The protein source in embryo culture media influences birthweight: a comparative study between G1 v5 and G1-PLUS v5

Jinliang Zhu1,2,3, Ming Li1,2,3, Lixue Chen1,2,3, Ping Liu1,2,3, and Jie Qiao1,2,3,*

1Department of Obstetrics and Gynecology, Reproductive Medical Center, Peking University Third Hospital, Beijing, China
2Key Laboratory of Assisted Reproduction, Ministry of Education, Beijing, China
3Beijing Key Laboratory of Reproductive Endocrinology and Assisted Reproduction, Beijing, China

*Correspondence address. Department of Obstetrics and Gynecology, Reproductive Medical Center, Peking University Third Hospital, No. 49 North Huayuan Road, Haidian District, Beijing 100191, China. Tel: +86-10-82265080; E-mail: jie.qiao@263.net

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STUDY QUESTION: Does protein source or human serum albumin (HSA) in embryo culture media influence the subsequent birthweight?

SUMMARY ANSWER: A significant difference was observed in gestational age- and gender-adjusted birthweight (Z scores) and the proportion of large-for-gestational age (LGA) babies between embryos cultured in G1 v5 and those cultured in G1-PLUS v5 media.

WHAT IS KNOWN ALREADY: It has been reported that the birthweights of singletons born from embryos cultured in Vitrolife are significantly higher than those cultured in the Cook group of media, and that G1-PLUS (Vitrolife, Gothenburg, Sweden) is associated with increased birth and placenta weights compared with Medicult ISMI.

STUDY DESIGN, SIZE, AND DURATION: This study was a retrospective analysis of neonatal birthweights, and included 1097 singletons born from fresh embryo transfer cycles at the Center for Reproductive Medicine of Peking University Third Hospital between January 2011 and August 2012. The number of singletons born from G1 v5 culture media was 489, and the number of singletons born from G1-PLUS v5 media was 608.

PARTICIPANTS/MATERIALS, SETTING, AND METHODS: Patients <40 years of age with a BMI <30 kg/m² were analysed. Only data from newborns from singleton pregnancies and born alive after the 28th week of gestation were included. Patients with a vanishing twin or with pregnancy-related complications, such as diabetes and hypertension, were excluded, as were patients who received preimplantation genetic diagnosis or used donor oocytes. Multiple linear regression analysis was performed to determine the influence of individual factors on birthweights of singleton newborns. The birthweights and Z scores of singletons and LGA babies were compared between the G1 v5 and G1-PLUS v5 media groups.

MAIN RESULTS AND THE ROLE OF CHANCE: The absolute birthweights for singletons resulting from G1-PLUS v5 were not different from singletons resulting from G1 v5 (3375.9 ± 479.6 g versus 3333.2 ± 491.6 g, respectively; P = 0.14). However, the Z scores for singletons from embryos cultured in G1-PLUS v5 were significantly higher than for singletons cultured in G1 v5 (0.28 ± 1.12 versus 0.09 ± 1.15, respectively; P = 0.04), and more LGA babies were born from G1-PLUS v5 culture compared with G1 v5 (16.8 versus 12.1%, respectively; P = 0.03) culture. Finally, multiple linear regression analysis suggested that female weight (P = 0.00), male height (P = 0.04), gestational age at birth (P = 0.00), infant gender (P = 0.00) and culture media (P = 0.04) all had significant effects on the birthweights of singleton newborns.

LIMITATIONS AND REASONS FOR CAUTION: This study was limited by its retrospective design.

WIDER IMPLICATIONS OF THESE FINDINGS: Our study suggests that protein source/HSA has a significant effect on birthweights of singleton newborns.

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**Introduction**

Systematic reviews and meta-analyses have indicated that singletons and twins born after assisted reproduction technology (ART) are at high risk of preterm birth and low birthweight (LBW) compared with spontaneously conceived newborns (Jackson et al., 2004; McDonald et al., 2009, 2010; Pandey et al., 2012; Pinborg et al., 2013). Patient-related factors are a major reason for adverse perinatal outcomes in ART singletons. However, even in the same mother, adverse outcomes are still observed more in ART siblings (Henningsen et al., 2011). Hence, in vitro fertilization (IVF) techniques appear to play an important role in the poor perinatal outcomes of such singletons. The trend towards LBW has been observed with ovarian stimulation as part of standard IVF compared with modified natural cycle IVF (Pelink et al., 2010). In contrast, singletons born after frozen embryo transfer have a decreased risk of preterm birth and LBW compared with fresh embryo transfer singletons, but when compared with spontaneous pregnancy, the occurrence of preterm birth, LBW and small-for-gestational age (SGA) after frozen embryo transfer is still observed (Wennerholm et al., 2009; Pelkonen et al., 2010; Henningsen et al., 2011). A systematic review and meta-analysis showed that an extended embryo culture period (5–6 days versus 3 days) is associated with an increased risk of preterm birth and LBW in singleton pregnancies (Maheshwari et al., 2013). In contrast, it has also been reported that prolonged in vitro culture causes more large-for-gestational age (LGA) babies when compared with in vitro culture of 2–3 days duration (Makin et al., 2013; Zhu et al., 2014). Thus, the length of in vitro culture may have a negative effect on birthweight and the duration of gestation.

Another important factor, widely-used commercial culture media, may have an influence on perinatal outcome, including gestational age and birthweight. In animal studies, it has been shown that a component of culture media is linked to aberrant fetal growth (Khosla et al., 2001; Rooke et al., 2007). In humans, controversy exists regarding the effect of culture media on neonatal birthweight. Dumoulin et al. (2010) reported that Vitrolife G1 version 3 media is significantly associated with increased birthweight compared with Cook K-SFM media, and this was confirmed by Nelissen et al. in 2012 using the same dataset. Other studies have not confirmed a relationship between culture media and neonatal birthweight (Eaton et al., 2012; Vergouw et al., 2012; Carrasco et al., 2013; Lin et al., 2013). However, it was recently reported that Vitrolife G1-PLUS is associated with increased birthweight and placenta weights compared with Medicult ISMI (Eskild et al., 2013).

Discrepancies in results could be partially explained by different types of commercial culture medium used in IVF laboratories. The effect of the protein source/HSA (human serum albumin) in the media has not been investigated before in humans. Therefore the purpose of the current study was to investigate the effect of protein source/HSA on neonatal birthweights and the incidence of LGA newborns following use of G1 v5 or G1-PLUS v5 media between January 2011 and August 2012.

**Methods**

**Patients**

This retrospective study was approved by the Ethics Committee of Peking University Third Hospital. Women who underwent IVF-embryo transfer cycles between January 2011 and August 2012 at the Reproductive Medical Centre of Peking University Third Hospital were analysed. Women underwent controlled ovarian hyperstimulation with a GnRH agonist or GnRH antagonist protocol. Ovarian follicle development was monitored based on serum estradiol (E2) levels and transvaginal ultrasonographic measurements. When at least one follicle reached a mean diameter of 18 mm and the E2 concentration exceeded 500 pg/ml, 10 000 units of urinary hCG (Serono, Aubonne, Switzerland) were administered before ultrasonography-guided oocyte retrieval. Luteal support was initiated on the day after oocyte retrieval using 60 mg of progesterone (Xianju Pharmacy, Zhejiang, China).

**Laboratory protocol**

The following two commercially available culture media were used: GS™ (Vitrolife, Gothenburg, Sweden) and GS™-PLUS (Vitrolife, Gothenburg, Sweden). HSA-solution™ (Vitrolife) as protein source supplemented the media. G1-PLUS v5 from the supplier was ready-to-use and included 5 mg/ml of HSA. G1 v5 was supplemented with 5 mg/ml pharmaceutical HSA solution before use. In our centre, the two types of media were used alternately: one kind of medium was used for 3 consecutive days, and then, the other medium was used for three days. Mineral oil was obtained from Sigma (St. Louis, MO, USA) and was used after washing and sterile filtration. IVF and intracytoplasmic sperm injection (ICSI) were performed according to the laboratory routine insemination procedures on the day of oocyte retrieval. In the case of IVF, oocytes were inseminated in IVF (Vitrolife) or IVF-PLUS (Vitrolife) media. After checking for the presence of pronuclei, the zygotes were transferred to G1 v5 or G1-PLUS v5. In the case of ICSI, oocytes were cultured in G1 v5 or G1-PLUS v5 after injection. Embryo morphology was evaluated with respect to cell number, fragmentation and symmetry 68–72 h after insemination. The number of embryos transferred was determined based on patient age, the number of previous IVF cycles and embryo quality.

**Data collection**

In the present study, only cycles with fresh embryo transfer were included. Patients <40 years of age with a BMI <30 kg/m² were analysed. Data from singletons born alive after the 28th week of gestation were included. Patients were excluded if preimplantation genetic diagnosis (PGD) or donor oocytes had been used. Patients with pregnancy-related complications, such as diabetes and hypertension, were also excluded. To neutralize the effect of the ‘vanishing twin,’ patients who had more than one fetal sac diagnosed by ultrasound, but who delivered singletons, were excluded.

**Definition**

Preterm birth was defined as delivery before 37 completed weeks gestation. LBW, very low birthweight (VLBW) and high birthweight (HBW) were defined as birthweights <2500 g, <1500 g and >4500 g, respectively.
SGA and very SGA were defined as birthweights < the 10th and < the 3rd percentiles, respectively. LGA was defined as a birthweight > the 90th percentile, and very LGA was defined as a birthweight > the 97th percentile.

Statistical analysis

All statistical analyses were performed with the Statistical Package for the Social Sciences software. The basic characteristics of patients were compared using analysis of variance (continuous variables), and categorical variables were evaluated with chi-squared tests. Multiple linear regression analyses were used to evaluate possible associations between birthweight and culture media with other potential confounding factors, including fertilization methods (IVF or ICSI), female age, female weight and height, male age, male weight and height, type of infertility, ovarian stimulation, duration of infertility, infertility factors, cycle number, gestational age, infant gender, and newborn complications (neonatal brain injury, congenital heart disease, Down syndrome, hypertrophic pyloric stenosis, icterus hepatitis, or congenital cartilage disease).

For calculating the proportion of SGA and LGA, we used the latest relevant publication regarding birthweight references for gestational ages, including standard deviations (Yuan et al., 2011). We also calculated gestational age- and gender-adjusted birthweights (also known as the Z score) for all IVF babies using the following equation: \[ Z = (x - \mu) / \sigma \], where \( x \) is the IVF birthweight, \( \mu \) is the mean birthweight at the same gestational age and same gender in the reference group, and \( \sigma \) is the standard deviation in the same reference group (Yuan et al., 2011). A P-value < 0.05 was considered statistically significant.

Results

A total of 1097 singletons born from fresh embryo transfer cycles were analysed between January 2011 and August 2012. There were 489 patients, with embryos cultured in G1 v5 who delivered singletons and 608 patients, with embryos cultured in G1-PLUS, v5 who delivered singletons. Every patient contributed one singleton in this study. Patient and cycle characteristics are shown in Table I. A significant difference existed in female BMI between the G1 v5 and G1-PLUS v5 media (22.23 ± 2.83 kg/m² versus 21.89 ± 2.85 kg/m², respectively; \( P < 0.05 \)). Smoking was not included in this study as a factor because very few women smoke in China, especially IVF patients.

The neonatal characteristics are shown in Table II. The mean birthweight in the G1 v5 media group was 3333 g and the mean birthweight in the G1-PLUS v5 media group was 3376 g. This difference was not significant (\( P = 0.14 \)). To control the confounding effect of gestational age and gender on birthweight, Z scores were compared between the G1 v5 and G1-PLUS v5 media groups. A significant difference in Z scores was detected between the G1 v5 and G1-PLUS v5 media groups (0.09 ± 1.15 versus 0.28 ± 1.12, respectively, \( P = 0.04 \)). In agreement with this finding, there were more LGA babies in the G1-PLUS v5 media group compared with that in the G1 v5 media group (16.8 versus 12.1%, \( P = 0.03 \)). A trend towards more very LGA babies in the G1-PLUS v5 media group was also observed, but the difference did not reach statistical significance (7.2 versus 4.7%, \( P = 0.09 \)). We also investigated the associations between culture media and SGA, very SGA, HBW, LBW, or VLBW, but no significant differences were observed (\( P > 0.05 \)).

Finally, multiple linear regression analysis was performed to determine the relationship between birthweight and culture media with other confounding factors, including duration of infertility, female factor, male factor, type of infertility, ovarian stimulation, cycles with ICSI, female age, male age, female height, male height, female weight, male weight, cycle number (>1 versus = 1), gestational age, infant gender and newborn complications (yes versus no). As shown in Table III, male height (\( P = 0.04 \)), female weight (\( P = 0.00 \)), gestational age (\( P = 0.00 \)), infant gender (\( P = 0.00 \)) and culture media (\( P = 0.04 \)) were significantly correlated with birthweight. Cycles with ICSI, and other possible confounding factors, duration of infertility, female factor, male factor, type of infertility, ovarian stimulation (agonist versus antagonist), female age, male age, female height, female weight, male weight, cycle number (>1 versus = 1), gestational age, infant gender, and newborn complications (yes versus no), were not significantly associated with birthweight (\( P > 0.05 \)).

Discussion

We have reported that gestational age and infant gender-adjusted birthweight (Z scores) derived from G1-PLUS v5 media were significantly higher than Z scores from G1 v5 media, although no significant difference in absolute birthweight existed between G1-PLUS v5 and G1 v5 media. Similarly, a higher proportion of LGA babies was observed after use of the G1-PLUS v5 media when compared with G-1 v5 media. The findings of the current study are in agreement with previously published findings.
This study, for the first time, has reported that the protein source/HSA affects birthweight in human. This finding is consistent with a previous study (Oken et al., 2005). To avoid the confounding effect of ‘vanishing twin’ pregnancy, which was reflected by the relatively small number of women with higher parity in our clinic. To control for gestational age and infant gender bias, Z scores for IVF babies were calculated and compared between the G1-PLUS v5 and G1 v5 media. The Z scores in the G1-PLUS v5 media group were higher, although no differences existed in the absolute birthweights between the two groups. Consistent with this data, multiple linear regression analysis indicated a significant effect of culture media on birthweight.

Some other studies have not demonstrated a relationship between culture media and birthweight. Eaton et al. (2012) reported no significant association between culture media and birthweight in a study in which 198 singletons (Vitrolife G1.3 [n = 102], Global [n = 53] and Vitrolife G1.5 [n = 43]) and 303 twins (Vitrolife G1.3 [n = 172], Global [n = 58] and Vitrolife G1.5 [n = 73]) were analysed. Vergouw et al. (2012) also showed no effect of culture media (HTF and SAGE) on birthweight in a study of 358 singletons derived from fresh embryo transfer and 159 singletons derived from frozen embryo transfer were analysed. Carrasco et al. (2013) reported no significant relationship between culture media and birthweight among 449 singletons in a prospective study (Vitrolife [n = 223] and Cook [n = 226]) and 2518 singletons in a retrospective study (Vitrolife [n = 943], Medicut [n = 821] and Cook [n = 754]). The same conclusion was obtained by Lin et al. (2013) when 1201 singletons and 445 twins were compared between different culture media (Vitrolife, Global and Quinn’s Advantage). The discrepancies between the studies may be partially explained by different commercial culture media used in the IVF centres.

A previous study has shown higher birthweights in blastocyst transfers compared with day 3 transfers (Zhu et al., 2014). The effect of extended

(Dumoulin et al., 2010; Nelissen et al., 2012; Eskild et al., 2013). This study, for the first time, has reported that the protein source/HSA affects birthweight in human.

It has been suggested that birthweights are related to maternal characteristics, such as BMI (Cogswell and Yip, 1995) and cigarette smoking (Pavic et al., 2011; Suter et al., 2013). We observed a trend towards a higher BMI in females who used the G1 v5 media, but this observation does not reflect bias as a higher female BMI is associated with a reduced risk of low birthweight (Cogswell and Yip, 1995). Cigarette smoking in pregnancy has a negative influence on the birthweights of newborns (Pavic et al., 2011). In the current study, however, we were not able to control the effect of cigarette smoking on birthweight, as this confounding factor was not available from our database. Of note, it has been reported that only 2.4% of Chinese women smoke (http://news.xinhuanet.com/fortune/2013–05/31/c_124794069.htm). This number would be lower among IVF patients who avoid smoking as it negatively influences pregnancy and the health of the baby. Not only maternal characteristics, but also the IVF technique is correlated with the birthweights of newborns (Henningsen et al., 2011). It has been reported that LBW in singletons derived from double embryo transfers is more frequent than singletons derived from single embryo transfers (De Sutter et al., 2006). Singletons derived from ‘vanishing twin’ pregnancies weigh less than singletons born from single embryo transfers (Pinborg et al., 2005). To avoid the confounding effect of ‘vanishing twin’ pregnancies, patients with more than one embryo implanted in the uterus, who delivered singletons, were excluded from the current study.

Multiple linear regression analysis indicated that gestational age and infant gender are the most influential factors determining birthweight. This finding is consistent with a previous study (Oken et al., 2003). We previously reported that parity does not significantly affect birthweight (Zhu et al., 2014), which was reflected by the relatively small number

### Table II Neonatal characteristics of live born singletons.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>G1 v5</th>
<th>G1-PLUS v5</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Singletons (n)</td>
<td>489</td>
<td>608</td>
<td>–</td>
</tr>
<tr>
<td>Male newborns</td>
<td>251 (51.3%)</td>
<td>317 (52.1%)</td>
<td>N.S</td>
</tr>
<tr>
<td>Gestational age</td>
<td>38.7 ± 1.38</td>
<td>38.6 ± 1.39</td>
<td>N.S</td>
</tr>
<tr>
<td>Preterm birth</td>
<td>30 (6.1%)</td>
<td>35 (5.8%)</td>
<td>N.S</td>
</tr>
<tr>
<td>Birthweight (g)</td>
<td>3333.2 ± 491.6</td>
<td>3375.9 ± 479.6</td>
<td>N.S</td>
</tr>
<tr>
<td>Average Z scores</td>
<td>0.09 ± 1.15</td>
<td>0.28 ± 1.12</td>
<td>0.04</td>
</tr>
<tr>
<td>Small for GA</td>
<td>55 (11.2%)</td>
<td>75 (12.3%)</td>
<td>N.S</td>
</tr>
<tr>
<td>Large for GA</td>
<td>59 (12.1%)</td>
<td>102 (16.8%)</td>
<td>0.03</td>
</tr>
<tr>
<td>Very small for GA</td>
<td>28 (5.7%)</td>
<td>31 (5.1%)</td>
<td>N.S</td>
</tr>
<tr>
<td>Very large for GA</td>
<td>23 (4.7%)</td>
<td>44 (7.2%)</td>
<td>N.S</td>
</tr>
<tr>
<td>Low birthweight (&lt; 2500 g)</td>
<td>15 (3.1%)</td>
<td>17 (2.8%)</td>
<td>N.S</td>
</tr>
<tr>
<td>Very low birthweight (&lt; 1500 g)</td>
<td>1 (0.2%)</td>
<td>4 (0.7%)</td>
<td>N.S</td>
</tr>
<tr>
<td>High birthweight (&gt; 4500 g)</td>
<td>4 (0.8%)</td>
<td>5 (0.8%)</td>
<td>N.S</td>
</tr>
</tbody>
</table>

Data are presented as numbers (%) or the mean ± SD. Continuous variables were compared using analysis of variance, and categorical variables were evaluated with chi squared tests. N.S, no significance.

### Table III Results of multiple regression analysis among live born singletons.

<table>
<thead>
<tr>
<th>Birthweight (g)</th>
<th>Beta</th>
<th>t</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 v5 or G1-PLUS v5</td>
<td>0.06</td>
<td>2.07</td>
<td>0.04</td>
</tr>
<tr>
<td>Duration of infertility (years)</td>
<td>0.04</td>
<td>1.26</td>
<td>N.S</td>
</tr>
<tr>
<td>Female factor</td>
<td>−0.04</td>
<td>−1.01</td>
<td>N.S</td>
</tr>
<tr>
<td>Male factor</td>
<td>−0.02</td>
<td>−0.66</td>
<td>N.S</td>
</tr>
<tr>
<td>Type of infertility</td>
<td>0.02</td>
<td>0.68</td>
<td>N.S</td>
</tr>
<tr>
<td>Ovarian stimulation (agonist versus antagonist)</td>
<td>0.04</td>
<td>1.59</td>
<td>N.S</td>
</tr>
<tr>
<td>Cycles with ICSI</td>
<td>−0.05</td>
<td>−1.35</td>
<td>N.S</td>
</tr>
<tr>
<td>Female age</td>
<td>−0.06</td>
<td>−1.37</td>
<td>N.S</td>
</tr>
<tr>
<td>Male age</td>
<td>0.01</td>
<td>0.17</td>
<td>N.S</td>
</tr>
<tr>
<td>Female height</td>
<td>0.00</td>
<td>0.01</td>
<td>N.S</td>
</tr>
<tr>
<td>Male height</td>
<td>0.06</td>
<td>2.03</td>
<td>0.04</td>
</tr>
<tr>
<td>Female weight</td>
<td>0.16</td>
<td>5.56</td>
<td>0.00</td>
</tr>
<tr>
<td>Male weight</td>
<td>0.05</td>
<td>1.55</td>
<td>N.S</td>
</tr>
<tr>
<td>Cycle number (&gt; 1 versus = 1)</td>
<td>0.02</td>
<td>0.72</td>
<td>N.S</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>0.41</td>
<td>15.18</td>
<td>0.00</td>
</tr>
<tr>
<td>Infant gender</td>
<td>0.17</td>
<td>6.15</td>
<td>0.00</td>
</tr>
<tr>
<td>Newborn complications (yes versus no)</td>
<td>−0.02</td>
<td>−0.58</td>
<td>N.S</td>
</tr>
</tbody>
</table>

Beta is the regression coefficient. N.S, no significance.
culture media on fetal growth may be related to the blastocyst culture media, although the birthweights in three culture media groups were comparable. G1 v5 and G1-PLUS v5 are sequential culture media; the current study only analysed the influence of the first-stage on birthweight and the incidence of LGA babies. It has been shown in animal studies that culture media supplemented with serum leads to heavier bovine and ovine offspring, and lighter murine offspring compared with culture media without serum (Khosla et al., 2001; Rooke et al., 2007). This finding was due to an epigenetic perturbation in embryos and placentas, which in turn affects fetal development. Aberrant methylation and expression of multiple growth-related imprinted genes have been detected in most of the fetuses derived from preimplantation embryo cultures (Young et al., 1998; Khosla et al., 2001; Mann et al., 2004; Hori et al., 2010).

Mammalian culture conditions have been improved over the past several decades by optimizing the components in culture media, such as the addition of amino acids and protein, and the use of sequential culture media to mimic the dynamic environment within the oviduct (Ho et al., 1995; Walker et al., 1996; Biggers and Summers, 2008; Sepulveda et al., 2009). Nevertheless, a complete understanding of human embryo requirements is lacking and no commercial culture media have been optimized for human embryo development. It has been reported in a human study that Vitrolife media provides a greater tendency for clinical pregnancy than Cook media (Van Langendonckt et al., 2001). Moreover, five commercial ART human embryo culture media were compared in a mouse model. Embryos cultured in any of the five commercial media had a decreased ability to maintain methylation of imprinted genes in comparison to in vivo generated embryos (Market-Velker et al., 2010). Furthermore, more types of commercial culture media were analysed in mouse model, and the results indicated that varying blastocyst formation rates and fetal development rates and specific gene expression patterns existed across the 13 tested ART culture media when compared with in vivo generated embryos (Schwarzer et al., 2012).

Subtle differences may exist between G1 v5 and G1-PLUS v5 owing to the higher Z scores and more LGA babies after use of the latter. The difference between the two media is that one is supplemented with HSA (G1 PLUS v5) and the other (G1 v5) needs the addition of HSA before use. G1 v5 supplemented with HSA was usually used within 3 days in our centre. The storage period for G1-PLUS v5 is ≈3 months as long as the storage period for G1 v5 after HSA supplementation. Whether or not a prolonged HSA supplementation period is associated with a higher Z score and LGA babies is not known. In addition, owing to the properties of commercial culture media, specific components and concentrations were not disclosed by the vendor. The composition of HSA solution could be different between G1 v5 and G1-PLUS v5, although both media come from the same manufacturer. It is speculated that protein stabilizer or more stable protein may be added to G1-PLUS v5 in order to extend its shelf life. Complete knowledge of the ingredients of culture medium would be of great importance to users to make choices about which culture medium to choose.

In conclusion, we found a relationship between culture media and Z scores and LGA babies. The current study provides important data in reviewing the safety of in vitro embryo cultures. Culture media giving higher birthweights than other media have been described in multiple studies. The composition of the media and protein sources vary among multiple culture media from different manufacturers. The underlying mechanism of higher birthweights and more LGA babies after the use of certain culture media could be related to the different protein sources, given that the human embryo is sensitive to these early culture condition. Nevertheless, we do not know which culture medium is the best for human embryogenesis. Therefore, more research is needed to investigate the impact of the ART culture systems on epigenetic changes in human embryos and placentas in the future.

**Authors’ roles**

J.Q., P.L. and J.L.Z. conceived and designed the study; J.L.Z., M.L. and L.X.C. coordinated data collection; J.L.Z. and L.X.C analysed the data; J.L.Z. drafted the manuscript; and all authors interpreted the data.

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**Conflict of interest**

None declared.

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