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IVF culture medium affects post-natal weight in humans during the first 2 years of life†

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STUDY QUESTION: Is post-natal growth during the first 2 years of life in IVF singletons affected by type of medium used for culturing human embryos during an IVF treatment?

SUMMARY ANSWER: The *in vitro* culture of human embryos in medium from Cook resulted in singletons with a lower weight during the first 2 years of life compared with singletons born after embryo culture in medium from Vitrolife.

WHAT IS KNOWN ALREADY: In a previous study, we reported that type of medium used for culturing human IVF embryos during the first few days after fertilization until fresh embryo transfer significantly affects fetal growth and consequently birthweight of the resulting singletons.

STUDY DESIGN, SIZE, DURATION: From July 2003 to December 2006, a total of 1432 IVF treatment cycles with fresh embryo transfer were randomly allocated to have all embryos cultured in medium from Vitrolife AB ($n = 715$) or from Cook ($n = 717$). Two years after delivery, questionnaires were sent to the parents of all children requesting data about weight, height and head circumference around 1, 2, 3, 4, 6, 7.5, 9, 11, 14, 18 and 24 months of age. These measurements were collected as part of the children's health programme at municipal infant welfare centres in the Netherlands by health professionals unaware of this study.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Patients requiring donor oocytes or applying for PGD were excluded from the study. From the 294 live born singletons that fulfilled our inclusion criteria, 29 were lost to follow-up. The remaining 265 singletons (Cook group: 117, Vitrolife group: 148) were included in the analysis. Data analysis included linear regression, to compare cross-sectionally weight standard deviation score (SDS), height SDS and head circumference, and the first order Berkey-Reed model for a longitudinal analysis of the growth data.

MAIN RESULTS AND THE ROLE OF CHANCE: Singletons in the Vitrolife group were heavier during the first 2 years of life compared with singletons in the Cook group. Cross-sectional analyses showed that adjusted weight SDS differed between groups at 1 (0.35 ± 0.14, $P = 0.010$), 2 (0.39 ± 0.14, $P = 0.006$), 3 (0.35 ± 0.14, $P = 0.011$), 4 (0.30 ± 0.13, $P = 0.020$), 11 (0.28 ± 0.13, $P = 0.036$), 14 (0.32 ± 0.13, $P = 0.014$) and 24 (0.39 ± 0.15, $P = 0.011$) months of age, while adjusted height SDS was only significantly different at 1 (0.21 ± 0.11, $P = 0.048$) month of age. Head circumference was similar between the two groups at all ages. Longitudinal analyses showed that both post-natal weight ($P = 0.005$) and height ($P = 0.031$) differed between the groups throughout the first 2 years of life, while the growth velocity was not significantly different between the two groups.

LIMITATIONS, REASONS FOR CAUTION: Factors that might influence post-natal growth were included in the analysis; however, it was not possible to include all such factors, for example childhood diseases or nutrition, as this information was not available.

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WIDER IMPLICATIONS OF THE FINDINGS: The effect of culture medium during the first few days after fertilization on prenatal growth and birthweight persists during the first 2 years of life. This suggests that the human embryo is sensitive to its very early environment, and that the culture medium used in IVF may have lasting consequences. Further monitoring of the long-term growth, development and health of IVF children is therefore warranted.

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Introduction

The first child conceived by IVF was born 35 years ago (Steptoe and Edwards, 1978). Since then, the use of assisted reproduction technologies (ART) has increased substantially. In 2009, the percentage of children conceived with *in vitro* techniques ranged from 0.6% up to 4.5% of the national births in Europe (Ferraretti *et al.*, 2013).

IVF singletons have a significantly increased risk of perinatal mortality, preterm birth, small for gestational age, low birthweight and birth defects (Helmerhorst *et al.*, 2004; Jackson *et al.*, 2004; Rimm *et al.*, 2004; McDonald *et al.*, 2009; Pandey *et al.*, 2012) compared with naturally conceived singletons. Both patient-related factors, for example older age of the mother, time to pregnancy, and subfertility of the parents, and specific aspects of ART, for example hormonal stimulation and culture conditions, may be risk factors for the adverse perinatal outcome in IVF children (Pinborg *et al.*, 2013).

Our group showed that the culture medium used during an IVF treatment affects birthweight (Dumoulin *et al.*, 2010; Nelissen *et al.*, 2012). The onset of the growth differentiation was already evident during the second trimester of pregnancy, as a more advanced fetus was seen in the Vitrolife group compared with the Cook group at 20 weeks of gestation, based on the differences between gestational age calculated by dating formulas using fetal biparietal diameter and the actual gestational age (Nelissen *et al.*, 2013). It is important to monitor the post-natal growth of these children to investigate whether this effect of culture medium disappears, is still present or increases later in life.

The aim of our present study was to compare post-natal weight, height and head circumference at several time points (cross-sectional analysis) and post-natal growth (longitudinal analysis) during the first 2 years of life in the same singletons as our previous study.

Materials and Methods

Study design

At the Maastricht University Medical Centre, The Netherlands, a total of 1432 IVF treatment cycles with fresh embryo transfer in the period from July 2003 to December 2006 were randomly allocated to culture in either medium from Cook (K-SICM, Brisbane, Australia; $n = 717$) or from Vitrolife AB (G1.3, Göteborg, Sweden; $n = 715$). Both culture media were commercially available and widely used. The media from both suppliers were ready to use and supplemented with 5 mg/ml human serum albumin. No serum was added to the media. Laboratory technicians strictly alternated between the two culture media for each cycle and this allocation was concealed from the clinicians involved in scheduling the order of ovum retrieval on a certain day, as well as from the patients. The alternate use of two culture media was part of an internal quality monitoring system that had been introduced to enable the identification of suboptimal batches of a particular medium. Besides type of culture medium, all other procedures in the IVF treatment, for example ovarian stimulation, fertilization, culture and embryo transfer procedures, were similar in both groups, as have been described earlier (Dumoulin *et al.*, 2010).

For the present study, all singleton live births after fresh embryo transfer were included. Patients requiring donor oocytes or applying for PGD were excluded from the study. In case couples had more than one child during the study, only data from the first singleton pregnancy were included in the analysis. This resulted in 126 live born singletons in the Cook group and 168 singletons in the Vitrolife group (Nelissen *et al.*, 2012). All participating couples gave written informed consent for the use of their data. The local medical ethics committee approved the collection of data for quality monitoring purposes as part of our IVF treatment protocol.

Study population and data collection

During the study period, only patients with a BMI of <30 kg/m^2 and ≤ 40 years of age, with the exception of a few cases, were admitted to our IVF programme. Parental weight, height and smoking habits were recorded at the start of each IVF treatment cycle. After delivery, the obstetricians and midwives were contacted to obtain information on pregnancy outcome. Two years after delivery, questionnaires were sent to the parents of all children requesting data about weight, height and head circumference around 1, 2, 3, 4, 6, 7.5, 9, 11, 14, 18 and 24 months of age. These measurements are part of the children's health programme at municipal infant welfare centres in the Netherlands, in which children are routinely measured by health professionals unaware of this study. Head circumference was only measured until the age of 11 months in most cases. Post-natal weight and height measurements are expressed as standard deviation score (SDS), normalized for age and gender, and were calculated by using the online TNO growth calculator, which is based on the data from the Fifth National Growth Study (TNO, 2010).

Statistical analysis

Cross-sectional differences in weight, height and head circumference between study groups were tested by use of the Student's *t*-test. Adjusted differences were analysed by use of linear multivariable regression analysis, while controlling for the following covariates: parental characteristics (height, weight, smoking habits and age), parity, gestational age at birth and gender. In case of weight SDS and height SDS, the covariate gender was omitted, as the SDS is normalized for gender.

To study whether the growth velocity of children from the Cook group was different from that of children from the Vitrolife group during the first 2 years of life, a variety of different growth models were applied on the pooled growth data from both groups. The goal was to find a (parametric) model that adequately captures the individual and overall growth trends. In particular, the Count, (first and second order) Berkey-Reed, and several non-linear

models (Jenss-Bayley, Gompertz, four-parameter logistic) were examined for their fitness (Jenss and Bayley, 1937; Berkey and Reed, 1987; Pinheiro and Bates, 2000; Hauspie and Molinari, 2004). In all cases, a mixed-effects model was used with random effects corresponding to all model variables with an unstructured variance-covariance matrix. Analyses were conducted with R (version 2.15.2) using the 'nlme' package (Pinheiro and Bates, 2000).

Among the various models considered, the first order Berkey-Reed model (Berkey and Reed, 1987) was given preference, for the following reasons. First, it is the model that fits the data best. Second, it is a linear model and therefore easier to fit than the non-linear models. In addition, the model automatically incorporates the gestation length into the model, as the age variable is counted starting at conception (which is exactly known in these cases). The basic model is described by:

$$y_{ij} = \beta_{0i} + \beta_{1i}\text{age}_{ij} + \beta_{2i}\log(\text{age}_{ij}) + \beta_{3i}(\text{age}_{ij})^{-1} + \beta_4\text{medium}_i + e_{ij},$$

where y_{ij} denotes the jth measurement for the ith individual, age_{ij} denotes the age of measurement (with age 0 corresponding to conception), and $\log(\text{age}_{ij})$ and $(\text{age}_{ij})^{-1}$ are the corresponding log and inverse transformed values of age_{ij}.

To avoid very small coefficients, the age variable was coded in years. Two-sided P-values of <0.05 were considered to reflect statistical significance.

Results

Of the 294 live born singletons (Cook group: 126, Vitrolife group: 168) that fulfilled our inclusion criteria, 29 were lost to post-natal follow-up (Cook group: 9, Vitrolife group: 20), because parents declined to participate or could not be traced. The remaining 265 (Cook group: 117 (92.9%), Vitrolife group: 148 (88.1%)) were included in the analysis. Parental and cycle characteristics are presented in Table I.

Table I Parental and cycle characteristics in a study of the effect of IVF culture medium on post-natal growth during the first 2 years of life.

Characteristic	Vitrolife group (n = 148)	Cook group (n = 117)
Primary indication for IVF treatment		
Tubal factor	15 (10.1)	16 (13.7)
Male factor	91 (61.5)	74 (63.2)
Unexplained	35 (23.6)	23 (19.7)
Other	7 (4.7)	4 (3.4)
Duration of subfertility (years)	3.36 ± 1.7	3.48 ± 1.9
Primary subfertility	114 (77.0)	86 (73.5)
Cycles with ICSI	93 (62.8)	75 (64.1)
Cycles with transfer on Day 2	114 (77.0)	87 (74.4)
Cycles with transfer on Day 3	34 (23.0)	30 (25.6)
Single embryo transfer	74 (50.0)	69 (59.0)
Maternal characteristics		
Age (years)	32.3 ± 4.0	32.6 ± 3.6
Age ≥ 38 years	10 (6.8)	8 (6.8)
Height (cm)	169.4 ± 6.0	167.8 ± 7.4
Weight (kg)	69.9 ± 10.2	67.6 ± 10.1
Smoking ≥10 cigarettes/day	21 (14.2)	16 (13.7)
Paternal characteristics		
Age (years)	35.2 ± 5.7	35.7 ± 4.9
Height (cm)	182.3 ± 8.1	181.2 ± 7.4
Weight (kg)	86.9 ± 14.4	83.2 ± 11.0
Smoking ≥10 cigarettes/day	25 (16.9)	22 (18.8)

Data are presented as n (%) or mean ± SD. No statistical comparisons between groups were performed.

Cross-sectional analysis of post-natal weight, height and head circumference

Post-natal weight and height measurements were expressed as SDS normalized for age and gender. The weight SDS, height SDS and head circumference measurements of the singletons are presented in Table II. Weight SDS was significantly lower in the Cook group compared with the Vitrolife group at all ages. The adjusted mean differences in weight SDS were significant at 1, 2, 3, 4, 11, 14 and 24 months of age. Height SDS was significantly greater in the Vitrolife group compared with the Cook group at 1, 3, 4, 6, 9, 14 and 24 months of age. However, adjusted mean differences in height SDS were only significant at 1 month of age. Head circumference differences and adjusted mean differences were not significant between groups at any of the ages.

Longitudinal analysis of post-natal weight and height

To compare the overall growth trajectories of the two groups, a longitudinal analysis was performed. The growth trajectories were constructed by using the first order Berkey-Reed model with the data at 1, 2, 3, 4, 6, 7.5, 9, 11, 14, 18 and 24 months of age from each individual child.

For post-natal weight, the regression coefficients and significance levels of four different Berkey-Reed models are presented in Table III. Model 1 is the basic model and shows a difference of 216 grams (P = 0.002) between the groups, which indicates that during the first 2 years of life, children in the Vitrolife group were heavier compared with children in the Cook group. After addition of covariates, a difference of 188 grams (P = 0.005) remained between the groups (model 2). Besides type of culture medium, maternal height (P = 0.003), parity (P = 0.003) and child's gender ($P < 0.001$) are significantly associated with post-natal weight. To study the effect of culture medium on the velocity of weight gain in the children, the interaction variable culture medium × age was included (model 3). The growth trajectories do not diverge significantly from each other ($\beta = 0.161$, $P = 0.062$), which indicates that children from the Vitrolife group do not gain weight at a faster rate than children from the Cook group. Model 4 shows that maternal weight ($P < 0.001$) and paternal height (P = 0.016) are significantly associated with the rate of post-natal weight gain.

For post-natal height, the regression coefficients and significance levels are presented in Table IV. Model 1 is the basic model and shows a difference of 0.74 cm (P = 0.003) between the groups, with taller children in the Vitrolife group compared with children in the Cook group. After addition of covariates, a difference of 0.49 cm (P = 0.031) remained between the groups (model 2). Besides type of culture medium, maternal

Table II Post-natal head circumference and standard deviation score (SDS) for weight and height.

Characteristic	Vitrolife group	Cook group	*P*-value	Adjusted mean difference	*P*-value
Weight SDS					
1 month	0.23 ± 0.10	−0.13 ± 0.10	0.013	0.35 ± 0.14	0.010
2 months	0.49 ± 0.10	0.06 ± 0.11	0.004	0.39 ± 0.14	0.006
3 months	0.53 ± 0.09	0.10 ± 0.11	0.003	0.35 ± 0.14	0.011
4 months	0.60 ± 0.09	0.21 ± 0.10	0.004	0.30 ± 0.13	0.020
6 months	0.48 ± 0.10	0.15 ± 0.09	0.019	0.24 ± 0.13	NS
7.5 months	0.44 ± 0.10	0.13 ± 0.09	0.025	0.23 ± 0.14	NS
9 months	0.38 ± 0.10	0.09 ± 0.10	0.042	0.18 ± 0.14	NS
11 months	0.34 ± 0.09	−0.02 ± 0.10	0.008	0.28 ± 0.13	0.036
14 months	0.18 ± 0.09	−0.22 ± 0.09	0.002	0.32 ± 0.13	0.014
18 months	0.02 ± 0.12	−0.35 ± 0.10	0.018	0.26 ± 0.15	NS
24 months	0.07 ± 0.10	−0.43 ± 0.12	0.001	0.39 ± 0.15	0.011
Height SDS					
1 month	−0.17 ± 0.08	−0.44 ± 0.08	0.021	0.21 ± 0.11	0.048
2 months	−0.05 ± 0.13	−0.32 ± 0.12	NS	0.07 ± 0.17	NS
3 months	0.17 ± 0.08	−0.13 ± 0.11	0.019	0.19 ± 0.11	NS
4 months	0.34 ± 0.12	−0.10 ± 0.10	0.006	0.26 ± 0.15	NS
6 months	0.24 ± 0.09	−0.01 ± 0.08	0.041	0.13 ± 0.11	NS
7.5 months	0.18 ± 0.13	−0.06 ± 0.12	NS	0.09 ± 0.17	NS
9 months	0.21 ± 0.08	−0.08 ± 0.09	0.019	0.15 ± 0.12	NS
11 months	0.23 ± 0.09	0.03 ± 0.10	NS	0.09 ± 0.12	NS
14 months	0.24 ± 0.08	−0.02 ± 0.09	0.043	0.16 ± 0.12	NS
18 months	0.19 ± 0.10	−0.03 ± 0.10	NS	0.10 ± 0.13	NS
24 months	0.25 ± 0.10	−0.14 ± 0.11	0.010	0.27 ± 0.14	NS
Head circumference (cm)					
1 month	37.20 ± 0.14	36.92 ± 0.14	NS	0.25 ± 0.17	NS
2 months	38.99 ± 0.14	38.80 ± 0.12	NS	0.14 ± 0.16	NS
3 months	40.32 ± 0.13	40.31 ± 0.14	NS	0.00 ± 0.16	NS
4 months	41.76 ± 0.13	41.63 ± 0.12	NS	0.13 ± 0.16	NS
6 months	43.44 ± 0.13	43.40 ± 0.13	NS	−0.02 ± 0.16	NS
7.5 months	44.50 ± 0.13	44.39 ± 0.13	NS	0.11 ± 0.16	NS
9 months	45.39 ± 0.14	45.27 ± 0.14	NS	0.02 ± 0.17	NS
11 months	46.26 ± 0.14	46.13 ± 0.13	NS	0.06 ± 0.17	NS

Data are presented as mean ± standard error (SE). Unadjusted mean differences are calculated using the Student's *t*-test and adjusted mean differences using linear multivariable regression analysis, while controlling for the following covariates: parental characteristics (height, bodyweight, smoking habits and age), parity, gestational age at birth and gender. In case of weight SDS and height SDS, the covariate gender was left out, as the SDS is normalized for gender.

height ($P < 0.001$), parity ($P = 0.015$) and child's gender ($P < 0.001$) are significantly associated with post-natal height. The interaction variable culture medium × age was included in model 3 to study the effect of culture medium on the velocity of height gain. The growth trajectories do not diverge significantly from each other ($\beta = 0.12$, $P = 0.466$), which indicates that children from the Vitrolife group do not gain height at a faster rate than children from the Cook group. In model 4 is shown that maternal height ($P < 0.001$) and parity ($P = 0.032$) are significantly associated with the rate of post-natal height gain.

The average growth trajectories for the children from the two culture medium groups are illustrated in Fig. 1 for post-natal weight and in Fig. 2 for post-natal height. Those are based on the models which include the interaction variable culture medium × age, and are described by:

$$y_{ij} = \beta_{0i} + \beta_{1i}\text{age}_{ij} + \beta_{2i}\log(\text{age}_{ij}) + \beta_{3i}(\text{age}_{ij})^{-1} + \beta_4\text{medium}_i + \beta_5\text{age}_{ij} \times \text{medium}_i + e_{ij}.$$

Based on these models, the predicted average weight at approximately 4 weeks after birth is 3.99 kg (95% confidence interval (CI) 3.89–4.10 kg) in the Cook group and 4.21 kg (95% CI 4.12–4.30 kg) in the Vitrolife group and at the age of 2 years the predicted average weights are 12.32 kg (95% CI 12.05–12.59 kg) and 12.85 kg (95% CI 12.61–13.08 kg), respectively. The predicted average height at 4 weeks after

Table III Results of the mixed model analysis using the first order Berkey-Reed model for post-natal weight during the first 2 years of life.

Variable	Model 1			Model 2			Model 3			Model 4		
	β	SE	*P*-value	*β*	SE	*P*-value	*β*	SE	*P*-value	*β*	SE	*P*-value
Intercept	14.056	0.476	<0.001	13.828	0.477	<0.001	14.133	0.477	<0.001	13.895	0.479	<0.001
Age	2.574	0.349	<0.001	2.583	0.350	<0.001	2.491	0.352	<0.001	2.544	0.353	<0.001
log (age)	−4.688	1.077	<0.001	−4.716	1.081	<0.001	−4.706	1.077	<0.001	−4.775	1.082	<0.001
inv (age)	−10.592	0.762	<0.001	−10.617	0.764	<0.001	−10.603	0.762	<0.001	−10.654	0.765	<0.001
Culture medium (Vitrolife versus Cook)	0.216	0.070	0.002	0.188	0.066	0.005	0.087	0.098	NS	0.097	0.097	NS
Maternal height (per cm)				0.017	0.006	0.003				0.017	0.006	0.003
Maternal weight (per kg)				−0.002	0.004	NS				−0.014	0.005	0.005
Paternal height (per cm)				−0.004	0.005	NS				−0.015	0.007	0.035
Paternal weight (per kg)				0.003	0.003	NS				0.003	0.003	NS
Maternal smoking (<10 versus ≥10 cig/day)				0.034	0.093	NS				0.034	0.093	NS
Parity (primiparous versus multiparous)				0.230	0.078	0.003				0.230	0.078	0.004
Child's gender (male versus female)				0.431	0.065	<0.001				0.430	0.065	<0.001
Culture medium × age							0.161	0.086	NS	0.108	0.084	NS
Maternal weight × age										0.015	0.004	<0.001
Paternal height × age										0.013	0.005	0.016

β is the regression coefficient . Model 1 is the basic model, which includes the variable 'Culture medium'. Model 2 includes several additional variables as possible predictors. Model 3 includes the interaction variable 'Culture medium × age' to study the effect of culture medium on weight gain. Model 4 includes the interaction variable 'Culture medium × age' and several additional variables as possible predictors.

birth is 53.2 cm (95% CI 52.8–53.6 cm) in the Cook group and 53.9 cm (95% CI 53.6–54.2 cm) in the Vitrolife group and at the age of 2 years the predicted average heights are 87.4 cm (95% CI 86.8–88.0 cm) and 88.4 cm (95% CI 87.9–88.9 cm), respectively.

Discussion

The main finding of this study is that *in vitro* culture of human embryos in medium from Cook resulted in singletons with a lower weight during the first 2 years of life compared with singletons born after embryo culture in medium from Vitrolife. This indicates that the effect of culture medium on fetal development and birthweight (Dumoulin *et al.*, 2010; Nelissen *et al.*, 2012, 2013) persists during the first 2 years of life.

Since the publication of our previous study (Dumoulin *et al.*, 2010; Nelissen *et al.*, 2012), there have been several other studies published on the effect of culture medium on birthweight. Several of these observational studies, in which other culture media were compared, found no differences in birthweight between the culture medium groups (Eaton *et al.*, 2012; Vergouw *et al.*, 2012; Carrasco *et al.*, 2013; Lin *et al.*, 2013). However, in these studies the culture media have been used in consecutive time periods. Carrasco *et al.* performed a small randomized study ($n = 98$), comparing newer versions of the Cook and Vitrolife media (Carrasco *et al.*, 2013). Although, the average birthweight was lower in the Cook group compared with the Vitrolife group, they found no significant difference in birthweight between the groups. Recently, Eskild *et al.* published a large study including the birthweights of 2435 singletons after the use of 3 different culture media and compared those with the birthweights of offspring from spontaneous conceptions ($n = 698\,359$) during the corresponding time periods (Eskild *et al.*, 2013). They found a significant effect of culture medium on birthweight of the offspring, also after adjustment for changes in birthweight after spontaneous pregnancies. To our knowledge, there are no prior studies on the effect of culture medium on post-natal growth in humans.

This study allows a valid comparison of the effect of two commercially available embryo culture media on post-natal growth in IVF children, since exactly the same ovarian stimulation, fertilization, culture and embryo transfer procedures were applied in each group. Furthermore, several factors that might influence post-natal growth were included in the analysis, for example parental weight and height, which are known to be contributors to post-natal child growth (Blair *et al.*, 2004; Griffiths *et al.*, 2007; Hindmarsh *et al.*, 2008; Mesman *et al.*, 2009). However, it was not possible to include all factors, e.g. childhood diseases or nutrition, as this information was not available.

Although the components in the media from this study are known, the exact concentrations of the different components were not disclosed by the suppliers. Therefore, it would be speculative to relate the differences found here to specific components in the culture media.

Since the first use of IVF, there has been a major interest in the health of IVF children. Several studies have investigated the growth of children born after IVF. The vast majority of these studies did not find differences in post-natal weight and height between IVF children and naturally conceived children (Ceelen *et al.*, 2009; Basatemur *et al.*, 2010; Beydoun

Table IV Results of the mixed model analysis using the first order Berkey-Reed model for post-natal height during the first 2 years of life.

Variable	Model 1			Model 2			Model 3			Model 4		
	β	SE	*P*-value	*β*	SE	*P*-value	*β*	SE	*P*-value	*β*	SE	*P*-value
Intercept	89.82	1.24	<0.001	89.13	1.24	<0.001	89.88	1.24	<0.001	89.10	1.24	<0.001
Age	13.07	0.89	<0.001	13.03	0.89	<0.001	13.01	0.89	<0.001	13.16	0.89	<0.001
log (age)	−22.51	2.74	<0.001	−22.37	2.74	<0.001	−22.54	2.73	<0.001	−22.55	2.73	<0.001
inv (age)	−41.92	1.98	<0.001	−41.82	1.98	<0.001	−41.94	1.97	<0.001	−41.92	1.98	<0.001
Culture medium (Vitrolife versus Cook)	0.74	0.25	0.003	0.49	0.23	0.031	0.64	0.29	NS	0.46	0.27	NS
Maternal height (per cm)				0.09	0.02	<0.001				0.03	0.02	NS
Maternal weight (per kg)				0.01	0.01	NS				0.01	0.01	NS
Paternal height (per cm)				0.03	0.02	NS				0.03	0.02	NS
Paternal weight (per kg)				0.01	0.01	NS				0.01	0.01	NS
Maternal smoking (<10 versus ≥10 cig./day)				−0.25	0.32	NS				−0.25	0.32	NS
Parity (primiparous versus multiparous)				0.66	0.27	0.015				1.05	0.33	0.001
Child's gender (male versus female)				1.39	0.23	<0.001				1.38	0.23	<0.001
Culture medium × age							0.12	0.16	NS	0.03	0.16	NS
Maternal height × age										0.05	0.01	<0.001
Parity × age										−0.40	0.19	0.032

β is the regression coefficient. Model 1 is the basic model, which includes the variable 'Culture medium'. Model 2 includes several additional variables as possible predictors. Model 3 includes the interaction variable 'Culture medium × age' to study the effect of culture medium on height gain. Model 4 includes the interaction variable 'Culture medium × age' and several additional variables as possible predictors.

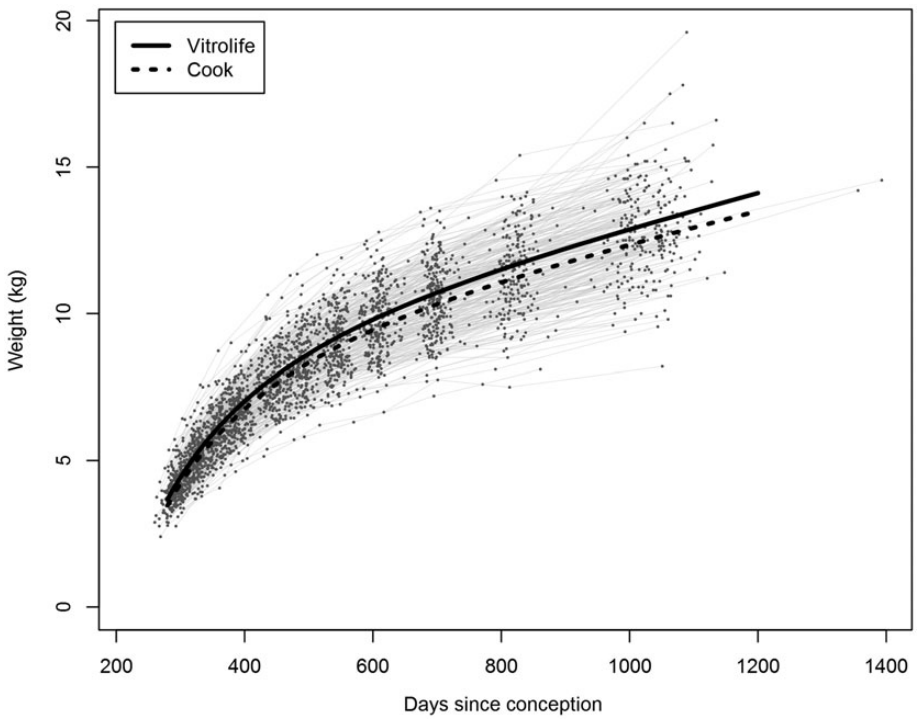

Figure 1 The average weight trajectories for the children from the culture medium groups superimposed on the observed individual trajectories during the first 2 years of life.

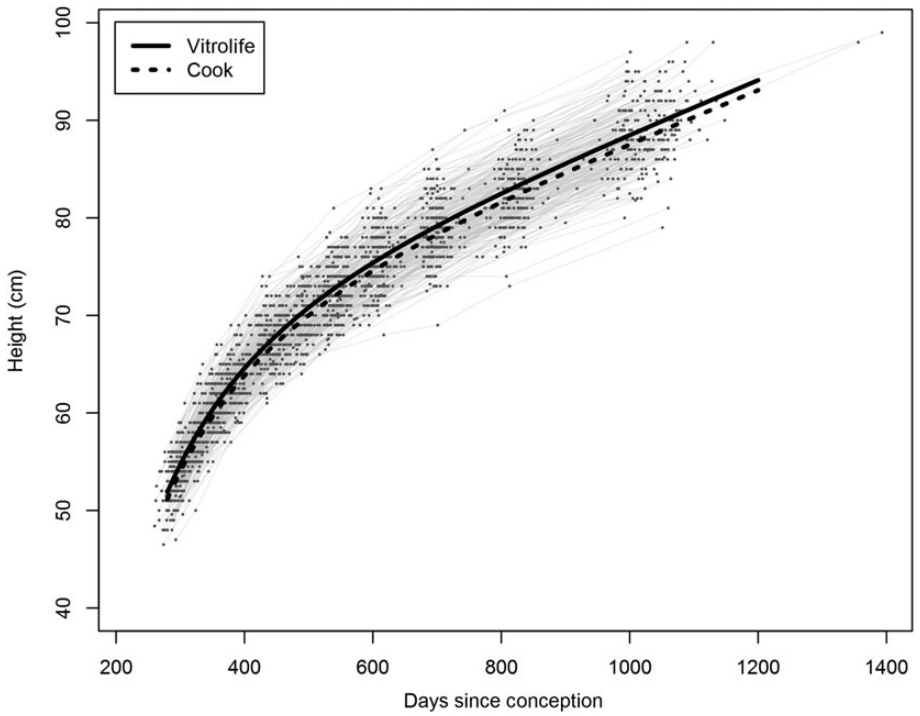

Figure 2 The average height trajectories for the children from the culture medium groups superimposed on the observed individual trajectories during the first 2 years of life.

et al., 2010; Lee et al., 2010; Woldringh et al., 2011). However, Ceelen et al. found that growth velocity was higher in IVF children compared with naturally conceived children and that rapid weight gain during early childhood was associated with increased cardiovascular risks in IVF children (Ceelen et al., 2009). Furthermore, it was found that healthy IVF children and adolescents displayed systemic and pulmonary vascular dysfunction, which appeared to be related to the IVF procedure itself (Scherrer et al., 2012).

It is too early to know if the differences that we have found in this study have any clinical significance. However, the findings are in line with the growing body of evidence that suggests that the fetus is sensitive to the uterine environment, and that adaptations of the fetus to its environment have lasting consequences for its development, growth and health. This was first proposed by Barker, who found an association between low birthweight and increased rates of coronary heart disease and the related disorders of stroke, hypertension and type 2 diabetes during adult life (Barker, 1997). In the Dutch famine study it has been observed that adults who had been exposed to famine in early gestation had higher rates of coronary heart disease, a more atherogenic lipid profile, disturbed blood coagulation, increased stress responsiveness and were more often obese than those exposed in mid- or late gestation and non-exposed individuals (Roseboom et al., 2006). Furthermore, it has been suggested that, even during the periconceptional period, gametes and preimplantation embryos adapt to their environment with long-term health consequences (Steegers-Theunissen et al., 2013). A maternal low-protein diet given to pregnant mice or rats exclusively during the preimplantation period resulted in offspring with increased weight, sustained hypertension, and abnormal anxiety-related behaviour (Kwong et al., 2000; Watkins et al., 2008). Maternal under-nutrition around the periconceptional and preimplantation period affects fetal and placental growth in sheep (MacLaughlin et al., 2005). Fernandez-Gonzalez et al. showed that female mice aged 31–70 weeks, that had been cultured during the preimplantation period in medium with serum, were significantly heavier compared with female mice cultured as an embryo in medium without serum (Fernandez-Gonzalez et al., 2004). Furthermore, mice sacrificed at 20 months of age showed large organs when cultured in medium with serum during the preimplantation period, as well as long-term neurodevelopmental and behavioural effects (Ecker et al., 2004; Fernandez-Gonzalez et al., 2004).

Several animal studies have shown that culture medium affects gene expression (van Montfoort et al., 2012). Rinaudo and Schultz (2004) compared global patterns of gene expression in mouse blastocysts cultured in either Whitten's medium or KSOM/AA with that of blastocysts that developed *in vivo*. Culture in Whitten's medium affected the expression of 114 genes, while only 29 genes were differently expressed after culture in KSOM/AA compared with *in vivo* counterparts. A side-by-side comparison of five commercial culture media showed that all five media had a varying but compromised ability to maintain genomic imprinting in comparison with *in vivo*-derived mouse embryos (Market-Velker et al., 2010). Khosla et al. showed that the presence of serum in culture medium reduces fetal weight and influences the regulation of multiple growth-related imprinted genes in mice (Khosla et al., 2001). In humans, the effect of culture medium on gene expression is currently unknown.

The culture medium is the direct environment of the preimplantation embryo for several days during an IVF treatment. It seems that the embryo makes adaptations, likely by epigenetic modifications, during

this short period of time, which will have long-lasting effects. In view of these results and the growing number of children conceived with ART, it is important that the effect of embryo culture medium is studied in the human more extensively, to select the optimal culture medium and to minimize short-term risks and perhaps even susceptibility to disease in later life.

Conclusion and Future Prospects

The effect of culture medium during the first few days after fertilization on human prenatal growth and birthweight persists during the first 2 years of post-natal life. This suggests that the human embryo is sensitive to its very early environment and that the culture medium used in IVF may have lasting consequences. This warrants further monitoring of the long-term growth, development and health of IVF children.

Authors' roles

J.C.M.D. initiated and designed the study. J.C.M.D., S.H.M.K. and A.P.A.V.M. coordinated data collection and quality control of data. L.J.M.S., W.V. and S.H.M.K. analysed the data. All authors interpreted the data. S.H.M.K. wrote the report with input from the other authors.

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Conflict of interest

None declared.

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