarean section, n (%)	42 (25)	28 (10)	< 0.001
Neonatal characteristics			
NICU admission, n (%)	7 (4)	0 (0)	0.001
Apgar score at 5 min < 7[b], n (%)	0 (0)	0 (0)	
Breastfed for > 6 weeks[b], n (%)	83 (50)	84 (30)	< 0.001
Parental characteristics			
Maternal age at conception, median (range)	33.2 (23.1–40.9)	29.5 (20.0–44.0)	< 0.001
Education level mother high[c], n (%)	71 (42)	72 (26)	< 0.001
Education level father high[b,c], n (%)	66 (40)	82 (32)	0.095

Student's *t*-tests, Fisher's exact tests and Mann-Whitney *U* tests were performed to investigate differences between the groups.
Subfertile group: COH-IVF, MNC-IVF and Sub-NC groups combined.
[a] Birthweight for gestational age is less than −2 standard deviations compared with the Dutch reference population.
[b] Missing data in the two groups: Apgar score < 7 — subfertile group: n = 2; breastfed > 6 weeks — subfertile group: n = 2; and education level father — subfertile group: n = 4, fertile group: n = 25.
[c] University education or vocational college.

CI]: 2.05 [1.06–3.99]), dyskinesia (adjusted OR [95% CI]: 4.23 [1.19–14.95]), and coordination (adjusted OR [95% CI]: 15.41 [6.82–34.81]; Tables II and III). Corresponding to the clinical outcome, the NOS was significantly lower in the subfertile group than in the fertile group (adjusted mean difference [95% CI]: −4.48 [−5.53 to −3.42]).

4. Discussion

This study showed that neurological outcome at 9 years of the COH-IVF, MNC-IVF and Sub-NC offspring did not significantly differ. Our findings suggest that ovarian hyperstimulation, the in vitro procedure and a combination of both do not affect neurological condition at 9 years. Second, neurological condition of the 9-year-old offspring of subfertile couples differed significantly from that of the 9-year-olds born to fertile couples. This suggests that parental subfertility may affect neurological outcome of offspring at 9 years.

Our findings on the potential effects of ART procedures are in line with the results of the Groningen ART cohort study at younger ages, when we also found no associations between the in vitro procedure and ovarian hyperstimulation and neurological outcome [6–9]. Knoester et al. [15] also studied the effect of IVF procedures on MND; they reported that IVF with ICSI was not associated with neurological outcome at 5 to 8 years. This finding was replicated and extended to the age of 9 years in the current study. Yet, Meijerink et al. [16] reported that 5-year-old children born after IVF with ICSI scored significantly below the norms of the general population on a standardized motor test. This difference may, however, be attributed firstly to the large proportion of twins studied (about one third of the study group), and secondly to the use of a specific form of ICSI, i.e., testicular sperm extraction ICSI. On the other hand, Zhu et al. [17] could not find an association between ART and motor outcome, in terms of DCD, at 7 years of age in the Danish National Birth Cohort.

Our study did not find associations between ART-components and neurological outcome, but it did observe a significantly higher prevalence of complex MND in the children born to subfertile parents than in the offspring of the fertile couples. At 3 months and 4 years, we also found a significant association between parental subfertility and non-optimal neurological condition [6,8]. In contrast, Ponjaert-Kristoffersen et al. [18] and Leunens et al. [19] showed that motor coordination evaluated at the ages of 5 and 8–10 years with standardized motor tests of singletons born after IVF with ICSI did not differ from that of singletons born after natural conception to fertile parents, implying that neither ART nor subfertility was associated with motor outcome at school-age. Yet, Knoester et al. [15] reported that the prevalence of MND (the simple and complex forms) in children born after IVF with ICSI tended to be higher than that in children born after natural conception in fertile parents. The trend was, however, not statistically significant. Conceivably, the differences in the various findings may be largely explained by the assessment techniques and the ages at which the children have been studied: the sensitive neuromotor measures used by Knoester et al. [15] and in the Groningen ART cohort study are more likely to detect group differences than the standardized motor tests used in the other studies. To detect subtle differences in neuromotor outcome, age-specific standardized tests have to be used. This means that the tests change with increasing age. In the study of Knoester et al. [15] the same MND-assessment was used as applied in the current study, which means that in the Knoester et al. [15] study, the children were tested in the lower age range of the test, as their children aged on average 6 years. This implies also that they assessed the children just before the period of substantial functional reorganisation in the school-aged brain, that occurs between 7 and 9 years [5]. In our study, outcome was assessed at 9 years, just after the transition, at an age at which minor dysfunctions generally 'bloom [5]. Another factor that may explain the differences in findings on potential differences in neuromotor outcome between offspring of subfertile couples and that of fertile couples is the size of the groups studied. This is illustrated by the study of Zhu et al. [17] who used the data of the large Danish National Birth cohort. This study showed that children born to subfertile couples – whether or not conceived with the help of

ART – had a slightly higher risk of DCD at the age of 7 years than children born to fertile couples.

Previously, we reported that a longer TTP – a proxy of the severity of subfertility – was associated with an increased risk of unfavourable neurological outcome at 4 years [6]. A similar association was absent in the current study. These findings are comparable to those of Zhu et al. [17,20] that indicated that an increased TTP was associated with a delayed achievement of motor milestones at 18 months, but not to motor outcome at 7 years. This may suggest that the effect of TTP fades away with increasing age. However, it is still conceivable that a very long TTP is associated with an unfavourable effect on neurological outcome [3]. Our study contained, however, too few participants with a TTP longer than 6 years to allow for the evaluation of this effect.

Our findings suggest that the presence of subfertility is associated with a fivefold increased risk for a less favourable neurological outcome. Interestingly, the analysis of the type of MND indicated that the children of the subfertile couples in particular had an increased risk of dysfunction in the neurological domains that preponderantly rely on subcortical networks (dysfunctional posture and muscle tone, dysfunctional reflexes, dyskinesia and dysfunctional coordination; Table II). Fine manipulative disability, a dysfunction mainly reflecting impairment of cortical networks, was not associated with subfertility [11]. The mechanisms that underlie the association between subfertility and MND are not well understood. In general, subfertility is caused by multifactorial conditions leading to a variety of the types of subfertility. Yet, it is conceivable that epigenetic changes in the male and female germ cells and the young embryo may induce a non-optimal development of the nervous system [21]. The association between subfertility and MND may also be induced and strengthened by increased maternal stress during pregnancy associated with the preceding fertility problems [22]. Increasing evidence suggests that prenatal stress is associated with mild impairments in the developing brain [23].

A major strength of the Groningen ART cohort study is its group composition that allows for a disentangling of the effects of ovarian hyperstimulation and the in vitro procedure. Another strength of the present study is the use of an age-specific, standardized and comprehensive neurological assessment allowing the detection of subtle deviations in neurological condition, that are associated with neurodevelopmental diagnoses such as DCD, autism and impaired learning [5,11].

The fertile reference group may be regarded as an additional strength. The participants of the subfertile and fertile reference group were tested with the same assessment, generally in the same assessment room, and the study was monitored by the same principal investigator (MHA). Yet, the nature of the fertile group is also a limitation of the study. The LCPUFA cohort was designed to study the effects of LCPUFA supplementation of infant formula. Formula feeding is associated with lower social class and a higher prevalence of smoking. In other words: the LCPUFA cohort study is not a random sample of the general fertile population, but a slightly disadvantaged group. This is reflected in the neurological condition of the 9-year-old children: the prevalence of complex MND was 13.6%, whereas in the general population it has been reported to be 6–7% for children born in the 70ies through the 90ies of last century [11,24]. This means that we may have underestimated the differences between the fertile group and the subfertile group. The stable prevalence of complex MND over the years makes it also unlikely that the fact that the fertile controls were born 7–8 years earlier than the children born to subfertile couples acted as a historical confounder. Another limitation of our study is the attrition rate of 21% and 19%, respectively, in the subfertile and fertile groups. This rate of attrition after 9 years is however considered as acceptable [25].

5. Conclusion

Our study shows that ovarian hyperstimulation and the in vitro procedure are not associated with a non-optimal neurological condition at school age. However, parental subfertility is associated with a less favourable neurological outcome, including a higher prevalence of complex MND. The first result is important for the counselling of subfertile couples. The second one has significance for society at large: couples should be aware that subfertility, which – in general – is associated with increased parental age at child conception, is associated with complex MND. Complex MND, in turn, is associated with an increased risk of developmental disorders, such as DCD, Attention Deficit Hyperactivity Disorder, Autism Spectrum Disorders and specific learning problems [24,26–28]. In addition, our results underscore the importance of long-term follow-up of offspring of subfertile couples born with and without ART. Future research needs to address the effect of factors underlying subfertility on long-term neurodevelopment.

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Conflict of interest statement

None.

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