Birthweight distribution in ART singletons resulting from embryo culture in two different culture media compared with the national population

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STUDY QUESTION: Is there a difference in birthweight distribution in ART singletons born after IVF culture in two different culture media?

SUMMARY ANSWER: There is no effect of culture media on both crude and adjusted birthweight distributions in ART singletons from nulliparous mothers.

WHAT IS KNOWN ALREADY: Studies on human ART singletons have reported a difference in birthweight in singletons following IVF culture in different culture media. However, other studies comparing different culture media have not shown any significant differences in birthweight.

STUDY DESIGN, SIZE, DURATION: This study was a retrospective comparison of birthweights in IVF/ICSI singletons conceived after fresh embryo transfer following embryo culture in Cook or Medicult medium and in a national cohort of naturally conceived singletons in nulliparous women. The study compares four independent groups consisting of singletons in nulliparous women from Cook-d2: 2-day culture in Cook medium at Rigshospitalet (n = 974), Medicult-d2: 2-day culture in Medicult EmbryoAssist medium at Rigshospitalet (n = 147), Medicult-d3: 3-day culture in Medicult EmbryoAssist medium with and without added GM-CSF (n = 204), and DK: pregnancies from the Danish birth registry (n = 106842).

PARTICIPANTS/MATERIALS, SETTING, METHODS: The study compares the birthweights of singletons from nulliparous women in the four independent groups mentioned above; Cook-d2: Medicult-d2: Medicult-d3: and DK. In addition, distributions of large and small for gestational age infants were compared between the groups and a multiple linear regression analysis was used to determine which factors determined birthweight.

MAIN RESULTS AND THE ROLE OF CHANCE: We found no significant difference in the crude birthweight distributions between singletons born after culture in Cook-d2 or Medicult-groups. Singleton girls from the Cook-d2 group weighed 3302 ± 28 g, versus 3252 ± 76 in the Medicult-d2 group (difference 50 g; P = 0.547). Singleton boys from the Cook-d2 group weighed 3430 ± 27 g, versus 3354 ± 56 in the Medicult-d2 group (difference 76 g; P = 0.279). In the background population, mean birthweights for singleton girls and boys were 3383 ± 2.4 g and 3494 ± 2.5 g, respectively. The mean birthweights of girls, 3315 ± 61 g, and boys, 3383 ± 64 g, in the Medicult-d3 group were not significantly different from that in the Medicult-d2 group. When pooling data from all culture media groups, we found the same slightly lower mean birthweight in IVF/ICSI singletons when compared with the national birth cohort as has been previously reported (Cook-d2 + Medicult-d2 + d3 versus birth cohort; girls: P < 0.001, boys: P < 0.001). We also pooled data on boys and girls and calculated the mean birthweight for the Cook-d2, Medicult-d2 and Medicult-d3 groups and found no significant differences.

LIMITATIONS, REASONS FOR CAUTION: The retrospective design and the inherent risk of confounding factors is a limitation. Selection bias cannot be excluded as the embryos cultured in Cook-d2 and Medicult-d2 were from single centre studies while data in Medicult-D3 group were derived from a multicentre study.

WIDER IMPLICATIONS OF THE FINDINGS: This large cohort of singletons born after IVF/ICSI shows no difference in crude mean birthweight after culture in two different culture media, indicating that if such a difference exists, this must be specific for certain culture media.
As expected we found a slightly lower mean birthweight in ART compared with naturally conceived singletons. This suggests that parental characteristics or IVF technique related factors other than type of culture medium may influence the birthweight in ART singletons.

**STUDY FUNDING/COMPETING INTEREST(s):** No external funding was used for this study. No conflicts of interest are declared.

**Key words:** culture media / birthweight / singleton / IVF/ICSI / outcome

## Introduction

In 2009, 0.5–5.0% of all births in Europe was associated with in vitro techniques (Ferraretti et al., 2013). Several meta-analyses and large cohort studies have shown that the mean birthweight in ART singletons is lower in than in naturally conceived (NC) singletons and that the adjusted risk of being born small-for-gestational age is higher after IVF than after natural conception (Jackson et al., 2004; Källén et al., 2005; Shih et al., 2008; McDonald et al., 2009). This lower mean birthweight is partly caused by the parental characteristics or the subfertility per se; however, an effect of the IVF procedures themselves cannot be excluded (Romundstad et al., 2008; Henningen et al., 2011; Pinborg et al., 2013).

Singletons conceived after transfer of cryopreserved/thawed embryos have a higher mean birthweight than singletons conceived after transfer of fresh embryo, which suggests an independent effect of the controlled ovarian stimulation or the IVF procedures on the early implantation and fetal development (Pelkonen et al., 2010; Sazonova et al., 2012; Wennerholm et al., 2013). Studies on the Developmental Origin of Health and Diseases (DoHaD) have shown that low birthweight is associated with cardiovascular diseases and type 2 diabetes later in life (Barker, 2004) and that preterm birth is associated with increased morbidity, mortality and affected reproduction (McIntire and Leveno, 2012; Swamy et al., 2008). Hence the importance of exploring the causal pathways of the differences in mean birthweight between IVF and non-IVF children is obvious. In animal studies, differences in mean birthweight in singletons born after IVF culture are associated with the culture media used (Thompson et al., 1995; Khosla et al., 2001). In humans, one Dutch group has reported a difference in mean birthweight for singletons conceived after IVF culture in Cook media compared with singletons born after embryos cultured in Vitrolife culture media and after fresh and frozen embryo transfer, respectively (Dumoulin et al., 2010; Nelissen et al., 2012). They found that singletons conceived after IVF with embryos cultured in Vitrolife were significantly heavier than singletons conceived after IVF culture in Cook media (Dumoulin et al., 2010). More recently a Norwegian group and an Iranian group showed significant higher birthweights in singletons conceived after culture in Medicult ISM1 medium compared with Medicult Universal and Vitrolife G1 medium (Eskild et al., 2013; Hassani et al., 2013) and a Chinese group found that the protein source in culture medium influenced z-scores but not absolute birthweights in IVF singletons (Zhu et al., 2014b). In contrast, four recent studies comparing culture in, respectively, G1.3/Global/G1.5 media, HTF/Sage media, GS™/Global/Quinn’s advantage media and Medicult/Cook/Vitrolife media did not show any significant differences in mean birthweight between singletons cultured in the different media (Eaton et al., 2012; Vergouw et al., 2012; Carrasco et al., 2013; Lin et al., 2013). This suggests that if a birthweight difference exists between singletons born after embryo culture in different media, this might be related to specific culture media. The primary aim of the present study was to compare crude and adjusted birthweights of singleton boys and girls cultured in Cook versus Medicult medium and, in addition, to compare them with the Danish background population. The second aim was to make adjusted risk estimates of being born small- and large for gestational age after embryo culture in Cook versus Medicult media and after natural conception.

## Materials and Methods

This study was a retrospective comparison of birthweight of IVF/ICSI singletons conceived after fresh embryo transfer following culture in Cook K-SICM (Cook, Sydney, Australia) or Medicult Embryo Assist (Origo, Målev, Denmark) medium, compared with naturally conceived pregnancies in nulliparous women. The study compared four independent groups consisting of singletons: Cook-d2 (2-day culture in Cook medium at the Rigshospital, 2004–2009, 474 girls and 503 boys), Medicult-d2 (2-day culture in Medicult EmbryoAssist medium without GM-CSF at Rigshospitalet, 2010, 87 boys, 60 girls), Medicult-d3 (3-day culture in Medicult EmbryoAssist medium with and without added GM-CSF, data from a randomized multicentre study (Ziebe et al., 2013), 2007–2010, 93 girls and 111 boys), and DK (singleton pregnancies in nulliparous women from the Danish Medical Birth Registry, 2003–2006, 52106 girls and 54736 boys). In Medicult-d3 ORIGO multicentre trial data, both control and GM-CSF exposed groups were combined, as the mean birthweights with and without GM-CSF were not statistically different (P = 0.679) and in previous animal studies GM-CSF was not shown to be linked to difference in birthweights (Sjoblom et al., 2005). Exclusion criteria were singletons with mothers who were HIV or hepatitis positive at time of conception and singletons born after frozen embryo transfer or PGD.

Children born before Week 22 (1 boy in the Cook-d2 group, 17 boys and 10 girls in the national birth cohort), children with a birthweight < 300 g (the same boy in the Cook-d2 group and 19 boys and 18 girls in the national birth cohort), or children with a birthweight above 5500 g (17 boys and 9 girls in the national birth cohort) were excluded. In addition, one girl in the Medicult-d3 group with unknown birthweight was not included in the study. In all, 233 boys and 263 girls with missing birthweight data and 101 boys and 81 girls with missing gestational age data were excluded from the national birth cohort data. For 78 singletons information on birth length was missing (49 in the Cook-d2 group, 1 in the Medicult-d3 group and 28 in the Medicult-d2 group).

Mean z-scores (birthweight adjusted for gestational age and gender) were calculated. We calculated a SD score (SD score, also known as z-score) for each newborn using the equation $z = (x - \mu) / \sigma$, where $x$ is the weight of a child, $\mu$, the national mean weight of the babies born at the same gestational age and gender (reference group: in this manuscript, the Marsål group) and $\sigma$, the SD of the reference group. The z-score or standard normal deviate is a measure of the variation in birthweight relative to the expected. A z-score of greater than or equal to +2 SD represents the upper 2.3% of the population. Gestational age was missing for three singletons in the Cook group so for the z-scores and multiple regression analysis, n = 974.

Large-for-gestational age (LGA) and small-for-gestational age (SGA) were calculated according to the Marsål standard growth curves if the z-score was greater than +2 standard deviations (SD) (LGA) or less than −2 SD (SGA) in the Marsål standard growth curves (Marsål et al., 1996). Marsål’s formula describes birthweight according to the child’s sex and gestational age at delivery.
Statistics

Statistical analyses were performed using IBM SPSS Statistics Version 19. For analyses on differences between means of continuous variables between groups, a 2-sided Student’s t-test was used. Differences between proportions of LGA and SGA in the study groups (binary variables) were assessed by chi-squared test. P-values of 0.05 or less were considered statistically significant.

Multiple linear regression analysis was performed to identify independent predictors of child’s birthweight and birth length. Only data from Day 2 cultures were included. The following variables were entered in the analyses: gender, maternal age at birth, type of culture medium and number of embryos transferred. In a separate analysis on z-scores, the variables included were maternal age at birth, type of culture medium and number of embryos transferred. In the analyses on birthweight and length as dependent variables, gestational age was also included as a covariate. A power analysis was performed to determine the risk for Type II error due to sample size differences. To show a difference of 150 g with 80% power and a 95% CI, we calculated that sample sizes of \( n = 104 \) and \( n = 625 \) would be adequate assuming a standard deviation of 637 g, which is the largest SD in our study groups.

Results

Demographics

Maternal and cycle characteristics of the three culture media groups are described in Table I. There were no differences in demographic data of embryos transferred. In the analyses on birthweight and length as dependent variables, gestational age was also included as a covariate. A power analysis was performed to determine the risk for Type II error due to sample size differences. To show a difference of 150 g with 80% power and a 95% CI, we calculated that sample sizes of \( n = 104 \) and \( n = 625 \) would be adequate assuming a standard deviation of 637 g, which is the largest SD in our study groups.

Table I: Patient and cycle characteristics of three cohorts of singletons born after IVF culture in different culture media.

<table>
<thead>
<tr>
<th></th>
<th>Cook-d2</th>
<th>Medicult-d2</th>
<th>Medicult-d3</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cook-d2 versus</td>
<td>Medicult-d2 versus</td>
<td>Medicult-d3 versus</td>
<td></td>
</tr>
<tr>
<td>Maternal age (mean ± SEM)</td>
<td>32.87 ± 0.1</td>
<td>32.77 ± 0.3</td>
<td>31.22 ± 0.3</td>
<td>0.786</td>
</tr>
<tr>
<td>Cycle no. (mean ± SEM)</td>
<td>1.85 ± 0.03</td>
<td>1.85 ± 0.08</td>
<td>1.69 ± 0.1</td>
<td>0.993</td>
</tr>
<tr>
<td>No. of oocytes picked up (mean ± SEM)</td>
<td>8.43 ± 0.12</td>
<td>8.24 ± 0.3</td>
<td>10.34 ± 0.3</td>
<td>0.606</td>
</tr>
<tr>
<td>No. of transferable embryos (mean ± SEM)</td>
<td>3.99 ± 0.08</td>
<td>3.69 ± 0.19</td>
<td>3.67 ± 0.2</td>
<td>0.185</td>
</tr>
<tr>
<td>No. of transferred embryos (mean ± SEM)</td>
<td>1.49 ± 0.02</td>
<td>1.45 ± 0.04</td>
<td>1.40 ± 0.03</td>
<td>0.331</td>
</tr>
<tr>
<td>No. of IVF cycles (%)</td>
<td>528 (54.2%)</td>
<td>71 (48.3%)</td>
<td>113 (55.67%)</td>
<td>0.214</td>
</tr>
<tr>
<td>No. of ICSI cycles (%)</td>
<td>447 (45.8%)</td>
<td>76 (51.7%)</td>
<td>65 (32.0%)</td>
<td>0.196</td>
</tr>
<tr>
<td>No. of mixed IVF and ICSI cycles (%)</td>
<td>25 (12.32%)</td>
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</table>

For analyses on differences between means of continuous variables between the groups a 2-sided Student’s t-test was used. Differences between binary variables were assessed by chi-squared test.

***Statistically significant difference of < 0.001.

Figure 1: Birthweight distribution in singleton boys and girls. Crude birthweight distribution of singleton boys and girls cultured in Cook medium for 2 days (Cook-d2), Medicult medium for 2 days (Medicult-d2) or Medicult medium for 3 days (Medicult-d3). The birthweight is divided in 250-g intervals and the percentage of newborn singletons in each birthweight category is given as a percentage of the total number of births in each group.
between the Cook-d2 and Medicult-d2 groups, while maternal age and number of oocytes retrieved differed significantly between the Medicult-d3 and Medicult-d2 groups.

Birthweight and birth length in singleton boys and girls

We found no significant difference in birthweight distribution between singletons born after culture in Cook-d2 or Medicult-d2 group (Fig. 1). Singleton girls from the Cook-d2 group weighed 3302 ± 28 g versus 3252 ± 76 in the Medicult-d2 group (difference 50 g; P = 0.547). Singleton boys from the Cook-d2 group weighed 3430 ± 27 g versus 3354 ± 56 in the Medicult-d2 group (difference 76 g; P = 0.279). In the background population, mean birthweights for singleton girls and boys were 3383 ± 2.4 g and 3494 ± 2.5 g, respectively. To explore the effect of culture length, we also included at a third cohort (Medicult-d3) in which the embryos were cultured for 3 days. The mean birthweights of girls 3315 ± 61 g and boys 3383 ± 64 g in the Medicult-d3 group were not significantly different from the Medicult-d2 group. Consistent with previous studies, we found a slightly lower birthweight in IVF/ICSI singletons when compared with the national birth cohort (Cook + Medicult versus birth cohort; girls: P < 0.001, boys: P < 0.001).

In the pooled analyses for boys and girls together, we found similar mean birthweights after culture in Cook-d2, Medicult-d2 and Medicult-d3 groups (Table II). There was a significant effect of culture medium on birth length of the children, with the shortest children in the Medicult-d2 group, significantly different from all of the other groups, Cook-d2, Medicult-d3 and DK. As described in the material and methods, for 78 singletons, information on birth length was missing.

Large- and small for gestational age

The percentages of LGA singletons in the Cook-d2, Medicult-d3 and Medicult-d2 group were 3.4, 2.0 and 2.0%, respectively, and for the national cohort it was 2.3%. The percentages of SGA singletons in the Cook-d2, Medicult-d3 and Medicult-d2 group were 2.8, 4.4 and 3.4%, respectively, and for the national cohort it was 3.7%. There was a significant higher percentage of LGA in the Cook-d2 group compared with the national cohort otherwise there were no differences in the distribution of LGA/SGA over the four groups.

Multiple linear regression analyses

Multiple linear regression analysis showed that birthweight was dependent on the child’s gender and length of gestation but not on the type of culture media (Table III). This was confirmed by the results of a multiple linear regression performed on the z-scores showing no dependence type of culture medium. Birth length was dependent on the child’s gender, gestational length and on type of culture media. Gestational age at birth was found not to be dependent on any of the variables tested.

Discussion

This large cohort of singletons born after IVF/ICSI shows no difference in crude mean birthweight after culture in two different culture media, indicating that if such a difference exists, it must be specific for certain culture media. In addition we did not find any difference in birthweight after in vitro culture for 2 days compared with 3 days. The proportions of

<table>
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<tr>
<th>Table II: Neonatal outcome of singletons born after IVF culture in different culture media and one naturally conceived cohort.</th>
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<tr>
<td>------------------</td>
</tr>
<tr>
<td>Cook-d2</td>
</tr>
<tr>
<td>Medicult-d2</td>
</tr>
<tr>
<td>Medicult-d3</td>
</tr>
<tr>
<td>Birth Cohort (DK)</td>
</tr>
</tbody>
</table>

*Statistically significant difference of P < 0.05, **Statistically significant difference of P < 0.01, ***Statistically significant difference of P < 0.001.
singleton being born LGA or SGA in the three different culture media groups were similar.

Consistent with four previous studies (Eaton et al., 2012; Vergouw et al., 2012; Carrasco et al., 2013; Lin et al., 2013), we found no differences in mean birthweight between singletons cultured in different media. The mentioned studies comprise various culture medium comparisons. Eaton et al. compared Global to Vitrolife (G1.3 and G1.5) media, Vergouw et al. compared HTF to Sage media, Lin et al. compared Vitrolife G1.5, Global and Quinn’s media, and Carrasco compared Cook, Vitrolife (G1.3) and Medicult (ISM1). Two studies from The Netherlands using partly the same dataset (Dumoulin et al., 2010; Nelissen et al., 2012) and the Norwegian and Iranian studies (Eskild et al., 2013; Hassani et al., 2013) showed a significant difference in singleton birthweight after culture in Cook versus Vitrolife, and Medicult ISM1 versus Medicult Universal or Vitrolife medium.

As expected the mean birthweight was slightly lower in IVF/ICSI singletons compared with naturally conceived singletons. This is in line with previously published data and may suggest that parental characteristics or IVF technique related factors other than type of culture medium influence the birthweight in IVF/ICSI singletons (Henningsen et al., 2011; Hayashi et al., 2012; Cooper et al., 2013; Pinborg et al., 2013). Pelinck et al. compared birthweight in singleton pregnancies after ovarian stimulation to singletons after modified natural cycles and concluded that the major factor attributing to birthweight differences was patient characteristics (Pelinck et al., 2010). Similar to previous large cohort studies and meta-analyses with odds ratio of low birthweight lingering between 1.4 and 1.8, the birthweight difference between ART and naturally conceived singletons was modest in our study (Helmerhorst et al., 2004; Jackson et al., 2004). Previous studies on birthweight in singletons after in vitro culture have been based on heterogeneous study groups as they include both singletons delivered by nulliparous and multiparous women (Dumoulin et al., 2010; Eaton et al., 2012; Nelissen et al., 2012; Eskild et al., 2013) or information on parity is not specified (Carrasco et al., 2013; Lin et al., 2013), and some reports have groups with different culture lengths (Dumoulin et al., 2010; Nelissen et al., 2012; Eskild et al., 2013). These studies also have a varying sample sizes, from 78 versus 108 in the Dumoulin article (Dumoulin et al., 2010), 43 up to 293 in the following five papers (Eaton et al., 2012; Nelissen et al., 2012; Vergouw et al., 2012; Carrasco et al., 2013; Hassani et al., 2013), 792/619/235 births in the groups in the paper by Lin et al., and finally with 402/449/1584 in the most recent publication (Eskild et al., 2013). Only the latest paper by Eskild et al. includes a comparison to a local background population (Eskild et al., 2013). The strength of our study is the size of the study groups, the fact that only singletons from nulliparous women were included, and the aim to compare the data with data from the background population. Furthermore, we calculated the proportions of small- and large-for-gestational age babies.

The limitations of this study are its retrospective design and the risk of residual confounding. Embryo culture in Cook-d2 was exclusively done in one clinic while data in Medicult-d3 derived from a multicentre study, while the group Medicult-d2 was cultured in the same clinic but in a different time period. Furthermore, there was a significant difference in age and the number of oocytes retrieved between the Medicult-d2 and Medicult-d3 groups.

In the multiple linear regression analysis, child’s gender and gestational age at birth were the only independent predictors of birthweight while culture medium did not influence birthweight. This is consistent with the findings of Vergouw et al. (2012), Lin et al. (2013) and Nelissen et al. (2012), where gender and gestational age were associated with birthweight. Only Nelissen et al. in their multiple regression analysis have shown that culture medium is associated with birthweight. We found a significant difference between the children’s body length from the culture in Medicult-medium for 2 days compared both with the children from embryos cultured in Cook for 2 days and with the children from culture in Medicult for 3 days. Lin et al. (2013) did not find a significant effect of culture medium on birth length, but Hassani et al. (2013) found a difference in length at birth in children born after culture in ISM1 versus Vitrolife G1– version 5. The measurement of body length at birth is however a very imprecise measurement and differences should be taken with caution.

We showed no birthweight difference between duration of in vitro culture for 2 and 3 days, which might be due to the fact that there was only 1 day difference in culture length. Another possibility is that a possible difference of culture length on birthweight could be masked by other differences between the two Medicult study groups such as maternal age or number of oocytes at OPU. Two recent studies (Mäkinen et al., 2013; Zhu et al., 2014a) have shown a significant larger proportion of large for gestational age children born after blastocyst culture compared with cleavage stage culture while several other studies have reported no association between culture length and birthweight (Kausche et al., 2001; Schwärzler et al., 2004) or birthweight and LGA/SGA distribution (Fernando et al., 2012). There was no association between length of culture and gestational age at birth in our study, which is in contrast to Dar et al. (2013) who showed an increased risk of preterm birth after blastocyst

Table III  Multivariate linear regression analyses with inclusion of the Day 2 study populations, performed to identify predictors of birthweight and birth length among live born nulliparous singletons.

<table>
<thead>
<tr>
<th>Culture medium group</th>
<th>Birthweight (g)</th>
<th>Maternal age at birth</th>
<th>Child’s gender</th>
<th>Gestational length</th>
<th>No. of embryos transferred</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>t</td>
<td>P-value</td>
<td>B</td>
<td>t</td>
</tr>
<tr>
<td>Culture medium group</td>
<td>−0.018</td>
<td>−0.796</td>
<td>0.426</td>
<td>−0.063</td>
<td>−2.977</td>
</tr>
<tr>
<td>Maternal age at birth</td>
<td>0.009</td>
<td>−0.390</td>
<td>0.697</td>
<td>0.022</td>
<td>1.000</td>
</tr>
<tr>
<td>Child’s gender</td>
<td>0.109</td>
<td>4.894</td>
<td>&lt;0.001***</td>
<td>0.143</td>
<td>6.701</td>
</tr>
<tr>
<td>Gestational length</td>
<td>0.661</td>
<td>29.678</td>
<td>&lt;0.001***</td>
<td>0.713</td>
<td>33.45</td>
</tr>
<tr>
<td>No. of embryos</td>
<td>0.037</td>
<td>1.614</td>
<td>0.107</td>
<td>0.026</td>
<td>1.194</td>
</tr>
</tbody>
</table>

**Statistically significant difference of <0.01.  
***Statistically significant difference of <0.001.
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...changes in neonatal outcome, clarifying the mechanisms behind this is necessary. Barker DJ. The developmental origins of adult disease. J Am Coll Nutr 2006; 25:605–612. None declared.

Conflict of interest

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script drafting and critical discussion. J.G.L. participated in the study design, execution, analysis, manuscript drafting and critical discussion; S.Z. participated in the study design, manuscript drafting and critical discussion; S.R. participated in the study design, analysis, manuscript drafting and critical discussion; A.P. participated in the study design, analysis, manuscript drafting and critical discussion.

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Authors’ roles

J.G.L. participated in the study design, execution, analysis, manuscript drafting and critical discussion; A.P. participated in the study design, analysis, manuscript drafting and critical discussion; S.R. participated in the study execution and analysis; S.Z. participated in the study design, manuscript drafting and critical discussion.

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None declared.

References


