Birthweight and placental weight; do changes in culture media used for IVF matter? Comparisons with spontaneous pregnancies in the corresponding time periods

Anne Eskild1,2,3, Lars Monkerud4, and Tom Tanbo5,*

1Department of Gynecology and Obstetrics, Akershus University Hospital, University of Oslo, Lørenskog 1478, Norway 2Institute of Clinical Medicine, Akershus University Hospital, University of Oslo, Lørenskog 1478, Norway 3Division of Mental Health, Norwegian Institute of Public Health, Oslo 0304, Norway 4Department of Economics, BI Norwegian Business School, Oslo 0484, Norway 5Section for Reproductive Medicine, Department of Gynecology, Oslo University Hospital, Rikshospitalet and Institute of Clinical Medicine, University of Oslo, Oslo 0424, Norway

*Correspondence address. Department of Gynaecology, Oslo University Hospital, 0424 Oslo, Norway. E-mail: tom.tanbo@ous-hf.no

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STUDY QUESTION: Have changes in culture media used for IVF resulted in changes in offspring birthweight or placental weight that differed from the trends in offspring from spontaneous conceptions during the corresponding time periods?

SUMMARY ANSWER: Changes in culture media used for IVF were associated with significant differences in offspring birthweight and in placental weight to birthweight ratio when compared with the trend in offspring from spontaneous conceptions during the time periods.

WHAT IS KNOWN ALREADY: The effect of culture media used for IVF on offspring birthweight has varied between studies. There is a large variation in birthweight between newborns, and birthweight may vary across populations and over time. Such variations may therefore have influenced previous results.

STUDY DESIGN, SIZE, DURATION: We included all singleton births from IVF at one treatment center in Norway during the years 1999–2011 (n = 2435) and all singleton births from spontaneous conceptions in Norway during the same years (n = 698,359).

PARTICIPANTS/MATERIALS, SETTING, METHODS: Three different media were used for embryo culture; Medicult Universal IVF (1999 through 2007, n = 1584), Medicult ISM1 (2008 until 20 September 2009, n = 402) and Vitrolife G-1 PLUS (21 September 2009 through 2011, n = 449). We estimated mean birthweight and placental weight in IVF pregnancies by culture media. We also estimated mean weights in IVF and in spontaneous pregnancies by year of birth. Thereafter, we studied whether the changes in mean weights in IVF pregnancies differed from the changes in weight in spontaneous pregnancies in the periods corresponding to culture media changes by applying a grouped difference-in-difference analysis. Adjustments were made for parity, maternal age and gestational age at birth.

MAIN RESULTS AND THE ROLE OF CHANCE: In singleton offspring from IVF the mean birthweight was 3447.6 g with Medicult Universal, 3351.7 g with Medicult ISM1 and 3441.4 g with Vitrolife G-1 PLUS (P < 0.05). The corresponding mean placental weights were 684.1, 693.4 and 704.3 g (P < 0.05). In offspring from spontaneous conceptions the mean birthweight decreased (56.9 g) and the placental weight increased (9.3 g) during the study period. The adjusted difference in birthweight in offspring from IVF decreased with 35.0 g by the change from Medicult Universal to Medicult ISM1 (P = 0.16) and increased with 79.9 g by the change from Medicult ISM1 to Vitrolife G-1 PLUS (P = 0.01) when compared with changes in offspring after spontaneous conceptions. We also found a significant increase in placental weight in relation to birthweight by the change from Medicult ISM1 to Vitrolife G-1 PLUS (P = 0.02).

LIMITATIONS, REASONS FOR CAUTION: There may be underlying factors that have influenced both birthweight and the use of culture media in IVF pregnancies. Lack of adjustment for such possible factors may have biased our results.

WIDER IMPLICATIONS OF THE FINDINGS: We found a significant effect of culture media used for IVF on birthweight and on placental weight in relation to birthweight. Also the population changes over time should encourage identification of factors in very early embryonic life that may influence birthweight and placental weight.
Introduction

Pregnancies from IVF are at increased risk of adverse outcomes, and offsprings from IVF are usually smaller than children born from natural conception (Helmerhorst et al., 2004). Pregnancies from IVF have, however, enlarged placentas and high placental weight in relation to birthweight (Haavaldsen et al., 2012). The differences in growth may be related to the techniques used for fertilization, but also to factors associated with infertility (Ghazi et al., 1991; Romundstad et al., 2008). Conversely, in cattle enlargement of offspring from IVF is well known and has been related to epigenetic modifications during embryo culture (Sinclair et al., 2000). Furthermore, epigenetic changes that cause syndromes in humans with growth disturbances, such as Beckwith-Wideman and Silver Russell syndrome, are seen more often in offspring from IVF (Allen and Reardon, 2005). Thus, speculations that IVF may induce epigenetic changes in the embryo have caused concern (Niemitz and Feinberg, 2004).

Recent studies suggest that the culture medium used for fertilization and growth of the embryo during IVF may influence fetal growth and thereby birthweight (Dumoulin et al., 2010; Nelissen et al., 2012). Up to 250 g difference in mean birthweight according to the culture medium used has been reported (Dumoulin et al., 2010). The findings are, however, inconsistent and others have reported no effect of culture medium on birthweight (Eaton et al., 2012; Vergouw et al., 2012; Lin et al., 2013).

Placental function is a determinant for fetal growth, and a large placenta relative to birthweight has been associated with IVF pregnancies and with complications in pregnancy (Eskild et al., 2009; Haavaldsen et al., 2012; Haavaldsen et al., 2013). To our knowledge, possible differences in placental weight by culture media used for IVF have not been studied.

Studies on the association of the different culture media used in IVF and birthweight have been of observational nature and with study periods ranging from 3–10 years. In the general population of newborns there is a large variation in birthweight, and there may be differences in birthweight between groups and over time (Weightman et al., 2012; Morisaki et al., 2013). Thus, an estimated effect of culture media on birthweight may have been biased by differences in the selection of women to IVF treatment according to the different culture media used.

At a large university hospital in Norway three different culture systems were used for fertilization and culture media were used for IVF or ICSI during the years 1999–2011. In all singleton births from IVF/ICSI, we compared mean birthweight, placental weight and placental to birthweight ratio according to the culture medium used. Thereafter, we compared these outcomes of IVF/ICSI pregnancies with the outcomes of all singleton pregnancies from spontaneous conception in Norway in the time periods corresponding to use of the different culture media. In the data analysis we made adjustments for possible differences between the pregnancy groups in the distribution of maternal age, number of previous births and gestational age at birth.

Methods

Populations and data collection

We performed a population-based observational study. The study population included all singleton births from IVF, with and without ICSI for fertilization of the oocytes, during the years 1999 through 2011 at the Section for Reproductive Medicine, Oslo University Hospital Rikshospitalet in Norway. Rikshospitalet is a public hospital, and ~60% of all births from IVF in Health Region South East in Norway were conceived at this hospital during the study period. Health Region South East offers health care to ~60% of the total population of Norway. We excluded pregnancies from transfer of frozen–thawed embryos since the delay from fertilization and embryo culture to transfer may cause alterations in fetal growth that is difficult to adjust for in data analyses.

For comparisons, we used all singleton births from spontaneous conception in Norway during the same years as the births from IVF. Pregnancies from IVF conceived at other treatment centers than Oslo University Hospital, Rikshospitalet were excluded since we had no information on the culture media used for these pregnancies.

We obtained information on pregnancy characteristics for all pregnancies from the Medical Birth Registry of Norway to which all births after 16 weeks of gestation are reported shortly after the delivery (Irgens, 2000). Information on the type of culture media used in IVF pregnancies was obtained from the electronic patient records at the Oslo University Hospital Rikshospitalet, and we linked this patient registry with the Medical Birth Registry of Norway by using the women’s unique person identification number.

We included singleton births after 22 weeks of gestation, and a total of 2495 births from IVF and 724 540 births from spontaneous conception were eligible. We excluded births with missing information on placental weight, birthweight or maternal age. Births with placental weight <25 g or >2500 g or birthweight <150 g or >6500 g were considered as having outlying values and were therefore excluded. Thus, we could include 2435 births from IVF and 698 359 births from spontaneous conception.

Study factors

Information on all study factors, except the culture medium used in IVF, was obtained from the Medical Birth Registry of Norway. Our outcome measures were placental weight, birthweight and the placental to birthweight ratio. Placental weight was measured in grams by the midwife, and according to routines in Norway the placentas were weighed fresh with membranes and umbilical cord attached within 1 h after delivery. Birthweight was based on routine weighing of the newborn shortly after delivery. By using both placental weight and birthweight in grams, we calculated placental to birthweight ratio. Hence, pregnancies with a high ratio have a large placenta relative to offspring size.

Three different culture systems were used for fertilization and culture during our study period. All media were developed for culture of human embryo in vitro to the 8-cell stage. Medicul Universal IVF Medium (MediCult AS Company, Jyllinge, Denmark) was used for monoculture during 1999 through 31 December 2007. From 1 January 2008 until 20 September 2009 a change was made to sequential culturing using Medicul Universal for fertilization and Medicul ISM1 (MediCult AS Company) for embryo culture until transfer. In the last part of our study period, from 21 September 2009
through 2011 another sequential system was implemented using Vitrolife G-IVF PLUS for fertilization and Vitrolife G-I PLUS (Vitrolife AB, Gothenburg, Sweden) for embryo culture. The changes in culture systems were based on publications indicating improvement in results with the latter system compared with the culture system already in use (Sifer et al., 2009; Xella et al., 2010). The components of the various media used for embryo culture is shown in Supplementary data, Table. Most embryos were cultured for 1 day or occasionally for 2 days after fertilization until transfer to the uterus. Our procedures for IVF have previously been reported (Opaien et al., 2012), and apart from changes in embryo culture media no other major changes were undertaken in the laboratory during our study period. In particular, the oxygen concentration in the incubators was kept constant at 5%.

The following other study factors were used; maternal age at delivery (years), parity (number of previous births after 16 weeks of gestation, coded 0 or ≥ 1) and gestational age at birth (days). In the Medical Birth Registry, gestational age at birth was based on the estimation of term at routine fetal ultrasound examination in gestational weeks 17–19 for both IVF/ICSI and spontaneous pregnancies. This examination is a part of the public antenatal program in Norway. For 2.7% of the pregnancies, estimation of term date was based on the first day of the last menstrual period, since estimations from the fetal ultrasound examination were not available. For 61 of the spontaneous pregnancies, information on maternal age was not available, and these pregnancies could not be included in the multivariable analyses.

Statistical analyses

For births from IVF we calculated mean placental weight, mean birthweight, mean placental to birthweight ratio and mean gestational age at birth according to the culture medium used. We also estimated mean weights in IVF and in spontaneous pregnancies by year of birth. Differences in means between groups were tested by applying the ANOVA test with Bonferroni correction.

To investigate whether fetal growth patterns associated with changes in culture media differed from the general trend during the time periods corresponding to the changes in culture media, we employed grouped difference-in-difference analysis (Wooldridge, 2009). Grouped difference-in-difference analysis is designed to estimate whether a trend in one population differs from a trend in another population, taking into account that the baseline levels between the populations may differ. For instance, baseline birthweight may differ between IVF pregnancies and pregnancies from spontaneous conception. We estimated the following model: \( Y = \beta_0 + \beta_1 \text{IVF} + \beta_2 \text{MV} + \beta_3 \text{VL} + \beta_4 \text{IVF MV} + \beta_5 \text{IVF VL} + u \), where \( Y \) is a growth measure of the individual conceptus (either birthweight, placental weight or placental to birthweight ratio); \( \text{IVF} = 1 \) if the pregnancy is the result of IVF and \( \text{IVF} = 0 \) if the pregnancy is a result of spontaneous conception. \( \text{VL} = 1 \) if the birth is the result of culture by Vitrolife G-I PLUS medium, or the birth was in 2010 or 2011 from spontaneous conception (corresponding to the year of IVF births from Vitrolife G-I PLUS culture). \( \text{VL} = 0 \) if otherwise. \( \text{MV} = 1 \) if the birth is a result of culture by Medicult Universal medium, or the birth was in 1999–2008 from spontaneous conception (corresponding to the years of IVF births from Medicult Universal culture). \( \text{MV} = 0 \) if otherwise. IVF-VL and IVF-MV represent the combination of treatment status (IVF) and treatment period (VL and MV), and \( u \) represents random error. The reference period was the period of births from Medicult ISM1 culture or births in 2009 from spontaneous pregnancy (corresponding to the year of births from Medicult ISM1 culture).

The following parameters are of particular interest: \( \beta_4 \), where \( \beta_4 \) gives the excess increase in weight associated with media change from Medicult Universal to Medicult ISM1 for IVF-treated patients when compared with births from spontaneous pregnancies in the same period. The estimate for \( \beta_5 \) gives the excess increase in weight associated with the change from Medicult ISM1 to Vitrolife G-I PLUS period for IVF pregnancies when compared with births from spontaneous pregnancies in the same period. In this way, we estimate whether the trend in growth by changes in culture media for IVF pregnancies differs from the growth trend in spontaneous pregnancies during the same period.

Births from treatment with Medicult Universal occurred in the years 1999–2008, while births from treatment with Medicult ISM1 occurred in 2008 (14.4%, 58/402), 2009 (62.9%, 253/402) and in 2010 (22.6%, 91/402). Births from treatment with Vitrolife G-I PLUS occurred in 2010 (42.1%, 189/449) and in 2011 (57.9%, 260/449). For data security reasons, as determined by the Medical Birth Registry, we obtained information about the year, but not month of delivery. Therefore, for the births in 2008 and 2010 we could not exactly match the month of birth from spontaneous conceptions according to months of births from use of the different culture media. Most of the births from the use of Medicult ISM1 occurred in 2009, we therefore chose to compare all births from Medicult ISM1 with births from spontaneous conception in 2009 (as mentioned in the equation above). A different approach would be to exclude all birth from 2008 and 2010 (both from IVF and spontaneous conception), thus assuring no year-to-year overlap of IVF with births from spontaneous conception, but also reducing the statistical power. We performed data analyses with both approaches. Adjustments were made for differences in maternal age at delivery (years), number of previous deliveries (0 or ≥ 1) and gestational age at birth (days) between spontaneous pregnancies and between IVF births according to the different culture media.

Ethical considerations

The study was approved by the Advisory Board of the Medical Birth Registry of Norway, the Data Protection Officer at Oslo University Hospital and the Regional Committee of Ethics in Medical Research.

Results

In singleton offspring from IVF, the mean birthweights were 3447.6 g from culture with Medicult Universal (\( n = 1584 \)), 3351.7 g from Medicult ISM1 (\( n = 402 \)) and 3441.4 g from Vitrolife G-I PLUS (\( n = 449 \); \( P = 0.020 \)). The corresponding mean placental weights were 684.1, 693.4 and 704.3 g (\( P = 0.038 \)) (Table I). Also, the mean placental to birthweight ratio differed across culture media (\( P = 0.001 \)) (Table I).

In singleton births from spontaneous conception during the years 1999 through 2011, there was a significant decrease in mean birthweight (56.9 g), an increase in placental weight (9.3 g) and thus an increase in placental to birthweight ratio (Table II). The trend for births after IVF during the same years showed a significant increase in mean placental weight, but no significant trend in mean birthweight. Overall, the mean placental weight was higher and birthweight was lower in IVF when compared with births from spontaneous conception. The mean length of gestation at birth was 39.2 weeks in IVF pregnancies, and it was 39.4 weeks in spontaneous pregnancies (Table II).

The decrease in crude birthweight in IVF pregnancies following the change from Medicult Universal to Medicult ISM1 was 61.9 g more than the decrease in birthweight from spontaneous conceptions during the same period (\( P = 0.06 \)). After adjustment for differences in maternal age, parity and gestational age at birth between IVF births and births from spontaneous conception, the difference in birthweight decrease was attenuated to 35.0 g (\( P = 0.16 \)) (Table III, Fig. 1). In IVF pregnancies, 71% were nullipara, and mean maternal age at delivery was 33.3 years. In spontaneous pregnancies, 40% were nullipara, and the mean maternal age was 29.4 years.
After the change from Medicult ISM1 to Vitrolife G-1 PLUS, the birthweight in IVF pregnancies decreased 93.0 g less than births from spontaneous conceptions during the same period (P < 0.05). After adjustment for the above study factors, the estimated difference in birthweight decrease was attenuated to 79.9 g (P < 0.05) (Table III, Fig. 1).

We found no significant difference in trends for placental weight in births from IVF when compared with births from spontaneous conceptions (Table III, Fig. 2). However, the increase in placenta to birthweight ratio in IVF births by the change from Medicult Universal to Medicult ISM1 was significantly higher than in births from spontaneous conception during the same period (P = 0.01) (Table III, Fig. 3). After the change from Medicult ISM1 to Vitrolife G-1 PLUS period there was no significant difference in trends in placenta to birthweight ratio (Table III, Fig. 3). The alternative approach with exclusion of all births in 2008 and 2010 to assure no year-to-year overlap of births from IVF and spontaneous conception showed essentially the same results (data not shown).

We also made additional analyses by restricting the sample for comparison to births from spontaneous conceptions in the Health Region South East only (n = 352,032 births), the catchment region for IVF treatment at our hospital, and we found very similar results (data not shown).

Finally, we made analyses (linear regression) including the IVF pregnancies only to explore if there could be additional factors within these pregnancies that had biased the association of culture media with offspring growth. We included in the analyses offspring year of birth as a proximate measure for any possible changes during the study period in the selection of patients or in laboratory routines. For 2393 of the IVF pregnancies we also had information on the number of embryos transferred and maternal pre-pregnancy body mass index. After adjustment for these factors, in addition to maternal age, parity and gestational age at birth, the results supported our main findings. The birthweight was 73.0 g (35.6 g standard deviation) less in pregnancies after culture in Medicult ISM1 when compared with Vitrolife G-1 PLUS. We estimated no significant associations of culture media with placental weight or with placenta to birthweight ratio.

**Discussion**

In singleton births from IVF, we found significant differences in birthweight, placental weight and placental weight to birthweight ratio according to culture media used for IVF. During our study period, 1999–2011, there were changes also in births from spontaneous conception in Norway; the mean birthweight decreased and the mean placental weight increased. By comparing trends between conceptions from IVF and conceptions from spontaneous conception, we found that in births from IVF the birthweight was lower and the placenta to birthweight ratio was higher when Medicult ISM1 was used when compared with periods when other culture media were used.

Our IVF patients were recruited from Rikshospitalet, Oslo University Hospital and represent ~60% of all births after IVF in the South-Eastern Health Region of Norway and ~30% of all births after IVF in Norway. We could not include all births after IVF since we had information on culture media from Rikshospitalet only. We repeated our analyses by using spontaneous pregnancies from the South-Eastern Health Region of Norway only as controls, and we found the same results. This finding suggests that different trends in offspring weight in Norway as a whole, when compared with our catchment area, are unlikely to have biased our results.

We included all singleton pregnancies in Norway, and by applying difference-in-difference data analyses, we made adjustments for underlying population trends in birthweight and placental weight. Additionally, we made adjustment for differences in maternal age, number of previous births and gestational age between birth after IVF and spontaneous conception. Such adjustment attenuated the associations, but the lower birthweight and the ratio of birthweight to placental weight associated with Medicult ISM1 when compared with Vitrolife G-1 PLUS and Medicult Universal, respectively, remained. However, insufficient control for factors that are linked to population trends, the use of culture media and offspring weight may still remain. Birthweight is positively associated with maternal body mass index (Stamnes Koepp et al., 2012). There was no difference in mean maternal body mass index by culture medium in women with IVF pregnancies (data not shown). We had no data on maternal body mass index for the women with spontaneous pregnancies. Therefore, we cannot rule out that differential changes over time in body mass index in mothers with spontaneous births when compared with IVF mothers have biased our estimate.

In IVF pregnancies there may have been changes over time that has influenced weight other than changes in culture media. Such factors may be changes in duration of the infertility and the number over embryos transferred (Pinborg et al., 2005; Grady et al., 2012). Birthweight has been reported higher in singleton IVF pregnancies from transfer of one when compared with two embryos (De Sutter et al., 2006). During our study period there was a gradual decrease in transfer of two embryos or more. In our data, however, we did not estimate any

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**Table I** Mean placental weight, birthweight, placenta to birthweight ratio, gestational age at birth with standard deviations (SD) and time of birth according to the different culture media used in a total of 2435 singleton births after IVF at Rikshospitalet, Norway.

<table>
<thead>
<tr>
<th>Culture media</th>
<th>n</th>
<th>Placental weight in grams (SD)</th>
<th>Birthweight in grams (SD)</th>
<th>Placenta to birthweight ratio (SD)</th>
<th>Gestational age (SD)</th>
<th>Time of birth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medicult Universal</td>
<td>1584</td>
<td>684.1 (151.8)</td>
<td>3447.6 (610.9)</td>
<td>0.2016 (0.04714)</td>
<td>39.27 (2.238)</td>
<td>January 1999–September 2008</td>
</tr>
<tr>
<td>Medicult ISM1</td>
<td>402</td>
<td>693.4 (146.0)</td>
<td>3351.7 (631.4)</td>
<td>0.2097 (0.03742)</td>
<td>39.13 (2.380)</td>
<td>August 2008–May 2010</td>
</tr>
<tr>
<td>Vitrolife G-1 PLUS</td>
<td>449</td>
<td>704.3 (154.9)</td>
<td>3444.1 (637.2)</td>
<td>0.2096 (0.04879)</td>
<td>39.20 (2.273)</td>
<td>May 2010–December 2011</td>
</tr>
<tr>
<td>Total</td>
<td>2435</td>
<td>689.3 (151.6)</td>
<td>3430.6 (620.0)</td>
<td>0.2044 (0.04879)</td>
<td>39.24 (2.273)</td>
<td>January 1999–December 2011</td>
</tr>
</tbody>
</table>

ANOVA 

<table>
<thead>
<tr>
<th></th>
<th>P</th>
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</thead>
<tbody>
<tr>
<td>Placental weight</td>
<td>0.038</td>
</tr>
<tr>
<td>Birthweight</td>
<td>0.020</td>
</tr>
<tr>
<td>Placenta to birthweight</td>
<td>0.001</td>
</tr>
<tr>
<td>Gestational age</td>
<td>0.697</td>
</tr>
</tbody>
</table>
Table II  Placental weight (grams), birthweight (grams), placenta to birthweight ratio (ratio) and gestational age in all singleton births after spontaneous conception in Norway ($n = 698\,359$) and in births after IVF at Rikshospitalet, Norway ($n = 2435$), during the years 1999–2011.

<table>
<thead>
<tr>
<th>Births after spontaneous conception</th>
<th>Births after IVF</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Year</strong></td>
<td><strong>n</strong></td>
</tr>
<tr>
<td>1999</td>
<td>52613</td>
</tr>
<tr>
<td>2000</td>
<td>53780</td>
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<tr>
<td>2001</td>
<td>51797</td>
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<tr>
<td>2002</td>
<td>50842</td>
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<tr>
<td>2003</td>
<td>52400</td>
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<tr>
<td>2004</td>
<td>52647</td>
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<tr>
<td>2005</td>
<td>51971</td>
</tr>
<tr>
<td>2006</td>
<td>53441</td>
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<tr>
<td>2007</td>
<td>53510</td>
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<tr>
<td>2008</td>
<td>56151</td>
</tr>
<tr>
<td>2009</td>
<td>57472</td>
</tr>
<tr>
<td>2010</td>
<td>57260</td>
</tr>
<tr>
<td>2011</td>
<td>54475</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>698359</td>
</tr>
</tbody>
</table>

ANOVA: P = 0.001, P = 0.001, P = 0.001, P = 0.001, P = 0.06, P = 0.43, P = 0.002, P = 0.91
significant difference in birthweight in singleton pregnancies from transfer of one when compared with two embryos (data not shown). Also, during the study period there was a gradual increase in the use of IVF with ICSI. Birthweight and placental weight after IVF with or without ICSI, however, have been reported to be similar (Bonduelle et al., 2002; Haavaldsen et al., 2012). Likewise, type and dose of ovarian stimulation do not seem to affect birthweight (Griesinger et al., 2008; Sazonova et al., 2011). Whether the number of days of embryo culture is associated with birthweight or placental weight is not clarified. However, in a study comparing outcomes from transfer of cleavage stage embryo or blastocyst transfer, there were no differences in large or small for gestational age newborns (Fernando et al., 2012). In our data there was no systematic differences in the number of days in culture over time or according to culture media used (data not shown).

To explore whether any changes over time in the selection of IVF patients or treatment routines could have confounded our results, we made additional analyses within the sample of IVF pregnancies and included offspring birth year as approximate measure for changes over time. We also included number of embryos transferred and maternal pre-pregnancy body mass index. The lower birthweight in offspring after culture in Medicult ISM1 when compared with Vitrolife G-1 PLUS was estimated also in these additional analyses. However, randomized controlled trials are necessary to further improve our understanding of the relation between culture media and offspring growth.

In pregnancies from spontaneous conception we observed a trend of decreasing birthweight and increasing placental weight. The decline in birthweight was almost 60 g, and this finding is in accordance with a study from the USA during 1990–2005 (Donahue et al., 2010).

| Table III | Differences in IVF birth outcomes after the use of different culture media when compared with the trends in births after spontaneous conception (reference) in the corresponding time periods. |
|-----------|-------------------------------------------------|----------------|-----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
|           | Birthweight crude | Birthweight adjusted | Placental weight crude | Placental weight adjusted | Ratio crude | Ratio adjusted |
| Universal period to Medicult ISM1 period | -61.93 (32.66) | -35.04 (25.16) | 6.73 (8.52) | 9.99 (8.10) | 0.574 (0.217)** | 0.470 (0.203)* |
| Medicult ISM1 period to Vitrolife G-1 PLUS period | 92.95 (40.15)* | 79.85 (30.93)** | 8.98 (10.47) | 8.18 (9.96) | -0.039 (0.267) | -0.002 (0.250) |
| Gestational length | 185.23 (0.27)** | 177.31 (1.18)** | 22.85 (0.09)*** | 27.48 (0.38)*** | -0.687 (0.002)*** | -0.223 (0.010)*** |
| Maternal age | 0.08 (0.12) | 0.59 (0.04)*** | 0.09552 | 0.001247 | 0.122003 |
| Parity | 177.31 (1.18)** | 175.06*** | 175.06*** | 121.34 *** |
| n | 700 794 | 700 794 | 700 794 | 700 794 | 700 794 | 700 794 |
| R² | 0.000865 | 0.407002 | 0.000130 | 0.000130 | 0.019 (0.001)*** |
| F | 121.34 *** | 60117.5*** | 18.27*** | 925.06,99*** | 175.06*** | 12171.3*** |

Medicult ISM1 period was used as the reference period. Difference-in-difference estimates in birthweight (grams), placental weight (grams) and placental to birthweight ratio (×100).

*P < 0.05, **P < 0.01, ***P < 0.001.
are not aware of any studies of changes over time in placental weight or placental to birthweight ratio. We cannot explain the population changes, but may speculate that the large increase in immigrants from developing countries among the women who give birth in Norway (Naimy et al., 2013) may have contributed to the decrease in mean birthweight and a concomitant increase in mean placental weight. Changes in maternal body mass index over time could also be an explanation.

We are aware of five prior studies on the possible influence of culture media on birthweight. Two of these studies reported an influence of culture media, but these studies used data from study samples that were almost completely overlapping (Dumoulin et al., 2010; Nelissen et al., 2012). In these quasi-randomized studies, the embryos were allocated to be cultured in Cook K-SIFM (Cook, Brisbane, Australia) or Vitrolife G1TM Version 3 media (Vitrolife AB, Gothenburg, Sweden) every other week, and the observation period lasted 3.5 years. In the first study, comparing birthweights after successful pregnancy in the first IVF treatment cycle (Dumoulin et al., 2010), an increase of 250 g in birthweight was reported in births after culture with Vitrolife G1TM Version 3 when compared with Cook K-SIFM. In the second study from the same group, comparing successful singleton pregnancies after any treatment cycle, an increase of 112 g was observed with culture in Vitrolife G1TM Version 3 when compared with Cook K-SIFM (Nelissen et al., 2012). The three other studies on the effect of culture media on birthweight were purely observational. Other culture media than those above were compared, and no differences in birthweight according to culture media were observed (Eaton et al., 2012; Vergouw et al., 2012; Lin et al., 2013). To our knowledge, no prior study has investigated the changes in birthweight or placental weight according to culture media in comparison with changes in weight in spontaneous pregnancies.

The changes in birthweight and placenta to birthweight ratio by the change of culture media in our study may represent a true influence of culture media on offspring growth. If such influence truly exists, our findings suggest that a growth medium may cause epigenetic changes in the embryo. Although the exposure to growth media takes place in very early embryonic life and for a short period of time (1–5 days), our findings suggest that factors in the culture media may influence subsequent intrauterine growth. It has been reported that different culture media may result in differences in embryo scoring prior to embryo transfer (Sifer et al., 2009; Xella et al., 2010). It has also been demonstrated that changes in culture media may result in different gene expression and genomic imprinting. These findings support the notion that culture media may influence fetal and placental growth (Ho et al., 1995; Mann et al., 2004). Such findings support an effect of culture media on embryo quality, and it may be speculated that embryo score is linked to subsequent birthweight. Thus, it cannot be ruled out that culture media truly affect birthweight and placental to birthweight ratio in IVF pregnancies. Also, the lower birthweight and higher placental weight in births from IVF when compared with spontaneous conceptions (Haavaldsen et al., 2012) could therefore be explained by differences in early embryo exposure and not by factors associated with the infertile couple only (Romundstad et al., 2008).

Small changes in birthweight or placental weight as a possible function of culture media used for IVF may not be clinically relevant. Nevertheless, low birthweight has been associated with several adverse outcomes for the infant, such as increased perinatal mortality and increased morbidity and mortality later in life (Gennser et al., 1988; Mathews and MacDorman et al., 2011). Also, a high placental to birthweight ratio has been associated with adverse pregnancy outcomes such as pre-eclampsia (Eskild et al., 2009), preterm fetal death (Haavaldsen et al., 2013), excess post-partum bleeding (Eskild and Vatten, 2011) and admission of the newborn to neonatal intensive care unit (Shehata et al., 2011). Thus, our findings of changes in placental to birthweight ratio in births from IVF, but also in births from spontaneous conceptions, should encourage studies of the underlying causes of such changes.

Conclusion

In this population study of 2435 singleton births from IVF and 698 359 births from spontaneous conceptions in Norway during 1999–2011, we found significant changes in birthweight and placental weight according to culture media used for IVF. Also in births from spontaneous conceptions changes in birthweight and placental weight occurred during our study period, but the trend differed from births from IVF.

Supplementary data

Supplementary data are available at http://humrep.oxfordjournals.org/.

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Authors’ roles

A.E. contributed to designing the study, collection, analysis and interpretation of data, drafting the article and final approval. L.M. contributed to substantial contributions to design; analysis and interpretation of data, drafting the article and final approval of the version to be published. T.T. contributed to design, analysis and interpretation of data, drafting...
the article, revising it critically for important intellectual content and final approval of the version to be published.

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**Conflict of interest**

None of the authors has any conflicts of interest to declare.

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