Human Reproduction Open, pp. 1–13, 2020
doi:10.1093/hropen/hoz037

human reproduction open

ORIGINAL ARTICLE

Increased blood pressure and impaired endothelial function after accelerated growth in IVF/ICSI children

H. Zandstra [1,†,*], APA van Montfoort [1,†], JCM Dumoulin[1], LJI Zimmermann[2], and RNH Touwslager[2]

[1]Department of Obstetrics and Gynaecology, GROW School for Oncology and Developmental Biology, Maastricht University Medical Center, Maastricht, The Netherlands [2]Department of Pediatrics, GROW School for Oncology and Developmental Biology, Maastricht University Medical Center, Maastricht, The Netherlands

*Corresponding address. Department of Obstetrics and Gynaecology, Maastricht University Medical Centre, PO Box 5800, 6202 AZ Maastricht, The Netherlands. E-mail: heleen.zandstra@mumc.nl https://orcid.org/0000-0003-1281-2711

Submitted on December 2, 2018; resubmitted on September 5, 2019; editorial decision on October 2, 2019

STUDY QUESTION: What is the effect of growth velocity (height and weight) in early infancy on metabolic end-points and endothelial function in children born after ART?

SUMMARY ANSWER: Neonatal, infant and childhood growth is positively related to blood pressure in 9-year-old IVF/ICSI offspring, while growth in childhood was negatively associated with endothelial function.

WHAT IS KNOWN ALREADY: Offspring of pregnancies conceived after ART are at risk for later cardiometabolic risk factors. It is well established that early growth is related to numerous later cardiometabolic risk factors such as high blood pressure. This concept is known as the Developmental Origin of Health and Disease theory.

STUDY DESIGN, SIZE, DURATION: The relation between early growth and later cardiometabolic risk profile was studied in the MEDIUM-KIDS study, a prospective observational cohort study in children born after an IVF/ICSI treatment. In 131 children (48.1% males) at the average age of 9.4 years, cardiometabolic outcomes were assessed and growth data from birth until age 9 years were collected from child welfare centers.

PARTICIPANTS/MATERIALS, SETTINGS, METHODS: The following cardiometabolic outcomes were assessed: blood pressure, skinfolds, lipid spectrum, hair cortisone and glucose and insulin levels. Data on maximum skin perfusion after transdermal delivery of acetylcholine as a measure of endothelial function were collected.
Growth charts were obtained electronically from child welfare centers, which offer free consultations and vaccinations to all Dutch children. At these centers, height and weight are recorded at predefined ages. Growth was defined as *z*-score difference in weight between two time points. Multivariable linear regression analysis was used to model the relation between growth and cardiometabolic outcomes. The following growth windows were –studied simultaneously in each model: 0–1 month, 1–3 months, 3–6 months, 6–11 months, 11–24 months and 2–6 years. The model was adjusted for height growth in all intervals except for 0–1 month.

MAIN RESULTS AND THE ROLE OF CHANCE: In multivariable linear regression analyses, multiple growth windows were positively associated with blood pressure, for example growth from 2–6 years was significantly related to systolic blood pressure: $B = 4.13$, $P = 0.005$. Maximum skin perfusion after acetylcholine was negatively associated with height-adjusted weight gain from 2 to 6 years: $B = -0.09$ (log scale), $P = 0.03$. Several growth windows (weight 1–3 months, 3–6 months, 6–11 months, 11–24 months, 2–6 years) were positively linked with total adiposity. Lipids, glucose tolerance indices and cortisone were not related to growth.

LIMITATIONS, REASONS FOR CAUTION: This study is of modest size and of observational nature, and we did not include a control group. Therefore, we cannot assess whether the observed associations are causal. It is also not possible to analyze if our observations are specific for, or exacerbated in, the ART population. Ideally, a control group of naturally conceived siblings of IVF/ICSI children should simultaneously be studied to address this limitation and to assess the impact of the ART procedure without the influence of parental (subfertility) characteristics.

WIDER IMPLICATIONS OF THE FINDINGS: The results of this study contribute to our understanding of the reported increased risk for hypertension in ART offspring. We speculate that early, accelerated growth may be involved in the reported increased risk for hypertension

†Both authors contributed equally

in ART offspring, with endothelial dysfunction as a possible underlying mechanism. However, additional research into the mechanisms involved is required.

STUDY FUNDING/COMPETING INTEREST(S): The study was financially supported by the March of Dimes, grant number #6-FY13-153. The sponsor of the study had no role in study design, data collection, data analysis, data interpretation or writing of the paper. The authors have no conflict of interest to declare.

TRIAL REGISTRATION NUMBER: NTR4220

Key words: IVF/ICSI outcome / cardiovascular / endothelium / infant growth / blood pressure

WHAT DOES THIS MEAN FOR PATIENTS?

This study looked at how the speed with which children grow in early childhood is related to the health of these children at later age. We looked at health measures such as blood pressure and how the blood vessels function at 9 years of age.

Other research had shown that children who were very small at birth and grow fast at a young age have an increased risk for all sorts of diseases related to their heart and blood vessels at a later age.

Children born after an IVF or ICSI treatment are smaller when they are born than children born after natural conception. This means that these children might also be at risk for fast growth at a young age.

In this study, we measured whether the speed that children born after IVF and ICSI grow when they are very young is related to their blood pressure at 9 years of age. We looked at blood pressure and at the quality of the blood vessels and found that children who grew faster in their first month of life showed a higher blood pressure and a reduced quality of their blood vessels when they were 9 years old.

This study shows that fast growth at a young age might be involved in the reported increased risk for high blood pressure in children born after an IVF or ICSI treatment. Further research should be done to find out what the causes of these differences are.

Introduction

Offspring of ART-conceived pregnancies have an increased risk for several cardiometabolic risk factors. These associations probably originate through epigenetic mechanisms in the early embryonic stage (Vrooman and Bartolomei, 2017; Fleming *et al*., 2018). This fits into the developmental origins of health and disease (DOHaD) concept, which states that early life aspects have lifelong health implications. In IVF or ICSI treatments, the fertilization of the oocyte and early embryo development occur outside the human body and therefore under non-natural circumstances. These processes coincide with epigenetic reprogramming events within the gametes and embryo. During the IVF/ICSI procedure, several environmental variables, such as embryo culture medium, may impact epigenetic reprogramming.

From a vascular perspective, children born after ART display endothelial dysfunction and vascular stiffness compared to controls of similar (and normal) gestational age and birthweight (Scherrer *et al*., 2012). Furthermore, persistent early cardiac remodeling (Valenzuela-Alcaraz *et al*., 2013) and increased blood pressure were identified (Ceelen *et al*., 2008a; Sakka *et al*., 2010; Valenzuela-Alcaraz *et al*., 2013). Metabolic changes have also been found in ART children. Compared to naturally conceived controls, fasting glucose levels were higher (Ceelen *et al*., 2008a), as well as triglycerides (Sakka *et al*., 2010). A recent meta-analysis showed increased blood pressure and higher fasting insulin levels but a slightly more favorable lipid profile in ART offspring (Guo *et al*., 2017).

From a growth perspective, ART newborns have an increased risk for premature birth and for low birthweight (Pandey *et al*., 2012). One would expect that relatively low birthweight would be followed by relatively fast infant growth (regression to the mean), as was shown in a study by Ceelen *et al*. (2009), at least in late infancy. On the contrary, in another study no rapid weight gain was seen in ART singletons in the first year and no differences were seen in any growth parameter in the first 3 years (Yeung *et al*., 2016). Also, normal growth in IVF and ICSI children up to 12 years of age was reported in a paper by Basatemur and colleagues (Basatemur *et al*., 2010). Altogether, it remains unclear whether ART (the procedure itself, parental subfertility characteristics or an indirect effect of the ART procedure on birthweight) predisposes to altered growth in infancy and childhood.

It is well established that early growth is related to numerous later cardiometabolic risk factors (DOHaD concept), for example high blood pressure (Kagura *et al*., 2016; Taine *et al*., 2016). There is an ongoing debate about the exact timing of these effects, but early postnatal growth (<3 months) has consistently been linked to later cardiometabolic risk factors (Leunissen *et al*., 2009a,2009b; Fabricius-Bjerre *et al*., 2011; Khuc *et al*., 2012). Ceelen *et al*. (2009) analyzed the effects of postnatal growth in IVF children. They found early childhood growth, but not infant growth, to be positively related to later blood pressure (only in IVF) and skinfold thicknesses (both IVF and controls).

In the present study, we aimed to analyze neonatal, infant and childhood growth simultaneously, to study metabolic end-points and to examine endothelial function. We hypothesized a harmful effect of accelerated growth on our cardiometabolic outcome parameters. For reasons of generalizability, we only included healthy children, born at term.

Materials and Methods

Participants

Parents of all singletons born after an IVF treatment with fresh embryo transfer at Maastricht University Medical Centre between July 2003 and December 2006 were approached to participate in the MEDIUM-KIDS study. In this prospective observational cohort study, 9-year-old children were investigated with regard to cardiometabolic risk profile

in relation to IVF culture medium (Cook and Vitrolife) (Dumoulin *et al*., 2010). In total, 47.7% of the potential participants gave informed consent ($n = 136$). The baseline characteristics at the time of birth of the participating children were comparable to those of the children not participating in the study (Zandstra *et al*., 2018). Pregnancy and neonatal characteristics were retrieved from the hospital charts. Gestational age was determined by the following formula: (date of birth − oocyte retrieval date) + 14 days. Prematurely born children (<37 weeks gestational age) were excluded from the analysis ($n = 5$). Two mothers had diabetes of pregnancy. Eight mothers had a hypertensive disorder during pregnancy (one HELLP i.e. hemolysis, elevated liver enzymes, low platelet count, one pre-eclampsia and six gestational hypertension, defined as clinical diagnosis in the hospital charts). These mothers were not excluded from the study. There were no relevant birth defects or later diagnosed illnesses.

Details of the IVF procedures were published previously, as the MEDIUM-KIDS study was part of an earlier study (Dumoulin *et al*., 2010). Only singletons born after transfer of fresh embryos were included.

Setting

Between March 2014 and December 2016, the participants were invited for a 2.5-h visit to our hospital. Two experienced researchers performed the tests. Information on possible confounders was recorded using a questionnaire completed by the parents during the visit.

Collection of independent variables

Growth data were received electronically from child welfare centers, which offer free consultations and vaccinations to all Dutch children. At these centers, height and weight are recorded. In the present study, we analyzed the data obtained at the approximate ages of 1, 3, 6, 11, 24 and 72 months. The obtained values were converted into sex- and age-specific *z*-scores by an online calculator (https://groeiweb.pgdata.nl/calculator.asp), by use of data from the Dutch 3rd national growth study (weight) and the Dutch 5th national growth study (height) (Schönbeck *et al*., 2011; Schonbeck *et al*., 2013). Birth length was missing in 50.4% of the cases and was therefore not analyzed. Birthweight was collected from the hospital charts and converted into *z*-scores by use of sex- and parity-specific reference data accurate to a gestational age of 1 day (Visser *et al*., 2009). Growth was defined as difference in weight *z*-scores. The following growth windows were studied: 0–1 month, 1–3 months, 3–6 months, 6–11 months, 11–24 months and 2–6 years.

Maternal pre-pregnancy weight and height were retrieved from hospital charts, and pre-pregnancy BMI was calculated. The highest completed maternal education level was dichotomized, high education level being defined as the completion of higher professional education or university. Breastfeeding was reported by the parents and dichotomized (yes/no). Maternal smoking during pregnancy was self-reported and dichotomized (yes/no).

Measurement of dependent variables

Children were measured lightly clothed; height (to the nearest 0.1 cm) and body weight (to the nearest 0.1 kg) were measured using a stadiometer (DGI 250D, The Netherlands) and scale (Seca 704, Germany). Skinfold thicknesses (triceps, biceps, subscapular and suprailiac) were measured in triplicate on the non-dominant side of the body using a Harpenden skinfold caliper (Baty International, UK). Total adiposity was calculated as the sum of the four averaged skinfold thicknesses. The sum of triceps and biceps skinfolds represented peripheral adiposity, and the sum of subscapular and suprailiac skinfolds represented truncal adiposity.

Systolic and diastolic blood pressures were measured in triplicate on the non-dominant arm while the child was sitting after a minimum of 30 min of rest in supine position on a bed. It was measured using a calibrated automatic device (Accutorr Plus, Datascope Inc., Montvale, NJ, USA) and averaged for analysis. All but 26 children gave permission for a venipuncture. Participants who did not fast overnight were excluded from the analyses of blood samples ($n = 6$). Five participants had incomplete results because not enough blood could be drawn. Fasting plasma glucose was measured using an enzymatic colorimetric assay (Cobas 8000 instrument, Roche Diagnostics, Mannheim, Germany). Fasting insulin was measured in serum using an immunometric assay (XPi instrument, Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA). The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated by the following formula: ((insulin (pmol/l)/6.94)∗glucose (mmol/l))/22.5. Total cholesterol, high-density lipoprotein (HDL) cholesterol and triglycerides were determined using an enzymatic colorimetric assay (Cobas 8000 instrument, Roche Diagnostics, Mannheim, Germany). Low-density lipoprotein (LDL) was calculated by the Friedewald formula (Rifai *et al*., 2006).

Hair samples were collected when the child had hair >3 cm and when parents consented. The hair strands (~3 mm in diameter) were collected from the posterior vertex region, as close to the scalp as possible. Cortisone was measured by use of chromatography of the 3-cm hair segment closest to the scalp. This measurement was performed in 116 out of the 131 children.

Laser doppler flowmetry of the forearm was performed to assess vascular function (PeriFlux 5000 system, Perimed A.B., Sweden). Using iontophoresis, acetylcholine and nitroprusside were administered transdermally (Perilont system; Perimed A.B., Sweden) and vasodilatation curves were constructed, as described previously (Touwslager *et al*., 2012a). Acetylcholine induces endothelium-dependent vasodilatation via stimulation of the endothelium, while nitroprusside induces endothelium-independent vasodilatation as a direct nitric oxide donor. Maximum perfusion was recorded in perfusion units. The recordings were analyzed by H.Z. and R. T, who were blinded to the growth patterns and reached consensus about quality criteria, such as movement artifacts.

Ethical approval

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

Statistical analysis

Insulin values below the detection limit of 12 pmol/l were analyzed as 12 pmol/l. Hair cortisone values below the detection limit of 0.68 pmol/mg hair were similarly analyzed as 0.68 pmol/mg hair. In

case of a significant Kolmogorov–Smirnov test, a visual inspection of a variable was performed and a 10 log transformation was performed when necessary. Linear regression analysis was used as the primary statistical analysis method. All growth windows were analyzed simultaneously in one model to take regression to the mean into account. Model 1 was constructed with only weight gain as the predicting variable. The model was adjusted for birthweight *z*-score and age at measurement of the outcome variable. Model 2 was additionally adjusted for maternal pre-pregnancy BMI, culture medium, maternal smoking during pregnancy, maternal hypertension during pregnancy, maternal education level and breastfeeding. Model 3 equals Model 2, but was further adjusted for height growth (starting from 1 month), so the results for weight gain can be interpreted as being independent of gain in height. In Model 3, adjustments were made for height at day of measurement in the blood pressure models and for baseline perfusion in the maximum skin perfusion models. To avoid collider bias or over adjustment, we did not correct our models for current weight or current BMI (Chiolero *et al*., 2012; Woo, 2017). We considered a *P* value <0.05 to be statistically significant. All analyses were performed by use of SPSS Statistics 23 (IBM, New York, USA)

Results

The baseline characteristics of the study sample are shown in Table I. We used national reference values based on large cohort studies to compare our population with a general population (Schönbeck *et al*., 2013). Gestational age and birthweight were normal compared to the general population.

Growth from 0 to 1 month, 3 to 6 months and 2 to 6 years was positively associated with systolic blood pressure at 9 years in the final height-adjusted models, for example growth from 2 to 6 years: $B = 4.13$, $P = 0.005$ (Table II), indicating a 4.13-mmHg rise in blood pressure per 1-unit increase in weight *z*-score between 2 and 6 years. Growth in the first month was also positively related to diastolic blood pressure: $B = 4.26$, $P = 0.004$.

In a paired samples Student's *t* test, there was no difference between baseline perfusion in the acetylcholine and nitroprusside protocols ($P = 0.96$). The correlation between baseline perfusion in the acetylcholine and nitroprusside protocols was 0.71 ($P < 0.001$). Maximum perfusion in response to acetylcholine was negatively related to growth from 2 to 6 years: $B = -0.09$ (log scale), $P = 0.03$. This effect size corresponds to a 19.5% decrease in maximum perfusion per one-unit increase in weight *z*-score. There were no significant associations between growth and maximum perfusion after nitroprusside, e.g. Model 3, growth from 2 to 6 years: $B = -0.03$ (log scale), $P = 0.38$.

Total adiposity was positively related to growth in all age windows, except 0–1 month, for example growth from 11 to 24 months: $B = 0.13$ (log scale), $P < 0.001$. This effect size corresponds to a 40.3% increase in total adiposity per one-unit increase in weight *z*-score. In Model 1, truncal:peripheral adiposity ratio was positively related to growth from 11 to 24 months: $B = 0.05$ (log scale), $P = 0.04$; this association was no longer significant in Models 2 and 3 (data not shown).

No significant relationships were identified between growth and cortisone, HOMA-IR, insulin, glucose, triglycerides, total cholesterol: HDL ratio or LDL (data not shown).

Discussion

In this study, we showed that in 9-year-old children born after IVF/ICSI, weight gain from 0 to 1 month was positively related to both systolic and diastolic blood pressure. Moreover, height-adjusted weight gain from 3 to 6 months and 2 to 6 years were positively related to systolic blood pressure. We report clinically relevant effect sizes of 4–5.5 mmHg per one-unit increase in weight *z*-score. The association between growth in early childhood and systolic blood pressure is in line with a previous study in IVF children (Ceelen *et al*., 2009), although we now add the fact that also infant growth, particularly growth from 0 to 1 month, is an influential growth window. Therefore, we cannot support the notion of a healthy infant growth trajectory in IVF/ICSI children, as postulated by Ceelen *et al*. (2009). However, it should be noted that our population had somewhat higher (and normal) birthweight than the population analyzed by Ceelen *et al*. and that our population was born at term (Ceelen *et al*., 2009).

To our knowledge, we are the first to describe an inverse association between growth in childhood and maximum perfusion in response to acetylcholine in ART offspring. No relationships were shown between growth and maximum perfusion in response to nitroprusside. Therefore, it can be concluded that our results reflect endothelial dysfunction. Endothelial dysfunction precedes atherosclerosis and can be tested by acetylcholine-induced vasodilatation (Puissant *et al*., 2013). The identification of a relationship between growth and endothelial function is consistent with a study in non-ART individuals, although this study analyzed growth in the first 2 weeks (Singhal *et al*., 2004). The relationship is also consistent with a previous study in our hospital in non-ART children up to 2 years of age, although based on this study we expected early infant growth to be of importance (Touwslager *et al*., 2015). Perfusion measurement by laser doppler was used because of the suitability to use this technique in children. This can however be challenging in children because of movement artifacts and the high variability (Puissant *et al*., 2013) in these measurements. We have shown previously a good interobserver variability of the obtained signal (Touwslager *et al*., 2012b), and the high correlation and equality between baseline perfusion in both arms was reassuring. The exact clinical importance of maximum skin perfusion after acetylcholine at 9 years of age is not known, but a 19.5% decrease per one-unit increase in *z*-score appears to be a reasonably large effect size.

ART-conceived offspring are at increased risk for high blood pressure (Guo *et al*., 2017), possibly through endothelial nitric oxide synthase methylation changes (Rimoldi *et al*., 2014). ART offspring also display endothelial dysfunction (Scherrer *et al*., 2012). In turn, endothelial dysfunction plays a role in the development of hypertension (Brandes, 2014). Previously, it was shown that accelerated growth in early childhood in IVF children was associated with an increase in blood pressure, but this was not the case in spontaneously conceived controls (Ceelen *et al*., 2009). Combining these previous findings with our results, we speculate that early accelerated growth may be involved in the reported increased risk for hypertension in ART offspring, with endothelial dysfunction as a possible underlying mechanism. Accelerated growth could be a 'second' hit on an already increased risk for hypertension and endothelial dysfunction in ART offspring. Importantly, most of the studies identifying increased blood pressure in ART offspring did not control for growth, so growth could also be the 'only' hit. Alternatively, ART offspring may be extra susceptible to

Table 1 Baseline characteristics of the study population.

	n	Mean (SD)	Median (IQR)	%
General				
Males	131			48.1
High maternal education	131			38.2
Maternal pre-pregnancy BMI (kg/m^2)	131	24.47 (3.15)		
Obstetric				
Gestational age (weeks)	131	39.83 (1.20)		
Birthweight (SD)	131	3422.21 (461.60)		
Received breastfeeding	131			55.7
Gestational hypertension	131			6.1
Smoking during pregnancy	131			5.3
ART				
Cook medium	131			45.0
Maternal age (years)	131	32.92 (3.74)		
ICSI	131			64.9
Growth				
Age at measurement '1 month' (days)	123	29.46 (5.67)		
Age at measurement '3 months' (days)	123	93.19 (10.77)		
Age at measurement '6 months' (days)	122	181.02 (12.76)		
Age at measurement '11 months' (days)	127	345.11 (18.28)		
Age at measurement '24 months' (years)	123	2.09 (0.11)		
Age at measurement '6 years' (years)	121	5.88 (0.52)		
0–1 month (Δ weight *z*-score)	123	0.05 (0.81)		
1–3 months (Δ weight *z*-score)	123	0.37 (0.75)		
3–6 months (Δ weight *z*-score)	122	−0.08 (0.63)		
6–11 months (Δ weight *z*-score)	122	−0.18 (0.61)		
11–24 months (Δ weight *z*-score)	122	−0.28 (0.66)		
2–6 years (Δ weight *z*-score)	110	0.20 (0.76)		
1–3 months (Δ length *z*-score)	110	0.44 (0.56)		
3–6 months (Δ length *z*-score)	112	−0.03 (0.50)		
6–11 months (Δ length *z*-score)	117	0.02 (0.51)		
11–24 months (Δ length *z*-score)	119	−0.07 (0.68)		
2–6 years (Δ length *z*-score)	113	−0.24 (0.54)		
Cardiometabolic				
Age on measurement day (years)	131		9.43 (0.33)	
BMI on measurement day (kg/m^2)	131	17.14 (2.45)		
Baseline perfusion acetylcholine protocol (PU)	121		3.73 (8.60)	
Maximum perfusion acetylcholine protocol (PU)	121		80.36 (60.69)	
Baseline perfusion nitroprusside protocol (PU)	115		3.65 (8.22)	
Maximum perfusion nitroprusside protocol (PU)	115		101.51 (69.04)	
Systolic blood pressure (mmHg)	131	100.38 (7.43)		
Diastolic blood pressure (mmHg)	131	60.05 (6.70)		
Sum of skinfolds (mm)	131		33.66 (26.53)	
Total:truncal adiposity ratio	131		0.60 (0.22)	
Insulin (pmol/l)	96		30.65 (28.70)	
Glucose (mmol/l)	93	4.71 (0.34)		
HOMA-IR	91		0.94 (0.94)	
Total:HDL cholesterol ratio	97		2.47 (0.86)	
Triglycerides (mmol/l)	97		0.63 (0.41)	
LDL (mmol/l)	97	2.31 (0.56)		
Cortisone (pg/mg hair)	116	12.68 (7.23)		

IQR = interquartile range. Δ = delta. PU = perfusion units. HOMA-IR = homeostatic model assessment insulin resistance. HDL = high-density lipoprotein. LDL = low density lipoprotein

Table II Regression coefficients for growth in children born after ART.

	Model 1			Model 2			Model 3		
	n	*B*	*P*	*n*	*B*	*P*	*n*	*B*	*P*
Systolic blood pressure (mmHg)									
Δ weight *z*-score 0–1 month	103	3.58	0.001*	103	3.54	0.002*	85	5.51	0.001*
Δ weight *z*-score 1–3 months		1.65	0.15		1.91	0.12		3.71	0.05
Δ weight *z*-score 3–6 months		3.58	0.006*		3.26	0.02*		4.69	0.01*
Δ weight *z*-score 6–11 months		3.53	0.02*		3.79	0.01*		3.78	0.07
Δ weight *z*-score 11–24 months		1.93	0.09		2.03	0.08		0.42	0.80
Δ weight *z*-score 2–6 years		3.71	<0.001*		3.62	0.001*		4.13	0.005*
Diastolic blood pressure (mmHg)									
Δ weight *z*-score 0–1 month	103	1.62	0.11	103	1.41	0.18	85	4.26	0.004*
Δ weight *z*-score 1–3 months		−0.74	0.50		-1.17	0.32		0.06	0.97
Δ weight *z*-score 3–6 months		1.69	0.17		1.13	0.38		2.92	0.08
Δ weight *z*-score 6–11 months		0.65	0.65		0.98	0.50		1.13	0.54
Δ weight *z*-score 11–24 months		0.11	0.92		0.20	0.86		−0.29	0.84
Δ weight *z*-score 2–6 years		0.51	0.60		0.48	0.63		1.13	0.37
Maximum perfusion after acetylcholine (log PU)									
Δ weight *z*-score 0–1 month	94	0.03	0.52	94	0.03	0.48	78	0.01	0.84
Δ weight *z*-score 1–3 months		0.01	0.88		0.01	0.86		0.00	0.97
Δ weight *z*-score 3–6 months		0.03	0.61		0.03	0.60		−0.01	0.86
Δ weight *z*-score 6–11 months		0.01	0.92		0.02	0.77		0.04	0.53
Δ weight *z*-score 11–24 months		0.03	0.50		0.04	0.40		−0.01	0.85
Δ weight *z*-score 2–6 years		−0.02	0.57		−0.02	0.64		−0.09	0.03*
Total adiposity (log mm)									
Δ weight *z*-score 0–1 month	103	0.03	0.27	103	0.03	0.24	85	0.03	0.35
Δ weight *z*-score 1–3 months		0.05	0.03*		0.07	0.009*		0.09	0.01*
Δ weight *z*-score 3–6 months		0.08	0.002*		0.09	0.002*		0.12	0.001*
Δ weight *z*-score 6–11 months		0.06	0.06		0.06	0.06		0.10	0.01*
Δ weight *z*-score 11–24 months		0.08	0.001*		0.08	0.001*		0.13	<0.001*
Δ weight *z*-score 2–6 years		0.13	<0.001*		0.14	<0.001*		0.15	<0.001*

Model 1: adjusted for age at measurement day. Birthweight *z*-score and weight gain in the other time windows
Model 2: Model 1 further adjusted for maternal pre-pregnancy BMI. Culture medium. Maternal smoking during pregnancy. Maternal hypertension during pregnancy. Maternal education level and breastfeeding
Model 3: Model 2 further adjusted for height growth in all windows except 0–1 month. Blood pressure models were adjusted for height. The perfusion model was adjusted for baseline perfusion
*Indicates a significant association with $P < 0.05$

the deleterious effects of accelerated growth. Based on our study and the existing literature, we recommend cardiometabolic follow-up for fast-growing individuals after ART-conceived pregnancies: both for reasons of prevention and for possible pharmacological interventions (for example antioxidants) in the future (Rimoldi *et al.*, 2015).

Growth in all windows except 0–1 month was positively related to total sum of skinfolds. This was not surprising since we defined growth as difference in weight *z*-score and adjusted the analyses for difference in height *z*-score. Our results were in agreement with a previous study in IVF children (Ceelen *et al.*, 2009). Extensive evidence links early growth to later obesity in non-ART individuals (Baidal *et al.*, 2016). We did not identify a relationship between growth and an unfavorable ratio of truncal to peripheral adiposity.

Importantly, the identified associations were shown in a term, healthy, normal birthweight group, which was prospectively collected. Cardiometabolic effects of prematurity, a complication of ART, could therefore be excluded. We did not perform an analysis to assess individual accelerated growth, but from the average differences in *z*-score in each growth window, it can be expected that growth was likely normal compared to the Dutch population. This is in line with

the study by Yeung *et al*. (2016) which did not observe any risk for accelerated growth in the first year in ART children, rapid weight gain being defined as >0.50 SD. This apparently normal growth is important for reasons of generalizability (both towards the majority of ART conceived offspring and to the general population), which we consider to be good in our study. Given our simultaneous analyses of all infant growth windows and concurrent analysis of height gain in Model 3, our growth measures can be interpreted independently of each other, and as weight gain in excess of what can be expected based on height gain. Optimal growth trajectories remains to be elucidated (Singhal, 2017), especially for ART offspring. Promising interventions for prevention of fast infant growth include breastfeeding (Carling *et al*., 2015), the modification of protein content in formula (Patro–Golab *et al*., 2016) and responsive parenting intervention programs (Savage *et al*., 2016).

This study is of modest size and of an observational nature, and we did not include a control group. Therefore we cannot assess whether the observed associations are causal. It is also not possible to analyze if our observations are specific for, or exacerbated in, the ART population. Also, we are unable to report whether ART children have altered growth patterns. Furthermore, a possible increased risk for cardiovascular risk factors in ART offspring, regardless of growth, cannot be concluded. Ideally, a control group of naturally conceived siblings of IVF/ICSI children should simultaneously be studied to address this and to assess the impact of the ART procedure without the influence of parental (subfertility) characteristics. Data on birth length was poor, so we could not adjust weight gain in the first months for gain in length. However, it should be noted that birth length measurements are not reliable (Johnson *et al*., 1998).

In conclusion, our study showed that neonatal, infant and childhood growth was positively related to blood pressure in IVF/ICSI offspring. Growth in childhood was negatively associated with endothelial function. This is an important step in understanding the increased risk for hypertension in ART offspring.

Acknowledgements

We are very grateful to the parents and children who participated in our study for their effort and willingness to also take part in this 9-year follow-up investigation.

Authors' roles

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

Funding

March of Dimes (#6-FY13-153).

Conflict of interest

The authors have no conflict of interest to declare.

References

Baidal JAW, Locks LM, Cheng ER, Blake-Lamb TL, Perkins ME, Taveras EM. Risk factors for childhood obesity in the first 1,000 days: a systematic review. *Am J Prev Med* 2016;**50**:761–779.

Banrezes B, Sainte-Beuve T, Canon E, Schultz RM, Cancela J, Ozil JP. Adult body weight is programmed by a redox-regulated and energy-dependent process during the pronuclear stage in mouse. *PLoS One* 2011;**6**:e29388.

Barbuscia A, Mills MC. Cognitive development in children up to age 11 years born after ART-a longitudinal cohort study. *Hum Reprod* 2017;**32**:1482–1488.

Barker DJ. Maternal nutrition, fetal nutrition, and disease in later life. *Nutrition* 1997;**13**:807–813.

Barker DJ. Fetal origins of cardiovascular disease. *Ann Med* 1999;**31**:3–6.

Barker DJ. In utero programming of cardiovascular disease. *Theriogenology* 2000;**53**:555–574.

Barker DJ. Adult consequences of fetal growth restriction. *Clin Obstet Gynecol* 2006;**49**:270–283.

Barker DJP. The origins of the developmental origins theory. *J Intern Med* 2007;**261**:412–417.

Basatemur E, Shevlin M, Sutcliffe A. Growth of children conceived by IVF and ICSI up to 12years of age. *Reprod Biomed Online* 2010;**20**:144–149.

Bay B, Mortensen EL, Kesmodel US. Is subfertility or fertility treatment associated with long-term growth in the offspring? A cohort study. *Fertil Steril* 2014;**102**:1117–1123.

Belfort MB, Gillman MW. Healthy infant growth: what are the trade-offs in the developed world? *Nestle Nutr Inst Workshop Ser* 2013;**71**:171–184.

Belva F, Henriet S, Liebaers I, Van Steirteghem A, Celestin-Westreich S, Bonduelle M. Medical outcome of 8-year-old singleton ICSI children (born >or=32 weeks' gestation) and a spontaneously conceived comparison group. *Hum Reprod* 2007;**22**:506–515.

Belva F, Painter R, Bonduelle M, Roelants M, Devroey P, De Schepper J. Are ICSI adolescents at risk for increased adiposity? *Hum Reprod* 2012;**27**:257–264.

Bergh C, Wennerholm UB. Obstetric outcome and long-term follow up of children conceived through assisted reproduction. *Best Pract Res Clin Obstet Gynaecol* 2012;**26**:841–852.

Berry KA, Baron IS, Weiss BA, Baker R, Ahronovich MD, Litman FR. In vitro fertilization and late preterm preschoolers' neuropsychological outcomes: the PETIT study. *Am J Obstet Gynecol* 2013;**209**:e351–e356.

Bloise E, Feuer SK, Rinaudo PF. Comparative intrauterine development and placental function of ART concepti: implications for human reproductive medicine and animal breeding. *Hum Reprod Update* 2014;**20**:822–839.

Bloise E, Lin W, Liu X, Simbulan R, Kolahi KS, Petraglia F, Maltepe E, Donjacour A, Rinaudo P. Impaired placental nutrient transport in mice generated by in vitro fertilization. *Endocrinology* 2012;**153**:3457–3467.

Boivin J, Bunting L, Collins JA, Nygren KG. International estimates of infertility prevalence and treatment-seeking: potential need and demand for infertility medical care. *Hum Reprod* 2007;**22**:1506–1512.

Bouillon C, Léandri R, Desch L, Ernst A, Bruno C, Cerf C, Chiron A, Souchay C, Burguet A, Jimenez C *et al*. Does embryo culture medium influence the health and development of children born after in vitro fertilization? *PLoS One* 2016;**11**:e0150857.

Bowman P, McLaren A. Viability and growth of mouse embryos after in vitro culture and fusion. *J Embryol Exp Morphol* 1970;**23**:693–704.

Brandes RP. Endothelial dysfunction and hypertension. *Hypertension* 2014;**64**:924–928.

Calle A, Fernandez-Gonzalez R, Ramos-Ibeas P, Laguna-Barraza R, Perez-Cerezales S, Bermejo-Alvarez P, Ramirez MA, Gutierrez-Adan A. Long-term and transgenerational effects of in vitro culture on mouse embryos. *Theriogenology* 2012;**77**:785–793.

Carling SJ, Demment MM, Kjolhede CL, Olson CM. Breastfeeding duration and weight gain trajectory in infancy. *Pediatrics* 2015;**135**:111–119.

Carrasco B, Boada M, Rodríguez I, Coroleu B, Barri PN, Veiga A. Does culture medium influence offspring birth weight? *Fertil Steril* 2013;**100**:1283–1288.

Ceelen M, van Weissenbruch MM, Prein J, Smit JJ, Vermeiden JP, Spreeuwenberg M, van Leeuwen FE, Delemarre-van de Waal HA. Growth during infancy and early childhood in relation to blood pressure and body fat measures at age 8-18 years of IVF children and spontaneously conceived controls born to subfertile parents. *Hum Reprod* 2009;**24**:2788–2795.

Ceelen M, van Weissenbruch MM, Roos JC, Vermeiden JP, van Leeuwen FE, Delemarre-van de Waal HA. Body composition in children and adolescents born after in vitro fertilization or spontaneous conception. *J Clin Endocrinol Metab* 2007;**92**:3417–3423.

Ceelen M, van Weissenbruch MM, Vermeiden JP, van Leeuwen FE, Delemarre-van de Waal HA. Cardiometabolic differences in children born after in vitro fertilization: follow-up study. *J Clin Endocrinol Metab* 2008a;**93**:1682–1688.

Ceelen M, van Weissenbruch MM, Vermeiden JP, van Leeuwen FE, Delemarre-van de Waal HA. Growth and development of children born after in vitro fertilization. *Fertil Steril* 2008b;**90**:1662–1673.

Chen M, Wu L, Zhao J, Wu F, Davies MJ, Wittert GA, Norman RJ, Robker RL, Heilbronn LK. Altered glucose metabolism in mouse and humans conceived by IVF. *Diabetes* 2014;**63**:3189–3198.

Chiolero A, Kaufman JS, Paradis G. Why adjustment for current weight can bias the estimate of the effect of birth weight on blood pressure: shedding light using causal graphs. *J Hypertens* 2012;**30**:1042–1045.

Chronopoulou E, Harper JC. IVF culture media: past, present and future. *Hum Reprod Update* 2015;**21**:39–55.

Cole TJ, Lobstein T. Extended international (IOTF) body mass index cut-offs for thinness, overweight and obesity. *Pediatr Obes* 2012;**7**:284–294.

Cracowski JL, Minson CT, Salvat-Melis M, Halliwill JR. Methodological issues in the assessment of skin microvascular endothelial function in humans. *Trends Pharmacol Sci* 2006;**27**:503–508.

De Vos A, Janssens R, Van de Velde H, Haentjens P, Bonduelle M, Tournaye H, Verheyen G. The type of culture medium and the duration of in vitro culture do not influence birthweight of ART singletons. *Hum Reprod* 2015;**30**:20–27.

Delle Piane L, Lin W, Liu X, Donjacour A, Minasi P, Revelli A, Maltepe E, Rinaudo PF. Effect of the method of conception and embryo transfer procedure on mid-gestation placenta and fetal development in an IVF mouse model. *Hum Reprod* 2010a;**25**:2039–2046.

Delle Piane L, Lin W, Liu X, Donjacour A, Minasi P, Revelli A, Maltepe E, Rinaudo PF. Effect of the method of conception and embryo transfer procedure on mid-gestation placenta and fetal development in an IVF mouse model. *Hum Reprod* 2010b;**25**:2039–2046.

Doherty AS, Mann MRW, Tremblay KD, Bartolomei MS, Schultz RM. Differential effects of culture on imprinted H19 expression in the preimplantation mouse embryo. *Biol Reprod* 2000;**62**:1526–1535.

Donjacour A, Liu X, Lin W, Simbulan R, Rinaudo PF. In vitro fertilization affects growth and glucose metabolism in a sex-specific manner in an outbred mouse model. *Biol Reprod* 2014;**90**:80.

Draper ES, Kurinczuk JJ, Abrams KR, Clarke M. Assessment of separate contributions to perinatal mortality of infertility history and treatment: a case-control analysis. *Lancet* 1999;**353**:1746–1749.

Dumoulin JC, Land JA, Van Montfoort AP, Nelissen EC, Coonen E, Derhaag JG, Schreurs IL, Dunselman GA, Kester AD, Geraedts JP *et al*. Effect of in vitro culture of human embryos on birthweight of newborns. *Hum Reprod* 2010;**25**:605–612.

Dyrlund TF, Kirkegaard K, Poulsen ET, Sanggaard KW, Hindkjaer JJ, Kjems J, Enghild JJ, Ingerslev HJ. Unconditioned commercial embryo culture media contain a large variety of non-declared proteins: a comprehensive proteomics analysis. *Hum Reprod* 2014;**29**:2421–2430.

Eaton JL, Lieberman ES, Stearns C, Chinchilla M, Racowsky C. Embryo culture media and neonatal birthweight following IVF. *Hum Reprod* 2012;**27**:375–379.

Ecker DJ, Stein P, Xu Z, Williams CJ, Kopf GS, Bilker WB, Abel T, Schultz RM. Long-term effects of culture of preimplantation mouse embryos on behavior. *Proc Natl Acad Sci U S A* 2004;**101**:1595–1600.

Eckert JJ, Porter R, Watkins AJ, Burt E, Brooks S, Leese HJ, Humpherson PG, Cameron IT, Fleming TP. Metabolic induction and early responses of mouse blastocyst developmental programming following maternal low protein diet affecting life-long health. *PLoS One* 2012;**7**:e52791.

Eriksson J, Forsén T, Tuomilehto J, Osmond C, Barker D. Size at birth, childhood growth and obesity in adult life. *Int J Obes Relat Metab Disord* 2001;**25**:735–740.

Eriksson JG, Forsén T, Tuomilehto J, Osmond C, Barker DJ. Early adiposity rebound in childhood and risk of type 2 diabetes in adult life. *Diabetologia* 2003;**46**:190–194.

Eskild A, Monkerud L, Tanbo T. Birthweight and placental weight; do changes in culture media used for IVF matter? Comparisons with spontaneous pregnancies in the corresponding time periods. *Hum Reprod* 2013;**28**:3207–3214.

Evers JL. Female subfertility. *Lancet* 2002;**360**:151–159.

Fabricius-Bjerre S, Jensen RB, Faerch K, Larsen T, Molgaard C, Michaelsen KF, Vaag A, Greisen G. Impact of birth weight and early infant weight gain on insulin resistance and associated cardiovascular risk factors in adolescence. *PLoS One* 2011;**6**:e20595.

Faddy MJ, Gosden MD, Gosden RG. A demographic projection of the contribution of assisted reproductive technologies to world population growth. *Reprod Biomed Online* 2018;**36**:455–458.

Feelisch M. The use of nitric oxide donors in pharmacological studies. *Naunyn Schmiedebergs Arch Pharmacol* 1998;**358**:113–122.

Fernández-Gonzalez R, Moreira P, Bilbao A, Jiménez A, Pérez-Crespo M, Ramírez MA, Rodríguez De Fonseca F, Pintado B, Gutiérrez-Adán A. Long-term effect of in vitro culture of mouse embryos with

serum on mRNA expression of imprinting genes, development, and behavior. *Proc Natl Acad Sci U S A* 2004;**101**:5880–5885.

Feuer S, Rinaudo P. Preimplantation stress and development. *Birth Defects Res C Embryo Today* 2012;**96**:299–314.

Feuer S, Rinaudo P. From Embryos to Adults: A DOHaD Perspective on In Vitro Fertilization and Other Assisted Reproductive Technologies. *Healthcare (Basel)* 2016;**4**.

Feuer SK, Liu X, Donjacour A, Lin W, Simbulan RK, Giritharan G, Piane LD, Kolahi K, Ameri K, Maltepe E *et al*. Use of a mouse in vitro fertilization model to understand the developmental origins of health and disease hypothesis. *Endocrinology* 2014a; en20132081.

Feuer SK, Liu X, Donjacour A, Lin W, Simbulan RK, Giritharan G, Piane LD, Kolahi K, Ameri K, Maltepe E *et al*. Use of a mouse in vitro fertilization model to understand the developmental origins of health and disease hypothesis. *Endocrinology* 2014b;**155**: 1956–1969.

Fleming TP, Kwong WY, Porter R, Ursell E, Fesenko I, Wilkins A, Miller DJ, Watkins AJ, Eckert JJ. The embryo and its future. *Biol Reprod* 2004;**71**:1046–1054.

Fleming TP, Velazquez MA, Eckert JJ. Embryos, DOHaD and David Barker. *J Dev Orig Health Dis* 2015;**6**:377–383.

Fleming TP, Watkins AJ, Velazquez MA, Mathers JC, Prentice AM, Stephenson J, Barker M, Saffery R, Yajnik CS, Eckert JJ *et al*. Origins of lifetime health around the time of conception: causes and consequences. *Lancet* 2018.

Gardner DK, Hamilton R, McCallie B, Schoolcraft WB, Katz-Jaffe MG. Human and mouse embryonic development, metabolism and gene expression are altered by an ammonium gradient in vitro. *Reproduction* 2013;**146**:49–61.

Gardner DK, Kelley RL. Impact of the IVF laboratory environment on human preimplantation embryo phenotype. *J Dev Orig Health Dis* 2017;**8**:418–435.

Gardner DK, Lane M. Culture and selection of viable blastocysts: a feasible proposition for human IVF? *Hum Reprod Update* 1997;**3**:367–382.

Gardner DK, Leese HJ. Concentrations of nutrients in mouse oviduct fluid and their effects on embryo development and metabolism in vitro. *J Reprod Fertil* 1990;**88**:361–368.

Gibbons WE, Cedars M, Ness RB. Society for Assisted Reproductive Technologies Writing G. Toward understanding obstetrical outcome in advanced assisted reproduction: varying sperm, oocyte, and uterine source and diagnosis. *Fertil Steril* 2011;**95**:1645–1649 e1641.

Gillman MW, Barker D, Bier D, Cagampang F, Challis J, Fall C, Godfrey K, Gluckman P, Hanson M, Kuh D *et al*. Meeting report on the 3rd International Congress on Developmental Origins of Health and Disease (DOHaD). *Ped Res* 2007;**61**:625–629.

Giritharan G, Talbi S, Donjacour A, Di Sebastiano F, Dobson AT, Rinaudo PF. Effect of in vitro fertilization on gene expression and development of mouse preimplantation embryos. *Reproduction* 2007;**134**:63–72.

Gluckman PD, Hanson MA, Bateson P, Beedle AS, Law CM, Bhutta ZA, Anokhin KV, Bougneres P, Chandak GR, Dasgupta P *et al*. Towards a new developmental synthesis: adaptive developmental plasticity and human disease. *Lancet* 2009;**373**:1654–1657.

Godfrey KM, Gluckman PD, Hanson MA. Developmental origins of metabolic disease: life course and intergenerational perspectives. *Trends Endocrinol Metab* 2010;**21**:199–205.

Green MP, Mouat F, Miles HL, Hopkins SA, Derraik JG, Hofman PL, Peek JC, Cutfield WS. Phenotypic differences in children conceived from fresh and thawed embryos in in vitro fertilization compared with naturally conceived children. *Fertil Steril* 2013;**99**:1898–1904.

Griesinger G, Kolibianakis EM, Diedrich K, Ludwig M. Ovarian stimulation for IVF has no quantitative association with birthweight: a registry study. *Hum Reprod* 2008;**23**:2549–2554.

Gruber I, Klein M. Embryo culture media for human IVF: which possibilities exist? *J Turk Ger Gynecol Assoc* 2011;**12**:110–117.

Gu F, Deng M, Gao J, Wang Z, Ding C, Xu Y, Zhou C. The effects of embryo culture media on the birthweight of singletons via fresh or frozen-thawed embryo transfer: a large-scale retrospective study. *BMC Pregnancy Childbirth* 2016;**16**:270.

Guo XY, Liu XM, Jin L, Wang TT, Ullah K, Sheng JZ, Huang HF. Cardiovascular and metabolic profiles of offspring conceived by assisted reproductive technologies: a systematic review and meta-analysis. *Fertil Steril* 2017;**107**:622–631 e625.

Han HD, Kiessling AA. In vivo development of transferred mouse embryos conceived in vitro in simple and complex media. *Fertil Steril* 1988;**50**:159–163.

Hart R, Norman RJ. The longer-term health outcomes for children born as a result of IVF treatment: Part I–General health outcomes. *Hum Reprod Update* 2013a;**19**:232–243.

Hart R, Norman RJ. The longer-term health outcomes for children born as a result of IVF treatment. Part II–Mental health and development outcomes. *Hum Reprod Update* 2013b;**19**:244–250.

Hassani F, Eftekhari-Yazdi P, Karimian L, Rezazadeh Valojerdi M, Movaghar B, Fazel M, Fouladi HR, Shabani F, Johansson L. The effects of ISM1 medium on embryo quality and outcomes of IVF/ICSI cycles. *Int J Fertil Steril* 2013;**7**:108–115.

Helmerhorst FM, Perquin DA, Donker D, Keirse MJ. Perinatal outcome of singletons and twins after assisted conception: a systematic review of controlled studies. *Bmj* 2004;**328**:261.

Henningsen AK, Pinborg A. Birth and perinatal outcomes and complications for babies conceived following ART. *Semin Fetal Neonatal Med* 2014;**19**:234–238.

Henningsen AK, Pinborg A, Lidegaard O, Vestergaard C, Forman JL, Andersen AN. Perinatal outcome of singleton siblings born after assisted reproductive technology and spontaneous conception: Danish national sibling-cohort study. *Fertil Steril* 2011;**95**: 959–963.

Hentemann M, Bertheussen K. New media for culture to blastocyst. *Fertil Steril* 2009;**91**:878–883.

Hokken-Koelega AC, De Ridder MA, Lemmen RJ, Den Hartog H, De Muinck Keizer-Schrama SM, Drop SL. Children born small for gestational age: do they catch up? *Pediatr Res* 1995;**38**:267–271.

Imudia AN, Awonuga AO, Kaimal AJ, Wright DL, Styer AK, Toth TL. Elective cryopreservation of all embryos with subsequent cryothaw embryo transfer in patients at risk for ovarian hyperstimulation syndrome reduces the risk of adverse obstetric outcomes: a preliminary study. *Fertil Steril* 2013;**99**:168–173.

Jackson RA, Gibson KA, Wu YW, Croughan MS. Perinatal outcomes in singletons following in vitro fertilization: a meta-analysis. *Obstet Gynecol* 2004;**103**:551–563.

Johnson TS, Engstrom JL, Warda JA, Kabat M, Peters B. Reliability of length measurements in full-term neonates. *J Obstet Gynecol Neonatal Nurs* 1998;**27**:270–276.

Kagura J, Adair LS, Munthali RJ, Pettifor JM, Norris SA. Association between early life growth and blood pressure trajectories in Black South African children. *Hypertension* 2016;**68**:1123–1131.

Khosla S, Dean W, Reik W, Feil R. Culture of preimplantation embryos and its long-term effects on gene expression and phenotype. *Hum Reprod Update* 2001;**7**:419–427.

Khuc K, Blanco E, Burrows R, Reyes M, Castillo M, Lozoff B, Gahagan S. Adolescent metabolic syndrome risk is increased with higher infancy weight gain and decreased with longer breast feeding. *Int J Pediatr* 2012;**2012**:478610.

Kleijkers SH, Mantikou E, Slappendel E, Consten D, van Echten-Arends J, Wetzels AM, van Wely M, Smits LJ, van Montfoort AP, Repping S *et al*. Influence of embryo culture medium (G5 and HTF) on pregnancy and perinatal outcome after IVF: a multicenter RCT. *Hum Reprod* 2016a;**31**:2219–2230.

Kleijkers SH, van Montfoort AP, Bekers O, Coonen E, Derhaag JG, Evers JL, Dumoulin JC. Ammonium accumulation in commercially available embryo culture media and protein supplements during storage at 2-8 degrees C and during incubation at 37 degrees C. *Hum Reprod* 2016b;**31**:1192–1199.

Kleijkers SH, van Montfoort AP, Smits LJ, Coonen E, Derhaag JG, Evers JL, Dumoulin JC. Age of G-1 PLUS v5 embryo culture medium is inversely associated with birthweight of the newborn. *Hum Reprod* 2015;**30**:1352–1357.

Kleijkers SH, van Montfoort AP, Smits LJ, Viechtbauer W, Roseboom TJ, Nelissen EC, Coonen E, Derhaag JG, Bastings L, Schreurs IE *et al*. IVF culture medium affects post-natal weight in humans during the first 2 years of life. *Hum Reprod* 2014;**29**:661–669.

Koivurova S, Hartikainen AL, Sovio U, Gissler M, Hemminki E, Jarvelin MR. Growth, psychomotor development and morbidity up to 3 years of age in children born after IVF. *Hum Reprod* 2003;**18**:2328–2336.

Krishnaveni GV, Veena SR, Wills AK, Hill JC, Karat SC, Fall CH. Adiposity, insulin resistance and cardiovascular risk factors in 9-10-year-old Indian children: relationships with birth size and postnatal growth. *J Dev Orig Health Dis* 2010;**1**:403–411.

Kuiper D, Hoek A, la Bastide-van Gemert S, Seggers J, Mulder DJ, Haadsma M, Heineman MJ, Hadders-Algra M. Cardiovascular health of 9-year-old IVF offspring: no association with ovarian hyperstimulation and the in vitro procedure. *Hum Reprod* 2017;**32**:2540–2548.

Lane M, Gardner DK. Ammonium induces aberrant blastocyst differentiation, metabolism, pH regulation, gene expression and subsequently alters fetal development in the mouse. *Biol Reprod* 2003;**69**:1109–1117.

Lawitts JA, Biggers JD. Optimization of mouse embryo culture media using simplex methods. *J Reprod Fertil* 1991;**91**:543–556.

Lee SH, Lee MY, Chiang TL, Lee MS, Lee MC. Child growth from birth to 18 months old born after assisted reproductive technology–results of a national birth cohort study. *Int J Nurs Stud* 2010;**47**:1159–1166.

Lei X, Chen Y, Ye J, Ouyang F, Jiang F, Zhang J. The optimal postnatal growth trajectory for term small for gestational age babies: a prospective cohort study. *J Pediatr* 2015;**166**:54–58.

Lemmen JG, Pinborg A, Rasmussen S, Ziebe S. Birthweight distribution in ART singletons resulting from embryo culture in two different culture media compared with the national population. *Human reproduction* 2014;**29**:2326–2332.

Leunissen RW, Kerkhof GF, Stijnen T, Hokken-Koelega A. Timing and tempo of first-year rapid growth in relation to cardiovascular and metabolic risk profile in early adulthood. *JAMA* 2009a;**301**:2234–2242.

Leunissen RW, Stijnen T, Hokken-Koelega AC. Influence of birth size on body composition in early adulthood: the programming factors for growth and metabolism (PROGRAM)-study. *Clin Endocrinol (Oxf)* 2009b;**70**:245–251.

Lim D, Bowdin SC, Tee L, Kirby GA, Blair E, Fryer A, Lam W, Oley C, Cole T, Brueton LA *et al*. Clinical and molecular genetic features of Beckwith-Wiedemann syndrome associated with assisted reproductive technologies. *Hum Reprod* 2009;**24**741–747.

Lin S, Li M, Lian Y, Chen L, Liu P. No effect of embryo culture media on birthweight and length of newborns. *Hum Reprod* 2013;**28**:1762–1767.

Lv PP, Meng Y, Lv M, Feng C, Liu Y, Li JY, Yu DQ, Shen Y, Hu XL, Gao Q *et al*. Altered thyroid hormone profile in offspring after exposure to high estradiol environment during the first trimester of pregnancy: a cross-sectional study. *BMC Med* 2014;**12**:240.

MacLaughlin SM, Walker SK, Roberts CT, Kleemann DO, McMillen IC. Periconceptional nutrition and the relationship between maternal body weight changes in the periconceptional period and fetoplacental growth in the sheep. *J Physiol* 2005;**565**:111–124.

Makinen S, Soderstrom-Anttila V, Vainio J, Suikkari AM, Tuuri T. Does long in vitro culture promote large for gestational age babies? *Hum Reprod* 2013;**28**:828–834.

Malchau SS, Loft A, Larsen EC, Aaris Henningsen AK, Rasmussen S, Andersen AN, Pinborg A. Perinatal outcomes in 375 children born after oocyte donation: a Danish national cohort study. *Fertil Steril* 2013;**99**:1637–1643.

Marino JL, Moore VM, Willson KJ, Rumbold A, Whitrow MJ, Giles LC, Davies MJ. Perinatal outcomes by mode of assisted conception and sub-fertility in an Australian data linkage cohort. *PloS One* 2014;**9**:e80398.

Market-Velker BA, Fernandes AD, Mann MR. Side-by-side comparison of five commercial media systems in a mouse model: suboptimal in vitro culture interferes with imprint maintenance. *Biol Reprod* 2010;**83**:938–950.

McDonald SD, Han Z, Mulla S, Murphy KE, Beyene J, Ohlsson A, Knowledge Synthesis G. Preterm birth and low birth weight among in vitro fertilization singletons: a systematic review and meta-analyses. *Eur J Obstet Gynecol Reprod Biol* 2009;**146**:138–148.

Messerlian C, Maclagan L, Basso O. Infertility and the risk of adverse pregnancy outcomes: a systematic review and meta-analysis. *Hum Reprod* 2013;**28**:125–137.

Meyer JS, Novak MA. Minireview: Hair cortisol: a novel biomarker of hypothalamic-pituitary-adrenocortical activity. *Endocrinology* 2012;**153**:4120–4127.

Morbeck DE, Krisher RL, Herrick JR, Baumann NA, Matern D, Moyer T. Composition of commercial media used for human embryo culture. *Fertil Steril* 2014a;**102**:759–766 e759.

Morbeck DE, Paczkowski M, Fredrickson JR, Krisher RL, Hoff HS, Baumann NA, Moyer T, Matern D. Composition of protein supplements used for human embryo culture. *J Assist Reprod Genet* 2014b;**31**:1703–1711.

Nakashima A, Araki R, Tani H, Ishihara O, Kuwahara A, Irahara M, Yoshimura Y, Kuramoto T, Saito H, Nakaza A *et al*. Implications of

assisted reproductive technologies on term singleton birth weight: an analysis of 25,777 children in the national assisted reproduction registry of Japan. *Fertil Steril* 2013;**99**:450–455.

National. High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents. The fourth report on the diagnosis, evaluation, and treatment of high blood pressure in children and adolescents. *Pediatrics* 2004;**114**: 555–576.

Nelissen EC, Van Montfoort AP, Coonen E, Derhaag JG, Geraedts JP, Smits LJ, Land JA, Evers JL, Dumoulin JC. Further evidence that culture media affect perinatal outcome: findings after transfer of fresh and cryopreserved embryos. *Hum Reprod* 2012;**27**:1966–1976.

Nelissen EC, Van Montfoort AP, Smits LJ, Menheere PP, Evers JL, Coonen E, Derhaag JG, Peeters LL, Coumans AB, Dumoulin JC. IVF culture medium affects human intrauterine growth as early as the second trimester of pregnancy. *Hum Reprod* 2013;**28**:2067–2074.

Onal H, Ercan O, Adal E, Ersen A, Onal Z. Subclinical hypothyroidism in in vitro fertilization babies. *Acta Paediatr* 2012;**101**:e248–e252.

Palermo G, Joris H, Devroey P, Van Steirteghem AC. Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. *Lancet* 1992;**340**:17–18.

Pandey S, Shetty A, Hamilton M, Bhattacharya S, Maheshwari A. Obstetric and perinatal outcomes in singleton pregnancies resulting from IVF/ICSI: a systematic review and meta-analysis. *Hum Reprod Update* 2012;**18**:485–503.

Patro-Golab B, Zalewski BM, Kouwenhoven SM, Karas J, Koletzko B, Bernard van Goudoever J, Szajewska H. Protein concentration in milk formula, growth, and later risk of obesity: a systematic review. *J Nutr* 2016;**146**:551–564.

Pelinck MJ, Hadders-Algra M, Haadsma ML, Nijhuis WL, Kiewiet SM, Hoek A, Heineman MJ, Middleburg KJ. Is the birthweight of singletons born after IVF reduced by ovarian stimulation or by IVF laboratory procedures? *Reprod Biomed Online* 2010;**21**: 245–251.

Pinborg A, Henningsen AA, Loft A, Malchau SS, Forman J, Andersen AN. Large baby syndrome in singletons born after frozen embryo transfer (FET): is it due to maternal factors or the cryotechnique? *Hum Reprod* 2014;**29**:618–627.

Pinborg A, Wennerholm UB, Romundstad LB, Loft A, Aittomaki K, Söderström-Anttila V, Nygren KG, Hazekamp J, Bergh C. Why do singletons conceived after assisted reproduction technology have adverse perinatal outcome? Systematic review and meta-analysis. *Hum Reprod Update* 2013;**19**:87–104.

Pontesilli M, Painter RC, Grooten IJ, van der Post JA, Mol BW, Vrijkotte TG, Repping S, Roseboom TJ. Subfertility and assisted reproduction techniques are associated with poorer cardiometabolic profiles in childhood. *Reprod Biomed Online* 2015;**30**:258–267.

Pourteymour Fard Tabrizi F, Alipoor B, Mehrzad Sadaghiani M, Ostadrahimi A, Malek Mahdavi A. Metabolic syndrome and its characteristics among reproductive-aged women with polycystic ovary syndrome: a cross-sectional study in Northwest Iran. *Int J Fertil Steril* 2013;**6**:244–249.

Puissant C, Abraham P, Durand S, Humeau-Heurtier A, Faure S, Leftheriotis G, Rousseau P, Mahe G. Reproducibility of non-invasive assessment of skin endothelial function using laser Doppler flowmetry and laser speckle contrast imaging. *PLoS One* 2013;**8**: e61320.

Rexhaj E, Paoloni-Giacobino A, Rimoldi SF, Fuster DG, Anderegg M, Somm E, Bouillet E, Allemann Y, Sartori C, Scherrer U. Mice generated by in vitro fertilization exhibit vascular dysfunction and shortened life span. *J Clin Invest* 2013;**123**:5052–5060.

Rifai N, Warnick GR. *Tietz Textbook of Clinical Chemistry and Molecular Diagnosis*, 4th Edition: Measurement of lipids, lipoproteins, and apolipoproteins, 2006,938–952

Rimoldi SF, Sartori C, Rexhaj E, Bailey DM, de Marchi SF, McEneny J, Arx R, Cerny D, Duplain H, Germond M *et al*. Antioxidants improve vascular function in children conceived by assisted reproductive technologies: A randomized double-blind placebo-controlled trial. *Eur J Prev Cardiol* 2015;**22**:1399–1407.

Rimoldi SF, Sartori C, Rexhaj E, Cerny D, Von Arx R, Soria R, Germond M, Allemann Y, Scherrer U. Vascular dysfunction in children conceived by assisted reproductive technologies: underlying mechanisms and future implications. *Swiss Med Wkly* 2014;**144**: w13973.

Roberts SA, Vail A. On the appropriate interpretation of evidence: the example of culture media and birth weight. *Hum Reprod* 2017;**32**:1151–1154.

Romundstad LB, Romundstad PR, Sunde A, von During V, Skjaerven R, Gunnell D, Vatten LJ. Effects of technology or maternal factors on perinatal outcome after assisted fertilisation: a population-based cohort study. *Lancet* 2008;**372**:737–743.

Rooke JA, McEvoy TG, Ashworth CJ, Robinson JJ, Wilmut I, Young LE, Sinclair KD. Ovine fetal development is more sensitive to perturbation by the presence of serum in embryo culture before rather than after compaction. *Theriogenology* 2007;**67**:639–647.

Roseboom T, de Rooij S, Painter R. The Dutch famine and its long-term consequences for adult health. *Early Hum Dev* 2006;**82**:485–491.

Roseboom TJ, Painter RC, van Abeelen AF, Veenendaal MV, de Rooij SR. Hungry in the womb: what are the consequences? Lessons from the Dutch famine. *Maturitas* 2011;**70**:141–145.

Roseboom TJ, van der Meulen JH, van Montfrans GA, Ravelli AC, Osmond C, Barker DJ, Bleker OP. Maternal nutrition during gestation and blood pressure in later life. *J Hypertens* 2001;**19**: 29–34.

Rumball CW, Bloomfield FH, Oliver MH, Harding JE. Different periods of periconceptional undernutrition have different effects on growth, metabolic and endocrine status in fetal sheep. *Pediatr Res* 2009;**66**:605–613.

Rumbold AR, Moore VM, Whitrow MJ, Oswald TK, Moran LJ, Fernandez RC, Barnhart KT, Davies MJ. The impact of specific fertility treatments on cognitive development in childhood and adolescence: a systematic review. *Hum Reprod* 2017;**32**:1489–1507.

Sakka SD, Loutradis D, Kanaka-Gantenbein C, Margeli A, Papastamataki M, Papassotiriou I, Chrousos GP. Absence of insulin resistance and low-grade inflammation despite early metabolic syndrome manifestations in children born after in vitro fertilization. *Fertil Steril* 2010;**94**:1693–1699.

Sauvé B, Koren G, Walsh G, Tokmakejian S, Van Uum SH. Measurement of cortisol in human hair as a biomarker of systemic exposure. *Clin Invest Med* 2007;**30**:E183–E191.

Savage JS, Birch LL, Marini M, Anzman-Frasca S, Paul IM. Effect of the INSIGHT Responsive Parenting Intervention on rapid infant weight gain and overweight status at age 1 year: a randomized clinical trial. *JAMA Pediatr* 2016;**170**:742–749.

Sazonova A, Kallen K, Thurin-Kjellberg A, Wennerholm UB, Bergh C. Obstetric outcome in singletons after in vitro fertilization with cryopreserved/thawed embryos. *Hum Reprod* 2012;**27**: 1343–1350.

Schellong K, Schulz S, Harder T, Plagemann A. Birth weight and long-term overweight risk: systematic review and a meta-analysis including 643,902 persons from 66 studies and 26 countries globally. *PLoS One* 2012;**7**:e47776.

Schendelaar P, La Bastide-Van Gemert S, Heineman MJ, Middelburg KJ, Seggers J, Van den Heuvel ER, Hadders-Algra M. Subfertility factors rather than assisted conception factors affect cognitive and behavioural development of 4-year-old singletons. *Reprod Biomed Online* 2016;**33**:752–762.

Scherrer U, Rimoldi SF, Rexhaj E, Stuber T, Duplain H, Garcin S, de Marchi SF, Nicod P, Germond M, Allemann Y *et al*. Systemic and pulmonary vascular dysfunction in children conceived by assisted reproductive technologies. *Circulation* 2012;**125**:1890–1896.

Schieve LA, Meikle SF, Ferre C, Peterson HB, Jeng G, Wilcox LS. Low and very low birth weight in infants conceived with use of assisted reproductive technology. *N Engl J Med* 2002;**346**:731–737.

Schonbeck Y, Talma H, van Dommelen P, Bakker B, Buitendijk SE, HiraSing RA, van Buuren S. The world's tallest nation has stopped growing taller: the height of Dutch children from 1955 to 2009. *Pediatr Res* 2013;**73**:371–377.

Schönbeck Y, Talma H, van Dommelen P, Bakker B, Buitendijk SE, Hirasing RA, van Buuren S. Increase in prevalence of overweight in Dutch children and adolescents: a comparison of nationwide growth studies in 1980, 1997 and 2009. *PLoS One* 2011;**6**:e27608.

Schwarzer C, Esteves TC, Arauzo-Bravo MJ, Le Gac S, Nordhoff V, Schlatt S, Boiani M. ART culture conditions change the probability of mouse embryo gestation through defined cellular and molecular responses. *Human reproduction* 2012;**27**:2627–2640.

Scott KA, Yamazaki Y, Yamamoto M, Lin Y, Melhorn SJ, Krause EG, Woods SC, Yanagimachi R, Sakai RR, Tamashiro KL. Glucose parameters are altered in mouse offspring produced by assisted reproductive technologies and somatic cell nuclear transfer. *Biol Reprod* 2010;**83**:220–227.

Sinclair KD, McEvoy TG, Carolan C, Maxfield EK, Maltin CA, Young LE, Wilmut I, Robinson JJ, Broadbent PJ. Conceptus growth and development following in vitro culture of ovine embryos in media supplemented with bovine sera. *Theriogenology* 1998;**49**: 218–218.

Singhal A. Long-term adverse effects of early growth acceleration or catch-up growth. *Ann Nutr Metab* 2017;**70**:236–240.

Singhal A, Cole TJ, Fewtrell M, Deanfield J, Lucas A. Is slower early growth beneficial for long-term cardiovascular health? *Circulation* 2004;**109**:1108–1113.

Singhal A, Lucas A. Early origins of cardiovascular disease: is there a unifying hypothesis? *Lancet* 2004;**363**:1642–1645.

Skovlund E, Fenstad GU. Should we always choose a nonparametric test when comparing two apparently nonnormal distributions? *J Clin Epidemiol* 2001;**54**:86–92.

Stalder T, Kirschbaum C, Alexander N, Bornstein SR, Gao W, Miller R, Stark S, Bosch JA, Fischer JE. Cortisol in hair and the metabolic syndrome. *J Clin Endocrinol Metab* 2013;**98**:2573–2580.

Steptoe PC, Edwards RG. Birth after the reimplantation of a human embryo. *Lancet* 1978;**2**:366.

Summers MC, Biggers JD. Chemically defined media and the culture of mammalian preimplantation embryos: historical perspective and current issues. *Hum Reprod Update* 2003;**9**:557–582.

Sunde A, Brison D, Dumoulin J, Harper J, Lundin K, Magli MC, Van den Abbeel E, Veiga A. Time to take human embryo culture seriously. *Hum Reprod* 2016;**31**:2174–2182.

Taine M, Stengel B, Forhan A, Carles S, Botton J, Charles MA, Heude B, Group EM-CCS. Rapid early growth may modulate the association between birth weight and blood pressure at 5 years in the EDEN cohort study. *Hypertension* 2016;**68**:859–865.

Thompson JG, Gardner DK, Pugh PA, McMillan WH, Tervit HR. Lamb birth weight is affected by culture system utilized during in vitro pre-elongation development of ovine embryos. *Biol Reprod* 1995;**53**:1385–1391.

Thouas GA, Jones GM, Trounson AO. The 'GO' system–a novel method of microculture for in vitro development of mouse zygotes to the blastocyst stage. *Reproduction* 2003;**126**:161–169.

Touwslager RN, Gerver WJ, Tan FE, Gielen M, Zeegers MP, Zimmermann LJ, Houben AJ, Blanco CE, Stehouwer CD, Mulder AL. Influence of growth during infancy on endothelium-dependent vasodilatation at the age of 6 months. *Hypertension* 2012a;**60**:1294–1300.

Touwslager RN, Houben AJ, Gielen M, Zeegers MP, Stehouwer CD, Zimmermann LJ, Kessels AG, Gerver WJ, Blanco CE, Mulder AL. Endothelial vasodilatation in newborns is related to body size and maternal hypertension. *J Hypertens* 2012b;**30**:124–131.

Touwslager RN, Houben AJ, Tan FE, Gielen M, Zeegers MP, Stehouwer CD, Gerver WJ, Westerterp KR, Wouters L, Blanco CE *et al*. Growth and endothelial function in the first 2 years of life. *J Pediatr* 2015;**166**:666–671 e661.

Turner J, Belch JJ, Khan F. Current concepts in assessment of microvascular endothelial function using laser Doppler imaging and iontophoresis. *Trends Cardiovasc Med* 2008;**18**:109–116.

Valenzuela-Alcaraz B, Crispi F, Bijnens B, Cruz-Lemini M, Creus M, Sitges M, Bartrons J, Civico S, Balasch J, Gratacos E. Assisted reproductive technologies are associated with cardiovascular remodeling in utero that persists postnatally. *Circulation* 2013;**128**: 1442–1450.

Veena SR, Hegde BG, Ramachandraiah S, Krishnaveni GV, Fall CH, Srinivasan K. Relationship between adiposity and cognitive performance in 9-10-year-old children in South India. *Arch Dis Child* 2014;**99**:126–134.

Velazquez MA, Sheth B, Smith SJ, Eckert JJ, Osmond C, Fleming TP. Insulin and branched-chain amino acid depletion during mouse preimplantation embryo culture programmes body weight gain and raised blood pressure during early postnatal life. *Biochim Biophys Acta* 2017;**1864**:590–600.

Vergouw CG, Kostelijk EH, Doejaaren E, Hompes PG, Lambalk CB, Schats R. The influence of the type of embryo culture medium on neonatal birthweight after single embryo transfer in IVF. *Hum Reprod* 2012;**27**:2619–2626.

Vermeiden JP, Bernardus RE. Are imprinting disorders more prevalent after human in vitro fertilization or intracytoplasmic sperm injection? *Fertil Steril* 2013;**99**:642–651.

Vidal M, Vellve K, Gonzalez-Comadran M, Robles A, Prat M, Torne M, Carreras R, Checa MA. Perinatal outcomes in children born after fresh or frozen embryo transfer: a Catalan cohort study based on 14,262 newborns. *Fertil Steril* 2017;**107**:940–947.

Visser GH, Eilers PH, Elferink-Stinkens PM, Merkus HM, Wit JM. New Dutch reference curves for birthweight by gestational age. *Early Hum Dev* 2009;**85**:737–744.

Vrooman LA, Bartolomei MS. Can assisted reproductive technologies cause adult-onset disease? Evidence from human and mouse. *Reprod Toxicol* 2017;**68**:72–84.

Watkins AJ, Lucas ES, Wilkins A, Cagampang FR, Fleming TP. Maternal periconceptional and gestational low protein diet affects mouse offspring growth, cardiovascular and adipose phenotype at 1 year of age. *PLoS One* 2011;**6**:e28745.

Watkins AJ, Platt D, Papenbrock T, Wilkins A, Eckert JJ, Kwong WY, Osmond C, Hanson M, Fleming TP. Mouse embryo culture induces changes in postnatal phenotype including raised systolic blood pressure. *Proc Natl Acad Sci U S A* 2007;**104**:5449–5454.

Wennerholm UB, Henningsen AK, Romundstad LB, Bergh C, Pinborg A, Skjaerven R, Forman J, Gissler M, Nygren KG, Tiitinen A. Perinatal outcomes of children born after frozen-thawed embryo transfer: a Nordic cohort study from the CoNARTaS group. *Human reproduction* 2013;**28**:2545–2553.

Woldringh GH, Hendriks JC, van Klingeren J, van Buuren S, Kollee LA, Zielhuis GA, Kremer JA. Weight of in vitro fertilization and intracytoplasmic sperm injection singletons in early childhood. *Fertil Steril* 2011;**95**:2775–2777.

Woo JG. Fast, slow, high, and low: infant and childhood growth as predictors of cardiometabolic outcomes. *J Pediatr* 2017;**186**:14–16.

Wunder D, Ballabeni P, Roth-Kleiner M, Primi MP, Senn A, Chanson A, Germond M, Leyvraz C. Effect of embryo culture media on birthweight and length in singleton term infants after IVF-ICSI. *Swiss Med Wkly* 2014;**144**:w14038. http://www.openepi.com.

Yeung EH, Sundaram R, Bell EM, Druschel C, Kus C, Xie Y, Buck Louis GM. Infertility treatment and children's longitudinal growth between birth and 3 years of age. *Hum Reprod* 2016;**31**:1621–1628.

Yin TL, Zhang Y, Li SJ, Zhao M, Ding JL, Xu WM, Yang J. Culture media influenced laboratory outcomes but not neonatal birth weight in assisted reproductive technology. *J Huazhong Univ Sci Technolog Med Sci* 2015;**35**:932–937.

Young LE, Fernandes K, McEvoy TG, Butterwith SC, Gutierrez CG, Carolan C, Broadbent PJ, Robinson JJ, Wilmut I, Sinclair KD. Epigenetic change in IGF2R is associated with fetal overgrowth after sheep embryo culture. *Nat Genet* 2001;**27**:153–154.

Zander DL, Thompson JG, Lane M. Perturbations in mouse embryo development and viability caused by ammonium are more severe after exposure at the cleavage stages. *Biol Reprod* 2006;**74**:288–294.

Zandstra H, Van Montfoort AP, Dumoulin JC. Does the type of culture medium used influence birthweight of children born after IVF? *Hum Reprod* 2015;**30**:2693.

Zegers-Hochschild F, Masoli D, Schwarze JE, Iaconelli A, Borges E, Pacheco IM. Reproductive performance in oocyte donors and their recipients: comparative analysis from implantation to birth and lactation. *Fertil Steril* 2010;**93**:2210–2215.

Zhan QT, Pan PP, Xu XR, Lou HY, Lou YY, Jin F. An overview of studies on psychological well-being in children born following assisted reproductive technologies. *J Zhejiang Univ Sci B* 2013;**14**:947–960.

Zhu J, Li M, Chen L, Liu P, Qiao J. The protein source in embryo culture media influences birthweight: a comparative study between G1 v5 and G1-PLUS v5. *Hum Reprod* 2014a;**29**:1387–1392.

Zhu J, Lin S, Li M, Chen L, Lian Y, Liu P, Qiao J. Effect of in vitro culture period on birthweight of singleton newborns. *Hum Reprod* 2014b;**29**:448–454.

Ziebe S, Loft A, Povlsen BB, Erb K, Agerholm I, Aasted M, Gabrielsen A, Hnida C, Zobel DP, Munding B *et al.* A randomized clinical trial to evaluate the effect of granulocyte-macrophage colony-stimulating factor (GM-CSF) in embryo culture medium for in vitro fertilization. *Fertil Steril* 2013;**99**:1600–1609.