Early pregnancy

IVF culture medium affects human intrauterine growth as early as the second trimester of pregnancy†

Ewka C.M. Nelissen1,*, Aafke P.A. Van Montfoort1, Luc J.M. Smits2, Paul P.C.A. Menheere3, Johannes L.H. Evers1, Edith Coonen4, Josien G. Derhaag1, Louis L. Peeters1, Audrey B. Coumans1, and John C.M. Dumoulin1

1Department of Obstetrics & Gynaecology, GROW School for Oncology and Developmental Biology, Maastricht University Medical Centre, Maastricht, The Netherlands 2Department of Epidemiology, Maastricht University Medical Centre, Maastricht, The Netherlands 3Department of Clinical Chemistry, Maastricht University Medical Centre, Maastricht, The Netherlands 4Department of Clinical Genetics, Maastricht University Medical Centre, Maastricht, The Netherlands

*Correspondence address. E-mail: e.nelissen@mumc.nl

Submitted on September 7, 2012; resubmitted on March 7, 2013; accepted on March 19, 2013

STUDY QUESTION: When does a difference in human intrauterine growth of singletons conceived after IVF and embryo culture in two different culture media appear?

SUMMARY ANSWER: Differences in fetal development after culture of embryos in one of two IVF media were apparent as early as the second trimester of pregnancy.

WHAT IS KNOWN ALREADY: Abnormal fetal growth patterns are a major risk factor for the development of chronic diseases in adult life. Previously, we have shown that the medium used for culturing embryos during the first few days after fertilization significantly affects the birth-weight of the resulting human singletons. The exact onset of this growth difference was unknown.

STUDY DESIGN, SIZE AND DURATION: In this retrospective cohort study, all 294 singleton live births after fresh embryo transfer in the period July 2003 to December 2006 were included. These embryos originated from IVF treatments that were part of a previously described clinical trial. Embryos were allocated to culture in either Vitrolife or Cook commercially available sequential culture media.

PARTICIPANTS/MATERIALS, SETTING, METHODS: We analysed ultrasound examinations at 8 (n = 290), 12 (n = 83) and 20 weeks’ (n = 206) gestation and used first-trimester serum markers [pregnancy-associated plasma protein-A (PAPP-A) and free β-hCG]. Differences between study groups were tested by the Student’s t-test, χ² test or Fisher’s exact test, and linear multivariable regression analysis to adjust for possible confounders (for example, parity, gestational age at the time of ultrasound and fetal gender).

MAIN RESULTS AND THE ROLE OF CHANCE: A total of 294 singleton pregnancies (Vitrolife group nVL = 168, Cook group: nC = 126) from 294 couples were included. At 8 weeks’ gestation, there was no difference between crown-rump length-based and ovum retrieval-based gestational age (ΔGA) (nVL = 163, nC = 122, adjusted mean difference, −0.04 days, P = 0.84). A total of 83 women underwent first-trimester screening at 12 weeks’ gestation (nVL = 45, nC = 38). ΔGA, nuchal translucency (multiples of median, MoM) and PAPP-A (MoM) did not differ between the study groups. Free β-hCG (MoM) ± SEM differed significantly (1.55 ± 0.19 in Vitrolife versus 1.06 ± 0.10 in Cook; P = 0.031, Student’s t-test). At 20 weeks’ gestation, a more advanced GA, reflecting an increased fetal growth, was seen at ultrasound examination in the Vitrolife group (n = 115) when compared with the Cook group (n = 91). After adjustment for confounding factors, both the difference between GA based on three biparietal diameter dating formulas minus the actual (ovum retrieval based) GA (adjusted mean difference + 1.14 days (P = 0.04), +1.14 days (P = 0.04) and +1.36 days (P = 0.048), as well as head circumference (HC) and trans-cerebellar diameter (TCD) were significantly higher in the Vitrolife group (HCvl 177.3 mm, HCc 175.9 mm, adjusted mean difference 1.8, P = 0.03; TCDvl 20.5 mm, TCDc 20.2 mm, adjusted mean difference 0.4, P = 0.008).

†Presented in part at the 27th annual meeting of the European Society of Human Reproduction and Embryology (ESHRE), Stockholm, 2011.

© The Author 2013. Published by Oxford University Press on behalf of the European Society of Human Reproduction and Embryology. All rights reserved.
For Permissions, please email: journals.permissions@oup.com
Introduction

In our previous studies, we have shown that the medium used for culturing IVF embryos during the first few days of preimplantation development affects birthweight of the resulting human singletons (Dumoulin et al., 2010; Nelissen et al., 2012). Even after correction for a large range of possible confounders, such as gestational age (GA) at delivery, fetal gender, number of transferred embryos, day of embryo transfer (second or third), fertilization method (IVF or ICSI), parity, parental height, weight and age, cause and duration of infertility, pregnancy related factors and life-style factors (e.g. smoking), the significant association between human birthweight and type of culture medium persisted (Dumoulin et al., 2010; Nelissen et al., 2012). The time of onset of this growth difference is, as yet, unknown.

Increasing evidence indicates that pace and pathway of fetal growth is a major risk factor for the development of chronic diseases in adult life, such as hypertension, type 2 diabetes, coronary heart disease and stroke, often referred to as the ‘fetal origins hypothesis’ (Barker et al., 2009). Although the general IVF progeny is still too young to determine the prevalence of these adult diseases, in IVF children parameters of metabolic syndrome, like increased systolic and diastolic blood pressures and glucose levels, are reported (Ceelen et al., 2008).

Evidence is accumulating that factors in the peri-conceptional period, such as maternal undernutrition, can modify the fetal growth trajectories, sometimes even with negligible effect on birthweight but nevertheless with adverse consequences for long-term health (Bloomfield et al., 2006). This suggests that the presence of appropriate nutrients, and other factors, in IVF culture media will be vital for embryonic development during this high-risk period. In the light of increasing numbers of children born worldwide after a pregnancy established by assisted reproduction technologies (ARTs), exploration of this topic is of pivotal importance.

The aim of the present study was to increase our insight into the impact of embryo culture media on subsequent fetal growth patterns, with particular focus on the onset of growth divergence. We hypothesized that the effect of in vitro culture of embryos on fetal growth will increase during the first trimester and will be detectable at the end of the first trimester or during the second trimester, because these differences will be the result of modified growth trajectories of the fetus itself after in vitro culture in different media, rather than differences in parental or other external factors. To examine this, we evaluated ultrasound examinations performed at 8, 12 and 20 weeks’ gestation in patients who participated in our previous study (Nelissen et al., 2012) and used first-trimester serum markers [free β-hCG (β-hCG) and pregnancy-associated plasma protein-A (PAPP-A)], as indicators of placental function.

Materials and Methods

Study population

At the Maastricht University Medical Centre (MUMC), we studied 294 singleton live births after fresh embryo transfer, from a previously reported comparative study (Nelissen et al., 2012). In this cohort, IVF (or IVF with ICSI) was applied in the period between July 2003 and December 2006 during which two widely used commercially available sequential culture media ([Vitrolife G1.3, Göteborg, Sweden] or Cook K-SICM, Brisbane, Australia) were used in alternating order. For this study, all singletons born alive after the 20th week of gestation, who were a couple’s first child from an IVF treatment performed during the study period, were included in the analysis. Data on pregnancy outcome including complications, such as gestational diabetes, hypertension and pre-eclampsia, and perinatal outcome, were collected from their obstetricians or midwives. Exclusion criteria for the study were patients who applied for PGD or required donor oocytes. All participating couples gave written informed consent for the use of their anonymized data.

Culture medium allocation and IVF procedure

We strictly alternated treatment allocation to either of the two media on the day before the ovum retrieval. This was performed by laboratory technicians who were unaware of patient characteristics. The order of ovum retrievals on a certain day, which determined the allocation to one of the study groups, was planned by clinical personnel who were unaware of the laboratory allocation procedure. This allocation to one of the two media was part of our internal quality monitoring system in order to be able to identify suboptimal batches of a particular medium. Except for the media, all other IVF procedures (clinical as well as laboratory) were similar in both groups. Detailed ovarian stimulation, fertilization, culture and fresh embryo transfer procedures have been described previously (Dumoulin et al., 2010).

Fetal growth data collection

GA was calculated from the day of oocyte retrieval, which was defined as Day 14 of the cycle. In the Netherlands, all pregnant women are offered ultrasound dating during the first trimester and are counselled for fetal ultrasound examination at 20 weeks’ gestation to have fetal biometry performed and be screened for structural abnormalities. For the present study, the results of ultrasonic measurements were collected retrospectively and all
sonographers were unaware of the randomization procedure and its outcome. Furthermore, all sonographers were experienced and specially trained for these ultrasound examinations.

In viable pregnancies, fetal crown-rump length (CRL) was measured at 7–8 weeks’ gestation in a mid-sagittal section of the embryo using transvaginal ultrasonography with care being taken to avoid inclusion of the yolk sac. The difference between the CRL-based GA calculated with the formula of (Hadlock et al., 1992) minus the actual (ovum retrieval based) GA, was expressed as the difference in days of gestation (ΔGA).

Only a subset of women participated in (elective) first-trimester screening for Down syndrome (information about the possibility for screening has only been given to all pregnant women in the Netherlands since June 2004). In these cases, serum was sampled at a gestational interval ranging from 11 to 13 weeks plus 6 days (NICE, 2008). The concentrations of maternal serum β-hCG and PAPP-A were analysed with commercially available kits and the AutoDELFIA analyser (PerkinElmer, Turku, Finland) at the National Institute for Public Health and the Environment (Bilthoven, The Netherlands) during the years 2003–2005 and at the authorized clinical chemistry laboratory of the MUMC during the years 2006–2007. CRL and fetal nuchal translucency (NT) thickness were obtained during abdominal ultrasonography. The values of β-hCG, PAPP-A and NT were expressed as multiples of the median (MoM) for GA with corrections for maternal weight according to the guidelines of the national first trimester prenatal screening programme (Schielen et al., 2003).

Furthermore, several sonographic parameters were measured during the mid-trimester ultrasound scan at ~20 weeks’ gestation to estimate GA and to calculate estimated fetal weight (EFW). The fetal biparietal diameter (BPD), head circumference (HC), abdominal circumference (AC), femur length (FL) and trans-cerebellar diameter (TCD) were measured using standardized ultrasound procedures. BPD dating formulas of Mul et al. (#1 and #2) (1996) and Selbing and Kjessler (1985) were used to calculate the GA (Salvèved et al., 2004). The difference between GA calculated by BPD dating formulas minus the actual (ovum retrieval based) GA was expressed in days (ΔGA). EFW was calculated using the formulas of Hadlock I (BPD, HC, AC, FL), Hadlock II (BPD, AC, FL) and Hadlock IV (HC, AC, FL) (Hadlock et al., 1985; Hoopman et al., 2010).

Statistical analysis

Crude differences between study groups were tested using the Student’s t-test for continuous variables and the χ² test for binary variables (Fisher’s exact test in case of more than five observations per cell). P-values of <0.05 (two-sided testing) were considered to reflect statistical significance. Linear regression analysis was applied in order to control for any between-group differences with respect to other determinants of fetal growth. In one of our previous articles (Dumoulin et al., 2010), we found a difference between study groups in height and weight but not BMI. Therefore, we used height and weight instead of BMI in our multiple regression analyses. The following factors were included in all multivariable analyses: parity, GA at the time of ultrasound, characteristics of both parents (age, weight, height, daily number of cigarettes smoked), duration of infertility, cause of infertility, pregnancy complications (gestational diabetes, hypertension and pre-eclampsia), day of embryo transfer (second or third), number of transferred embryos (one or two) and fetal gender. The residuals were normally distributed in all regression analyses. Residual versus fitted plots did not indicate heteroscedasticity. Furthermore, there was no evidence of collinearity.

Results

A total of 294 singleton pregnancies (168 in the Vitrolife group versus 126 in the Cook group) from 294 different couples were included. Neonatal characteristics have been reported previously (Nelissen et al., 2012). Parental characteristics are listed in Table I. The distribution of the confounding variables is described in the Supplementary data, Table SI (available online). The rate of pregnancy complications (such as gestational diabetes, hypertension and pre-eclampsia), was similar in both groups (14 (8.3%) in the Vitrolife group versus 9 (7.1%) in the Cook group).

8 weeks’ gestation

Fetal CRL was measured around 8 weeks’ gestation (range 6 weeks and 5 days to 9 weeks and 2 days) in 290 singleton pregnancies in 167 in the Vitrolife group and 123 in the Cook group. Four couples were lost to follow-up. The ΔGA was comparable in the two groups (unadjusted mean difference −0.08 days, P = 0.68, adjusted −0.04 days, P = 0.84) (Table II).

12 weeks’ gestation

A total of 83 women chose to undergo first-trimester screening around 12 weeks’ gestation (range 11 weeks and 3 days to 13 weeks and 6 days): 45 in the Vitrolife group (27%) and 38 in the Cook group (30%). The difference in ΔGA (adjusted mean difference −0.59 days, P = 0.22) (Table II) and that in NT and PAPP-A was statistically insignificant (Table III). However, β-hCG and β-hCG (MoM) in the Vitrolife group were significantly higher than in the Cook group (P = 0.029, P = 0.031, respectively, Table III).

20 weeks’ gestation

Fetal growth parameters were measured around 20 weeks’ gestation (range 18 weeks and 2 days to 22 weeks and 1 day) in 206 pregnancies, 113 (68%) in the Vitrolife group and 91 (72%) in the Cook group. The remaining 88 couples chose not to have the mid-trimester fetal ultrasound scan or had this examination elsewhere in the Netherlands. Using three distinct BPD dating formulas, the adjusted ΔGA differed significantly between groups in all three calculations. In the Vitrolife group, all ΔGA were more positive than in the Cook group (linear multivariable regression analysis; P = 0.04, P = 0.04 and P = 0.048) (Table IV). This is consistent with a more advanced GA, reflecting an increased fetal growth in the Vitrolife group. The adjusted mean values of several other sonographic parameters (BPD, HC, AC, FL and TCD) are shown in Table V. All these fetal sonographic parameters are useful to assess fetal growth. HC and TCD were significantly higher in the Vitrolife group compared with the Cook group (P = 0.03, P = 0.008, respectively), again reflecting an increased fetal growth in the Vitrolife group. EFW, as calculated with the Hadlock formulas I, III or IV, did not differ between the two groups at 20 weeks’ gestation (Table V).

Discussion

The main findings of our study of a series of singletons are that the Vitrolife group differed from the Cook group by significantly higher circulating levels of β-hCG (MoM) at 12 weeks and a more advanced GA, consistent with an increased fetal growth, at 20 weeks’ gestation. In previous studies, we observed a significant difference in birthweight between the two study groups of singletons resulting from culture in either Vitrolife or Cook medium (Dumoulin et al., 2010; Nelissen et al., 2012). Although in these studies, we controlled for many maternal and environmental co-variables specified in our analysis, theoretically it could be possible that our results were influenced by the effect of an as yet unknown
maternal or pregnancy related confounder. It is generally assumed that during the first half of pregnancy, the fetus’ own genetic programme is the primary determinant of growth, while during the second half of pregnancy, many external factors, such as maternal factors (pre-pregnancy weight or BMI, height, ethnic background), pregnancy-related factors (gestational diabetes, hypertension and pre-eclampsia) and environmental factors (maternal nutrition, disease, smoking, drugs) have an increasing impact on fetal growth (Mongelli and Gardosi, 1995; Rosenberg et al., 2010). As the results of the present study indicate that the growth differences between the study groups are already discernible in the second trimester of pregnancy, our findings of the differences in birthweight between the two study groups are more in line with our hypothesis that these differences are the result of modified growth trajectories of the fetus itself after in vitro culture in different media, rather than differences in parental or other external factors.

Recently, variability in first-trimester fetal growth by maternal factors was investigated in humans (Mook-Kanamori et al., 2010). Fetal CRL appeared positively associated with higher maternal age, parity ≥1, folic acid use, negatively associated with, for example, smoking and not associated with maternal anthropometrics (Mook-Kanamori et al., 2010). After adjustment for these and several other confounding factors (except folic acid because of standard use with IVF treatment), we found no significant ΔGA between groups at 8 weeks’ gestation.

Table I Parental characteristics of singletons born after fresh embryo transfers.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>8 weeks</th>
<th>12 weeks</th>
<th>20 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vitrolife group (n = 167)</td>
<td>Cook group (n = 123)</td>
<td>Unadjusted mean differences</td>
</tr>
<tr>
<td>Maternal characteristics</td>
<td>32.4 ± 4.0</td>
<td>32.6 ± 3.6</td>
<td>34.0 ± 4.1</td>
</tr>
<tr>
<td>Age (years)</td>
<td>11 (6.6)</td>
<td>6 (4.9)</td>
<td>7 (15.6)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>164.9 ± 5.8</td>
<td>167.4 ± 7.3</td>
<td>168.6 ± 6.2</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>70.1 ± 10.5</td>
<td>66.7 ± 9.8</td>
<td>69.0 ± 9.8</td>
</tr>
<tr>
<td>BMI (kg/m², range)</td>
<td>24.4 (17.5–33.3)</td>
<td>23.8 (18.0–31.4)</td>
<td>24.3 (3.0)</td>
</tr>
<tr>
<td>Smoking ≥ 10 cigarettes/day</td>
<td>25 (15.0)</td>
<td>17 (13.8)</td>
<td>6 (13.3)</td>
</tr>
<tr>
<td>Paternal characteristics</td>
<td>35.3 ± 5.7</td>
<td>35.7 ± 4.9</td>
<td>35.9 ± 6.9</td>
</tr>
<tr>
<td>Age (years)</td>
<td>182.1 ± 7.8</td>
<td>180.8 ± 7.4</td>
<td>182.3 ± 7.6</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>86.4 ± 13.9</td>
<td>83.0 ± 11.2</td>
<td>88.3 ± 14.7</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>26.0 (17.9–46.0)</td>
<td>25.3 (19.9–34.3)</td>
<td>26.6 (4.0)</td>
</tr>
<tr>
<td>BMI (kg/m², range)</td>
<td>29 (17.4)</td>
<td>25 (20.3)</td>
<td>5 (11.1)</td>
</tr>
<tr>
<td>Smoking ≥ 10 cigarettes/day</td>
<td>17 (10.2)</td>
<td>15 (12.2)</td>
<td>8 (17.8)</td>
</tr>
<tr>
<td>Tubal factor</td>
<td>107 (64.1)</td>
<td>79 (64.2)</td>
<td>29 (64.4)</td>
</tr>
<tr>
<td>Other</td>
<td>36 (21.6)</td>
<td>25 (20.3)</td>
<td>8 (17.8)</td>
</tr>
</tbody>
</table>

Data are presented as numbers (%) or mean ± SD.

Table II Difference in GA at 8 and 12 weeks’ gestation.

<table>
<thead>
<tr>
<th></th>
<th>8 weeks’ gestation</th>
<th>12 weeks’ gestation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitrolife group (n = 168)</td>
<td>−0.42 (n = 167)</td>
<td>1.80 (n = 45)</td>
</tr>
<tr>
<td>Cook group (n = 126)</td>
<td>−0.50 (n = 123)</td>
<td>1.38 (n = 38)</td>
</tr>
<tr>
<td>Unadjusted mean difference</td>
<td>−0.08</td>
<td>−0.42</td>
</tr>
<tr>
<td>P-value</td>
<td>0.68</td>
<td>0.58</td>
</tr>
<tr>
<td>Adjusted mean difference</td>
<td>−0.04</td>
<td>−0.59</td>
</tr>
<tr>
<td>P-value</td>
<td>0.84</td>
<td>0.22</td>
</tr>
</tbody>
</table>

Data are presented as days. Difference between the crown-rump length based GA minus actual (ovum retrieval based) GA was expressed as difference in days of gestation (ΔGA). A negative value indicates an underestimation of the GA, while a positive value indicates an overestimation of the GA. Unadjusted mean differences are calculated using the Student’s t-test and adjusted mean differences using linear multivariable regression analysis.
Measurement of fetal CRL using a high-frequency transvaginal ultrasound for early pregnancy dating is accurate (Verburg et al., 2008; Papaioannou et al., 2010). In our study, at 12 weeks’ gestation, no significant difference in ΔGA, PAPP-A and NT was found between the two study groups. Bukowski et al. (2007) found that ART pregnancies with appropriate fetal growth (ΔGA = 0) in the first trimester had lower birthweight and a higher risk of small-for-gestational age infants than the general population (Bukowski et al., 2007). Also Conway et al. (2011) did not find an appreciable difference in first-trimester fetal growth as measured by CRL between spontaneously conceived and ART pregnancies. The findings from both studies suggest that the generally reported impaired fetal growth in ART pregnancies (Helmerhorst et al., 2010) did not find an appreciable difference in first-trimester fetal growth as measured by CRL between spontaneously conceived and ART pregnancies. The variance in AC depends mainly on the abdominal fat tissue and furthermore on the volume of the liver, stomach or spleen (Kehl et al., 1996). Fat deposition begins from ~24 gestational weeks (Cunningham et al., 2010). At 20 weeks’ gestation, the amount of abdominal fat tissue is still low which could explain why we did not find a difference in AC at this stage in fetal development.

There is no preferred formula to estimate fetal weight and sonographic weight formulas generally show poor rates of accuracy (Dudley 2005). Nonetheless, fetal weight estimation is important for obstetric care management. Most formulas are derived from non-linear regression analysis using single or combined ultrasound measurements, whereas others are based on volumetric methods. Mostly used are the formulas of Hadlock (Hadlock et al., 1985; Hoopmann et al., 2010). We used the formulas of Hadlock I, III and IV, and showed no significant difference in EFW between groups. This was explainable as AC and FL, which were similar between groups, have a higher contribution in the EFW regression model of Hadlock than HC and/or BPD.

Our study has limitations with respect to the allocation procedure, being a strictly alternate-case, quasi-random one. However, as allocation was performed with two-sided allocation concealment and blinding, this alternate allocation to one of two media closely approaches optimal randomisation. Furthermore, all sonographers were unaware of the randomization procedure, because ultrasound measurements were collected retrospectively for this study. Although all sonographers were experienced and specially trained to perform these ultrasound examinations, we cannot totally rule out a possible intra- and inter-observer variability. Owing to the fact that a first-trimester (12 weeks) fetal screening was not yet offered routinely during the study period, only 28% of women in our study participated in this elective screening programme.

More and more studies are now investigating adverse influences in the preimplantation embryonic stage upon fetal growth and development. It appears that peri-conceptional undernutrition in sheep can alter fetal growth trajectories (Rumball et al., 2009). In rats, a maternal low-protein diet only during the preimplantation period resulted in lower cell numbers in the inner cell mass and trophoblast, reduced birthweight and hypertension in the offspring (Kwong et al., 2000) and altered expression of growth-regulating imprinted genes (Kwong et al., 2006). A maternal low-protein diet fed exclusively during mouse preimplantation development had no appreciable effect on fetal growth but was...
Table V Difference in sonographic parameters and estimated fetal weight (EFW) at 20 weeks’ gestation.

<table>
<thead>
<tr>
<th></th>
<th>Vitrolife group (n = 115)</th>
<th>Cook group (n = 91)</th>
<th>Unadjusted mean difference</th>
<th>P-value</th>
<th>Adjusted mean difference</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPD</td>
<td>50.2 (0.3)</td>
<td>49.8 (0.3)</td>
<td>0.4</td>
<td>0.27</td>
<td>0.5</td>
<td>0.07</td>
</tr>
<tr>
<td>AC</td>
<td>152.1 (0.9)</td>
<td>151.2 (1.0)</td>
<td>0.9</td>
<td>0.51</td>
<td>0.8</td>
<td>0.42</td>
</tr>
<tr>
<td>HC</td>
<td>177.3 (0.8)</td>
<td>175.9 (0.9)</td>
<td>1.4</td>
<td>0.24</td>
<td>1.8</td>
<td>0.03</td>
</tr>
<tr>
<td>FL</td>
<td>32.7 (0.2)</td>
<td>32.8 (0.3)</td>
<td>−0.1</td>
<td>0.80</td>
<td>−0.1</td>
<td>0.83</td>
</tr>
<tr>
<td>TCD</td>
<td>20.5 (0.1)</td>
<td>20.2 (0.1)</td>
<td>0.3</td>
<td>0.11</td>
<td>0.4</td>
<td>0.008</td>
</tr>
<tr>
<td>EFW (Hadlock I); BPD, HC, AC, FL</td>
<td>350.1 (4.1)</td>
<td>347.9 (4.9)</td>
<td>2.27</td>
<td>0.72</td>
<td>2.62</td>
<td>0.53</td>
</tr>
<tr>
<td>EFW (Hadlock III); BPD, AC, FL</td>
<td>357.3 (4.3)</td>
<td>354.8 (5.0)</td>
<td>2.53</td>
<td>0.70</td>
<td>2.83</td>
<td>0.51</td>
</tr>
<tr>
<td>EFW (Hadlock IV); HC, AC, FL</td>
<td>346.0 (4.1)</td>
<td>343.6 (4.8)</td>
<td>2.44</td>
<td>0.70</td>
<td>2.82</td>
<td>0.49</td>
</tr>
</tbody>
</table>

Data are presented as mean values (mm or gram) (± SEM). HC, head circumference; AC, abdominal circumference; FL, femur length; TCD, trans-cerebellar diameter; BPD, biparietal diameter. Unadjusted mean differences are calculated using the Student’s t-test and adjusted mean differences using linear multivariable regression analysis.

associated with excess post-natal growth and sustained hypertension (Watkins et al., 2008).

Also effects on fetal development related to in vitro culture were found in several animal models (Farin et al., 2006; Delle Piane et al., 2010). However, not only fetal development but also placental development is affected. In several animal models, ART has been shown to lead to a diverse set of placental abnormalities, such as an impaired placental steroid metabolism in mice (Collier et al., 2009), alterations in placental morphology and blood vessels in cattle (Farin et al., 2006) and a larger placental/fetal weight ratio in mice (Delle Piane et al., 2010). In human, an increased risk of placental abnormalities, such as placenta praevia, has been shown (Kallen et al., 2005; Romundstad et al., 2006). One can postulate that these placental abnormalities may partly explain the obstetric and neonatal complications associated with ART.

In our present series of IVF singletons, β-hCG (MoM) measured at around 12 weeks’ gestation was found to be significantly higher in the Vitrolife than in the Cook group. hCG is a glycoprotein produced by the syncytiotrophoblast cells (Kovalevskaya et al., 2002). Levels of hCG increase rapidly in early pregnancy until peak levels are reached at 7–9 weeks, followed by a progressive decline until around 20 weeks and then remain comparatively low and stable until term (Kletzky et al., 1985). hCG plays a major role in early human development, especially in the first trimester but also during the remainder of pregnancy. For instance hCG promotes progesterone production, angiogenesis in uterine vasculature, growth of the uterus, differentiation of growing cytотrophoblast cells, quiescence of myometrial contractions and has a function in growth and development of fetal organs (Keay et al., 2004; Ticconi et al., 2007; Cole, 2010). Furthermore, low first-trimester β-hCG levels are associated with fetal growth restriction, gestational hypertension and gestational diabetes (Ong et al., 2000), and with SGA (Dugoff et al., 2004; Krantz et al., 2004; Kirkegaard et al., 2011; Poon et al., 2011). The effect of ART on first-trimester β-hCG levels has been extensively studied indicating increased, decreased or unaltered levels (for review see Gjerris et al., 2012). Our increased β-hCG (MoM) level in the Vitrolife group is consistent with an increased fetal growth seen in the Vitrolife group. Oraşanu et al. (2006) investigated the effect of four different IVF culture media on serum hCG concentrations measured on Day 15 after embryo transfer in singleton viable pregnancies. Vitrolife G1.3 medium was associated with higher serum hCG levels on Day 15 after embryo transfer compared with the other media. However, the average of 3.5 embryos transferred could have led to undetected early implantation sites (Orașanu et al., 2006).

Since the level of β-hCG in early pregnancy is assumed to represent the mass of syncytiotrophoblast (Almog et al., 2011), it is possible that the number of trophoblast cells is increased after culture in Vitrolife medium compared with Cook medium. Effects of IVF on the number of trophoblast cells in humans have been suggested previously (Turan et al., 2010). Also, in mice and rat models, embryo culture or a maternal low-protein diet around conception leads to altered cell numbers in the trophoblast and inner cell mass (Kwong et al., 2000; Watkins et al., 2007). Changes in epigenetic regulation might explain the altered cell numbers as well as differences in hCG level and/or fetal growth as this plays an important role in placental development and function, and can be disturbed by ART (Nelissen et al., 2011; van Montfoort et al., 2012). From animal studies it is known that culture medium can affect the epigenetic regulation of imprinted genes, mainly in placental tissue, and to a lesser extent in the embryo (Mann et al., 2004; Rivera et al., 2008; Fauque et al., 2010). A possible explanation for our finding might be that Vitrolife G1.3 medium modulates the epigenetic regulation of the placenta leading to increased β-hCG levels. However, ours is a relatively small study and our observations need to be investigated in larger studies before firm clinical conclusions can be drawn.

Conclusion and future perspective

According to the fetal origins hypothesis, many adult diseases originate in utero owing to adaptations made by the fetus to the environment it encounters. Our study shows that IVF culture medium has the ability to influence fetal growth rate. This emphasizes the need for studies investigating fetal growth patterns after ART and the long-term health outcomes of these IVF children.
Supplementary data
Supplementary data are available at http://molehr.oxfordjournals.org/.

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

Funding
Part of this research was funded by an unrestricted research grant by Organon BV (now MSD BV). They had no role in the design, analysis, interpretation or reporting of findings.

Conflict of interest
None declared.

References
Krantz D, Goetzl E, Simpson JL, Thom E, Hallahan TW, Silver R, Pergament A, Platt LD, Filkins K et al. Association of extreme...


