




No effect of IVF culture medium on cognitive development of 9-year-old children

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STUDY QUESTION: Do embryo culture media used during an IVF/ICSI treatment have an effect on cognitive development of singleton IVF children at 9 years of age?

SUMMARY ANSWER: Cognitive development of children born after culture in two different embryo culture media is comparable.

WHAT IS KNOWN ALREADY: Previously, we have shown that the culture medium used in an IVF/ICSI treatment affects birthweight and weight at 2 years of age after alternating assignment to embryo culture in either K-SCICM (Cook) or G1™ Version 3 (Vitrolife). Children with low birthweight are known to have an increased risk for learning disabilities. Data on cognitive development in general of children born after ART are still conflicting, and the only study reporting on the effects of culture medium on cognitive development shows significant differences in cognitive development between two culture medium groups.

STUDY DESIGN, SIZE, DURATION: In this observational cohort follow-up study (MEDIUM-KIDS), parents of all singletons from our abovementioned study were approached after the ninth birthday of their child to participate in an additional follow-up study. Of the 294 eligible children included in the original study, 119 children (70 Vitrolife and 49 Cook) participated in the current study.

PARTICIPANTS/MATERIALS, SETTING, METHODS: All follow-up measurements were performed between March 2014 and December 2016. CITO (Dutch Central Institute for Test Development) developed the Dutch pupil monitoring system, which involves nationwide independent, standardized, academic achievement score tests to monitor the child's school performance twice a year at fixed time points from third grade onward. The tests include language skills (vocabulary and orthography), mathematics and reading capability and comprehension. Results from the tests performed between third and sixth grades, expressed as ability scores, were obtained from the school. To investigate school performance development over the years, we used a mixed effects multilevel model. The least complex model with the best fit was selected to analyze whether culture medium affects cognitive development in our cohort. The study had enough power to detect a difference in ability score that reflects at least one performance category between the two groups.

MAIN RESULTS AND THE ROLE OF CHANCE: No differences were seen in baseline characteristics between participants and non-participants (both parental and children characteristics). For all domains, the random intercept model was used. All analyses showed comparable results for the two culture medium groups. No significant differences were observed for any of the cognitive development domains, even after correction for potential confounders. Parental level of education was higher in the IVF group (45%) if compared to the national average level of education (35%), which most likely explains the higher CITO scores for the IVF children if compared to the National ability scores.

LIMITATIONS, REASONS FOR CAUTION: A limitation of the study was its pseudo-randomized design and the relatively low participation rate of 40.5%. This and the number of missing data prevent us from drawing robust causal conclusions. However, as this is the first and therewith oldest cohort of children where culture medium was allocated alternatingly and used in a blinded setting, in the same period, with all other conditions identical this study gives up until now the best available evidence.

WIDER IMPLICATIONS OF THE FINDINGS: Our study analyzes the effects of culture medium on school performance of children born after IVF/ICSI in a prospective cohort study. Although further research on long-term academic skills and also on behavior is essential, our results are reassuring and should make parents of children born after IVF/ICSI feel comfortable with their children's cognitive development.

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Key words: IVF/ICSI outcome / culture medium / child follow-up / cognitive development / ART

WHAT DOES THIS MEAN FOR PATIENTS?

This study looked at whether the type of culture media used in IVF/ICSI has an effect on the school performance of 9-year olds born after treatment. The culture medium is the liquid in the Petri dish that embryos are stored in during IVF/ICSI treatment.

Previous research had looked at two different types of culture media used in fertility treatment and found that this made a difference to the birthweight of the babies and continued to have an impact on those children's weight at the age of 2 years. This new study followed up the same children at the age of 9 years and looked at their school performance. Language skills, maths, reading and comprehension were all assessed.

The researchers found no differences between the children relating to the two different types of culture media. They say that more research is needed to assess their long-term academic skills and to look at their behavior, but that their findings should make parents of IVF/ICSI children feel more comfortable about any impact on their children's academic development.

Introduction

Children born after IVF are known to have an increased risk of adverse perinatal outcomes, such as low birthweight and preterm birth, compared to children born after natural conception (Jackson et al., 2004; Bergh and Wennerholm, 2012; Pinborg et al., 2013). Studies on long-term outcome of the children born after IVF are scarce. There is growing evidence that culture medium can affect birthweight of children born after ART, however the comparison of studies analyzing these effects is complicated by the fact that each study compares different culture media with different compositions (Zandstra et al., 2015).

It is known that adverse perinatal outcomes, such as preterm birth and low birthweight, are associated with a higher incidence of learning disabilities and attention deficit hyperactivity disorder (Murray et al., 2016). Also, it is known that intelligence quotient (IQ) is related to birthweight and that the childhood IQ increases within the normal birthweight ranges (Matte et al., 2001). It is also known that children born preterm are at increased risk of poor academic outcome (Twilhaar et al., 2018).

Only a limited number of studies have been published on cognitive outcome of children born after IVF, with conflicting results. In 2013, a study published by Berry et al. (2013) found similar cognitive functions at 3 years of age if late preterm (LPT) children born after IVF were compared with LPT non-IVF children, suggesting that IVF itself at least not worsens the diminished cognitive development in LPT born children (Berry et al., 2013). However, in children with normal perinatal outcome, a systematic review showed a possible negative effect on cognitive development caused by fertility treatments (Rumbold et al., 2017). Most high-quality studies did not find differences if IVF children were compared with naturally conceived children, but some studies

showed lower IQs specifically among ICSI children (Rumbold et al., 2017). A recent study by Schendelaar et al. (2016) analyzed the effects of hormonal stimulation on cognitive outcome of the offspring. They found no causal relation between ovarian stimulation or the *in vitro* procedure and the outcome. However, direct negative causal effects were found between severity of subfertility (defined by time to pregnancy) and cognition and behavior (Schendelaar et al., 2016). Only one study reporting on the effects of culture medium on cognitive development has been published (Bouillon et al., 2016). They compared development of the offspring after a randomized study where they allocated IVF treatments to the use of one of two different culture media [Single step Medium (SSM) and Global]. Neonatal and early childhood (up to 5 years) follow-up showed that the children in the Global group were significantly less likely than the children in the SSM group to show developmental problems (Bouillon et al., 2016).

In 2010, we published the results of our study where a corrected difference of 112 g in birthweight was observed in children born after IVF, if two different culture media were used alternatingly (Dumoulin et al., 2010). The children born after embryo culture in G1 version 3 (Vitrolife AB, Goteborg, Sweden) were heavier with an average birthweight of 3453 (SEM ± 53) grams compared to the average birthweight of 3208 (SEM ± 61) grams for the children born after embryo culture in Sydney IVF Cleavage Medium (K-SICM: Cook Medical, Brisbane, Australia) ($P = 0.003$) (Dumoulin et al., 2010). Further follow-up showed that the weight difference between the two culture medium groups was still present at 9 years of age, likely to be caused by a higher adiposity (Zandstra et al. 2018). The difference in weight and BMI associated with culture medium was 1.58 kg [95%CI 0.01–3.14] and 0.84 kg/m² [95%CI 0.02–1.67], respectively, with Vitrolife children being heavier (Zandstra et al., 2018). Knowledge

about the effects of culture medium on cognitive development of children born after IVF is scarce and this is the oldest cohort with children born after embryo culture in two different culture media used alternately. Therefore, this population is extremely valuable for evaluating possible effects of culture medium on long-term cognitive development of children born after ART. Therefore, the aim of our study was to analyze the effect of culture medium on cognitive development of the children from this cohort 9 years after IVF. Furthermore, we compared the IVF group with the average Dutch scores to gain insight into the cognitive performance of the IVF group compared to a non-IVF group.

Materials and Methods

Study design

The current study is a part of a prospective observational cohort study (MEDIUM-KIDS). In this prospective observational cohort study, parents of all singletons from our first culture medium study (Dumoulin *et al.*, 2010) were approached, after the ninth birthday of their child, to participate in a follow-up study (MEDIUM-KIDS). It concerned all singletons born after fresh embryo transfer of cleavage stage embryos resulting from an IVF or ICSI treatment between July 2003 and December 2006, when two

culture media (either G1 version 3 (Vitrolife AB, Goteborg, Sweden), or K-SICM (Cook Medical, Brisbane, Australia)) were used strictly alternately in our laboratory. Children resulting from a twin pregnancy were excluded from the study. The media from both suppliers were ready to use and supplemented with 5 mg/ml human serum albumin. Except for the media, all other IVF procedures (clinical as well as laboratory) were similar in both groups. Detailed information on ovarian stimulation, fertilization, culture and embryo characteristics has been described previously (Dumoulin *et al.*, 2010).

All follow-up measurements were performed between March 2014 and December 2016. Parents and their child were invited to attend our clinic for a single visit, lasting ~2.5 h. Detailed information on the complete follow-up study protocol and anthropometric and cardiovascular outcome has also been published (Zandstra *et al.*, 2018). Information on possible confounding factors, such as lifestyle, demographic data and medical history of the child and parents, was recorded by means of a questionnaire, completed by the parents on the day of examination. As a part of the follow-up, school performance data were analyzed.

Ethical approval

The study was registered in the Dutch Trial register (NTR4220) and the local ethics committee approved the study. Both parents of all children gave written informed consent.

Table 1 Baseline characteristics of the parents of 294 singleton children born after ART who were eligible for participation in the study.

Characteristics	Participants (n = 119) ^{b,c}	Non-participants ^a (n = 175) ^{b,c,d}	P-value
Duration of infertility (years)	3.5 (1.87)	3.4 (1.76)	0.849
Type of treatment			0.580
ICSI	79 (66.4%)	111 (63.4%)	
IVF	40 (33.6%)	64 (36.6%)	
Maternal characteristics (at ovum retrieval)			
Age (years)	32.8 (3.7)	32.3 (3.9)	0.242
Height (Cm)	168.5 (6.5)	168.7 (6.7)	0.380
Weight (Kg)	69.3 (10.0)	68.5 (10.7)	0.551
BMI (Kg/m ²)	24.3 (3.1)	23.9 (3.4)	0.130
Paternal characteristics (at ovum retrieval)			
Age (years)	35.6 (4.9)	35.5 (5.7)	1.000
Height (Cm)	181.8 (8.0)	181.4 (7.5)	0.675
Weight (Kg)	86.3 (11.8)	83.8 (13.5)	0.122
BMI (Kg/m ²)	26.1 (3.0)	25.2 (4.4)	0.062
Gestational age (weeks)	39.6 (1.8)	39.2 (3.5)	0.280
Preterm birth (<37 weeks)	4 (3.4%)	11 (6.3%)	0.271
Very preterm birth (<32 weeks)	1 (0.8%)	4 (2.3%)	0.367
Birthweight (g)	3403.2 (531.5)	3326.6 (596.7)	0.908
LBW (<2500 g)	5 (4.2%)	11 (6.3%)	0.442
Very LBW (<1500 g)	1 (0.8%)	4 (2.3%)	0.367
Gender (Male)	56 (47.1%)	85 (48.6%)	0.799

^aBaseline characteristics in this table represent characteristics at the time of the IVF/ICSI treatment, since characteristics of non-responders could not be measured at the time of the present study. Statistical analysis was performed using one-sided Student's *t*-test and logistic regression.

^bCategorical data presented as *n* (%).

^cContinuous data presented as mean (SD).

^dOf the 294 eligible participants, 9 were lost to follow-up and data were not retrieved for 166 children. LBW, low birthweight.

School performance

To measure cognitive development of the children in our cohort, school performances were analyzed. In the Netherlands, primary school usually starts in September after the child turns 4 years old. The vast majority of schools (95%) use the test and monitor system developed by CITO (Centraal Instituut voor TestOntwikkeling, Dutch Central Institute for Test Development), the Dutch national pupil monitoring system, to give parents and teachers insight into the study progression of a child during primary school. Within this monitoring system regular testing is done. The first test starts in the third year of primary school at the age of 6 years and tests are offered to all children twice a year on fixed time points. The test includes the following five domains: comprehensive reading, orthography, vocabulary, technical reading and mathematics. The test for comprehensive reading consists of a series of different texts with multiple-choice questions about the content of the text to test the child's understanding of the written text. Orthography is tested by a series of verbally presented words (read out loud by the teacher) that should be written down correctly by the child. Technical reading requires a 3-min reading test which measures fluency and speed of word reading by counting the number of correctly read words from three cards containing separate words with an increasing level of difficulty. Vocabulary is tested by presenting separate words to the child together with multiple-choice questions about the meaning of the word. Also, several mathematics skills including computational problems and problems on notion of time and the use of money are tested.

The raw test scores on each domain are converted to a standardized individual ability score, which corrects for test content and difficulty and allows teachers to monitor progression in performance. It also allows a

meaningful comparison between children, also in different classes and schools, which enables us to compare the two culture medium groups. Moreover, it allows a comparison with average results of all Dutch children. Average results for Dutch children were obtained from the CITO institution (CITO, 2016).

Statistical analysis

All analyses were conducted in SPSS version 23.0 (IBM-SPSS, Chicago, IL, USA). A Shapiro–Wilk test ($P < 0.05$) and a visual inspection of the histograms, normal Q-Qplots and box plots were used to test for normality of the data. For comparing baseline characteristics of the two groups of both parents and children, an unpaired Student's *t*-test (for continuous variables) and logistic regression analysis were performed. To investigate school performance development over the years, we used a mixed effects multilevel model. This model enables us to compare the longitudinal school performance of all children while taking into account the dependency of data within one child. Furthermore, it allows data to be unbalanced (i.e. participants do not have to be measured at the same time points). This analysis was conducted separately for each domain. The ability scores of all children were modeled against the number of months that the child had received education at that particular test moment, expressed by 'didactic age'. The culture medium as well as several potentially confounding factors (parental education, gender of the child, age of the mother, smoking behavior of the mother, ethnicity of the parents and the duration of being breastfed) were added to the model as a fixed factor and the children as a random factor to take into account the repeated measures. We used an unstructured variance–covariance matrix for the random effects

Table II Baseline characteristics of the 119 participating children.

Characteristics	ART culture medium Cook ($n = 49$) ^{a,b}	Vitrolife ($n = 70$) ^{a,b}	P-value
Boys (n)	24 (49.0%)	32 (45.7%)	0.725
Method of conception			0.604
ICSI	31 (63.3%)	48 (68.6%)	
IVF	18 (36.7%)	22 (31.4%)	
Embryo transfer			0.693
SET	27 (55.1%)	36 (51.4%)	
DET	22 (44.9%)	34 (48.6%)	
Gestational age at birth (weeks)	39.6 (2.2)	39.6 (1.4)	0.979
Preterm birth (<37 weeks)	1 (2.0%)	3 (4.3%)	0.504
Very preterm birth (<32 weeks)	1 (2.0%)	0 (0%)	0.230
Birthweight (grams)	3301.4 (529.5)	3460.2 (519.5)	0.166
LBW (<2500 g)	4 (8.2%)	1 (1.4%)	0.072
Very LBW (<1500 g)	1 (2.0%)	0 (0%)	0.230
Breastfeeding (Yes)	29 (59.2%)	38 (54.3%)	0.398
Duration of breastfeeding (months)	2.6 (3.3)	3.8 (7.8)	0.339
Performing sports (Yes)	43 (87.8%)	66 (94.3%)	0.351
Duration of sports/ week (hrs)	2.8 (1.8)	2.9 (1.9)	0.693
Medical history			
Autism-related disorders ^c	2 (4.1%)	5 (7.1%)	0.501
Allergic problems (including asthma, eczema)	3 (6.1%)	9 (12.9%)	0.253

^aCategorical data presented as n (%).

^bContinuous data presented as mean (SD).

^cAutism-related disorders: PDD-NOS (2x), ADD (1x), autism (2x) and ADHD (2x).

^dStatistical analysis was performed using one-sided Student's *t*-test.

SET, single-embryo transfer, DET, double-embryo transfer, PDD-NOS, pervasive developmental disorder-not otherwise specified, ADD, attention deficit disorder, ADHD, attention deficit hyperactivity disorder.

and tested two covariance structures (unstructured and autoregressive I) for the correlations of measures over time within children. The model fit was compared based on the restricted log likelihood (-2LL) and Akaike information criterion (AIC) using Chisquare. Standardized ability scores were compared for all domains between both culture media groups and the Dutch reference population using a one-sided *t*-test.

Results

The inclusion process is described in detail elsewhere (Zandstra *et al.*, 2018). A total of 294 singletons were eligible to participate in the MEDIUM-KIDS study, 126 children born after embryo culture in Cook medium and 168 after culture in Vitrolife medium. Out of the 294 parent couples, nine were lost to follow-up because of living abroad or failure to trace them. Of the remaining 285, a total of 136 (47.7%) parent couples agreed for their child to participate. They signed a written informed consent; 61 (50.8%) in the Cook group, and 75 (45.5%) in the Vitrolife group ($P = 0.523$). Of the 136 participating children, we were able to retrieve the available CITO results of 119 children; 49 children in the Cook group and 70 in the Vitrolife group. The schools of the remaining 17 children did not use the CITO system or were using a different version of the system with results not comparable to the earlier versions. Not all children had results for all measuring moments since some measuring moments are optional and the schools can decide to skip those. Also, depending on the age at inclusion, not all children had already performed the mid sixth-grade test (M6). The baseline biomedical characteristics of the participating children (gestational age, birthweight, gender) were comparable to those of the children not participating in the study (Table I). Neonatal outcome and baseline biomedical characteristics of the participating children were compared, suggesting a higher birthweight for the Vitrolife children, in keeping with the full cohort in the original study (Dumoulin *et al.*, 2010), however this was no longer significant due to the smaller subgroup sizes (Table II). Baseline biomedical characteristics of the parents were compared for both groups (Table III). Level of education of the fathers was significantly ($P = 0.032$) higher in the Vitrolife group. For the mothers, this was the opposite, although not statistically significant ($P = 0.089$). Next to other variables, having at least one higher educated parent or not was included in the models as a possible confounder.

For the linear mixed model, we tested several models and covariance structures. The least complex model with the best fit was selected to analyze whether culture medium affects cognitive development in our cohort. For all domains, the unstructured matrix with random intercept model was chosen as the model with the best fit. All analyses showed comparable results for the two culture medium groups. No significant differences were observed for any of the cognitive development domains, even after correction for confounders; results are presented in Fig. 1.

The average ability scores of the IVF children were also compared cross-sectionally with the national average ability scores for all five domains. Both IVF groups performed significantly better compared to the average Dutch scores on almost all measuring moments on all five domains. The difference was significant on all measuring moments except for end of third grade for technical reading and mid third grade for vocabulary. The results for mid fifth grade (= 25-month didactic age) are presented in Fig. 2, as an example. The presented differences

between the IVF children and the average Dutch population were comparable for the other measuring moments (data not shown).

Discussion

Comparison of all school performance (CITO) test scores of the children showed no significant differences between the two culture media groups. The results of our study are in contrast with the only other study published previously on the effects of culture medium on cognitive development: this study showed a lower general development and more developmental problems in children born after culture in SSM culture medium if compared to Global culture medium (Bouillon *et al.*, 2016). However, the endpoint of our study is slightly different, since we focused specifically on school performance, while the study by Bouillon *et al.* (2016) also compared motor development and social behavior. However, we checked for differences in major behavioral or developmental problems by asking the parents for the presence of attention deficit hyperactivity disorder or autism spectrum disorders, which was comparable between the two groups. Also the culture media used in the study of Bouillon and co-workers were different from the culture media we compared. Results of different studies focusing on cognitive development of IVF children compared to naturally conceived children have been published with conflicting results (Rumbold *et al.*, 2017). Different study designs, the use of different test instruments and population compositions, and small population

Table III Baseline characteristics of parents at the time their participating children were 9 years old.

Characteristics	ART culture medium Cook (n = 49) ^{a,b}	Vitrolife (n = 70) ^{a,b}	P-value
Maternal characteristics			
Age (years)	42.6 (3.2)	42.5 (4.2)	0.786
Height (cm)	167.4 (7.0)	169.2 (6.2)	0.141
Weight (kg)	68.8 (10.5)	73.1 (12.4)	0.129
BMI (kg/m ²)	24.5 (3.6)	25.5 (3.8)	0.309
Smoking (yes)	11 (22.4%)	6 (8.6%)	0.029
Higher Education	21 (42.9%)	20 (28.6%)	0.089
Caucasian ethnicity	45 (91.8%)	65 (92.9%)	0.613
Exercising regularly	30 (61.2%)	40 (57.1%)	0.723
Primary infertility	37 (75.5%)	54 (77.1%)	0.836
Paternal characteristics			
Age (years)	45.8 (5.2)	45.0 (4.6)	0.285
Height (cm)	180.3 (7.8)	182.8 (7.8)	0.089
Weight (kg)	86.9 (12.4)	90.7 (12.8)	0.120
BMI (kg/m ²)	26.7 (3.4)	27.1 (3.3)	0.619
Smoking (yes)	6 (12.2%)	12 (17%)	0.307
Higher education	13 (26.5%)	31 (44.3%)	0.032
Caucasian ethnicity	48 (97.9%)	68 (97.1%)	0.684
Exercising regularly	28 (57.1%)	37 (52.9%)	0.557

^aCategorical data presented as n (%).

^bContinuous data presented as mean (SD).

^cStatistical analysis was performed using a one-sided Student's *t*-test.

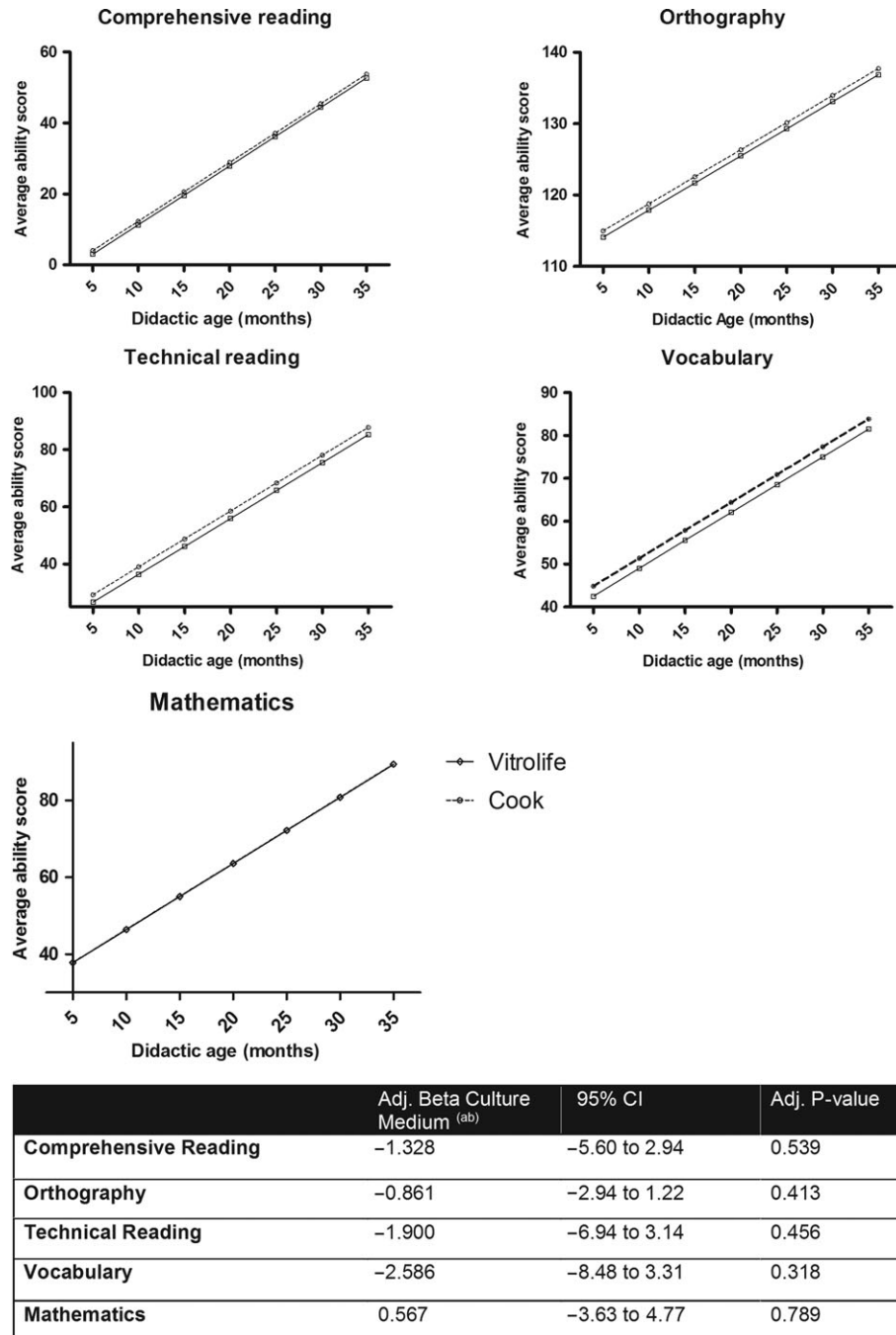
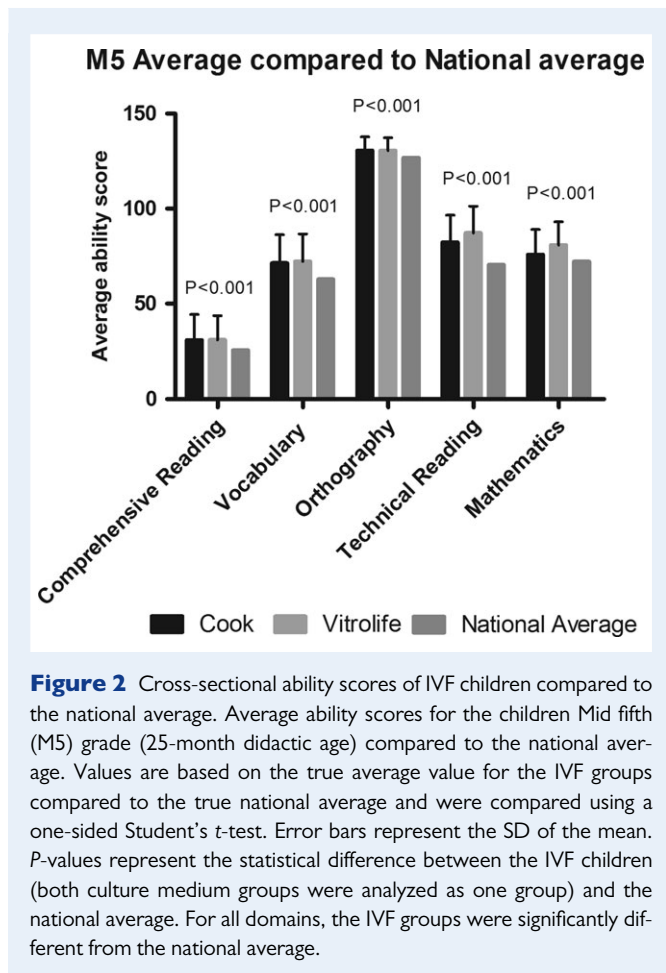


Figure 1 Longitudinal cognitive development. Results for the effect of IVF culture medium on cognitive development, using mixed effects multilevel analysis. For all domains, an unstructured matrix with random intercept was used. The graphs represent the results for the fixed effects of the multilevel analysis. (a) Cook culture medium was used as the reference in this analysis. Therefore, the adjusted Beta reflects the estimated difference for the Vitrolife group compared to the Cook group. The presented CIs reflect the 95%CI of the adjusted Beta. (b) Values are adjusted for educational level of the parents, gender of the child, age of the mother, smoking behavior of the mother, ethnicity of the parents and (duration of) being breastfed.

sizes have led to different outcomes in several studies comparing children born after ART with naturally conceived children.

A study by Spangmose et al. (2017) found lower cognitive scores for IVF singleton adolescents (15–16 years) if compared to naturally conceived controls using a large Danish administrative data register with

standardized scores of school performance. This was not seen in twins (Spangmose et al., 2017). Another study performed by Barbuscia and Mills (2017) reported the results of a study comparable to Spangmose et al. (2017) but found opposite results, with IVF children performing better than controls. At the ages of 3 and 5 years, children conceived



with the aid of IVF had statistically higher verbal cognitive abilities than children conceived naturally. However, this difference consistently decreased over time and had disappeared by age 11 years (Barbuscia and Mills, 2017).

Both groups in our study showed significantly higher academic performance if compared to the national average scores at the age of 9 years. Since it is known that parental education is a very important parameter in determining the academic performance of the child (Chin-Lun Hung *et al.*, 2015, Ruijsbroek *et al.*, 2015, Spangmose *et al.*, 2017), the most likely explanation for this difference is the higher level of parental education in our study population compared to the average Dutch population. The percentage of at least one higher educated parent was 45.9% for the Cook group and 45.3% for the Vitrolife group. This percentage was 35.2% for Dutch people from the same year of birth as the parents of the children from our cohort (CBS). Higher parental education in ART groups compared to a natural conception cohort is also described in several other studies (Mains *et al.*, 2010, Barbuscia and Mills, 2017, Spangmose *et al.*, 2017). Also our analyses showed that parental level of education was significantly associated with the ability scores of the child, which supports this theory.

Limitations of our study are the relatively low participation rate (40.5%) and hence the relatively small sample sizes. However, with the current sample size we were able to detect the clinically relevant difference in ability score between the two groups. The CITO tests are validated tests to monitor progression of children over time and to

compare school performance with a child's peers. Therefore, it allows for an objective, validated comparison between the two culture medium groups and gave us the opportunity to compare these results with the average Dutch children in the same age groups. Although further research on long-term academic skills and behavior is essential, the results of this study are reassuring and should make parents of children born after IVF feel comfortable with their child's cognitive development.

In conclusion, our study did not show any significant differences in cognitive development of children born after culture as an embryo for 2–3 days in one of the two different culture media compared. Significant differences were observed if the scores of the IVF children were compared with the general Dutch population, which is most likely explained by a higher parental education level in the IVF group.

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Authors' roles

All authors fulfill the criteria for authorship; A.v.M. and H.Z. initiated the study; H.Z. collected the data; A.v.M., L.S. and S.v.K. and H.Z. analyzed the data; A.v.M., J.D., J.E. and H.Z. interpreted the data. All authors commented on the draft, and have seen and approved the final version.

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Conflict of interest

The authors have no conflict of interest to declare.

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