Large baby syndrome in singletons born after frozen embryo transfer (FET): is it due to maternal factors or the cryotechnique?

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STUDY QUESTION: Are singletons born after frozen embryo transfer (FET) at increased risk of being born large for gestational age (LGA) and if so, is this caused by intrinsic maternal factors or related to the freezing/thawing procedures?

SUMMARY ANSWER: Singletons after FET have an increased risk of being born LGA. This cannot solely be explained by intrinsic maternal factors as it was also observed in sibling pairs, where the sibling conceived after FET had an increased risk of LGA compared with the sibling born after Fresh embryo transfer.

WHAT IS KNOWN ALREADY: FET singletons have a higher mean birthweight than singletons born after transfer of fresh embryos, and FET singletons may be at an increased risk of being born LGA.

STUDY DESIGN, SIZE, DURATION: The national register–based controlled cohort study involves two populations of FET singletons. The first population (A: total FET cohort) consisted of all FET singletons (n = 896) compared with singletons born after Fresh embryo transfer (Fresh) (n = 9480) and also with that born after natural conception (NC; n = 4510) in Denmark from 1997 to 2006. The second population (B: Sibling FET cohort) included all sibling pairs, where one singleton was born after FET and the consecutive sibling born after Fresh embryo transfer or vice versa from 1994 to 2008 (n = 666). The sibling cohort included n = 550 children with the sibling combination first child Fresh/second child FET and n = 116 children with the combination first child FET/second child Fresh.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Main outcome measures were LGA defined as birthweight of >2 SD from the population mean (z-score >2) according to Marsal’s curves. Macrosomia was defined as birthweight of >4500 g. Crude and adjusted odds ratios (ORs) of LGA and macrosomia were calculated for FET versus Fresh and versus NC singletons in the total FET cohort. Similarly, OR was calculated for FET versus Fresh in the sibling cohort. Adjustments were made for maternal age, parity, child sex, year of birth and birth order in the sibling analyses. Meta-analyses were performed by pooling our data with the previously published cohort studies on LGA and macrosomia.

MAIN RESULTS AND THE ROLE OF CHANCE: The ORs of LGA (z-score >2) and macrosomia in FET singletons versus singletons conceived after Fresh embryo transfer were 1.34 [95% confidence interval (95% CI) 0.98–1.80] and 1.91 (95% CI 1.40–2.62), respectively. The corresponding risks for FET versus NC singletons were 1.41 (95% CI 1.01–1.98) for LGA and 1.67 (95% CI 1.18–2.37) for macrosomia. The increased risk of LGA and macrosomia in FET singletons was confirmed in the sibling cohort also after adjustment for birth order. Hence, the increased risk of LGA in FET singletons cannot solely be explained by being the second born or by intrinsic maternal factors, but may also partly be related to freezing/thawing procedures per se. In the meta-analysis, the summary effects of LGA and macrosomia in FET versus singletons conceived after Fresh embryo transfer were AOR 1.54 (95% CI 1.31–1.81) and AOR 1.64 (95% CI 1.26–2.12), respectively. The corresponding figures for FET versus NC singletons were for LGA AOR 1.32 (95% CI 1.07–1.61) and macrosomia AOR 1.41 (95% CI 1.11–1.80), respectively.

LIMITATIONS, REASONS FOR CAUTION: Adjustment for body mass index as a possible confounder was not possible. The size of the FET/Fresh sibling cohort was limited; however, the complete sibling cohort was sufficiently powered to explore the risk of LGA. A bias is very unlikely as data coding was based on national registers.
Introduction

Worldwide >5 million children have been born after assisted reproductive technology (ART), and an increasing proportion of these are conceived after transfer of frozen embryo transfer (FET) (Ferraretti et al., 2012; Sullivan et al., 2013). Three systematic reviews have shown that singletons born after FET with the slow freeze technique and transfer of cleavage stage embryos carry less perinatal risks than those born after conventional ART with transfer of fresh embryos (Wennerholm et al., 2009; Maheshwari et al., 2012; Pinborg et al., 2013). Moreover, FET singletons have perinatal outcomes comparable with those observed in naturally conceived (NC) singletons (Pelkonen et al., 2010; Pinborg et al., 2010; Sazonova et al., 2012). With the intensified use of FET along with the elective single embryo transfer policy, it is reassuring that the perinatal outcomes in FET singletons mirror that of their naturally conceived peers.

In 2008, a large Australian study reported that singletons born after cryopreservation of embryos had a significantly higher birthweight z-score [standard deviation (SD) from the population mean] than those conceived by Fresh embryo transfer (Shih et al., 2008). Recently, larger cohort studies have shown a higher risk of being large for gestational age (LGA) in FET singletons compared with both singletons conceived after Fresh embryo transfer and the background population (Pelkonen et al., 2010; Sazonova et al., 2012). This was confirmed in a large Japanese study on perinatal outcomes after single embryo transfer of vitrified embryos. However, after adjustment, the difference in the proportion of LGA babies was no longer statistically significant when FET singletons were compared with singletons born after fresh single embryo transfer (Kato et al., 2012). In contrast to the other studies, the Japanese study included only children born after conception with vitrified embryos with cryopreservation of both cleavage stage embryos and blastocysts. Whether the higher risk of LGA in FET singletons compared with singletons born after fresh embryo transfer is related to parental factors or to the freezing/thawing procedure per se remains unknown.

From animal studies, it is well known that IVF can cause ‘large offspring syndrome’ also related to severe obstetric conditions and birth defects (Young et al., 1998; Grace and Sinclair, 2009). In humans, the results of the current literature are diverging, as the risk of being born small for gestational age (SGA) is increased in IVF singletons conceived after Fresh embryo transfer compared with children conceived after NC (Helmerhorst et al., 2004; Jackson et al., 2004; McGovern et al., 2004; McDonald et al., 2009). The recent findings of increased risks of LGA related to transfer of FET indicate that assisted reproduction may influence the early embryo and placental development and intrauterine growth environment even in humans. Moreover, a recent study revealed that independent contributing factors for LGA in singletons conceived by Fresh embryo transfer were mother’s body mass index (BMI), parity and embryo culture length approximately Day 2 versus Days 5–6 (Mäkinen et al., 2013). This further stresses that in vitro culture may influence intrauterine growth rates.

The first objective of this study was to assess the crude and adjusted risks of being LGA and macrosomia in FET singletons compared with singletons born after Fresh embryo transfer and NC. Furthermore, we included our adjusted risks on LGA in FET singletons in a meta-analysis with the results of previous studies.

The second aim was to distinguish intrinsic maternal factors from factors related to the freezing and thawing procedures by comparing the prevalence of LGA in consecutive singleton sibling pairs, where one sibling was born after transfer of FET and the other sibling after fresh embryo transfer. By keeping the intrinsic maternal factors constant, it is possible to explore the effect of parameters related to the freezing and thawing procedures.

Materials and Methods

The study involves two national study populations of singletons born after transfer of FET. The first population (A: total FET cohort) consists of all FET singletons born in Denmark during a 10-year period from 1997 to 2006. The second population (B: Sibling FET cohort) consists of all sibling pairs, where one singleton was born after FET and the consecutive sibling born after Fresh embryo transfer or vice versa from 1994 to 2007. Perinatal outcomes other than LGA, macrosomia and SGA in the two cohorts have been reported in two previous publications from our study group (Henningsen et al., 2011; Pinborg et al., 2010).

Total FET cohort

The total FET cohort was a national cohort consisting of n = 896 FET and n = 9480 singletons born after Fresh embryo transfer and a randomly selected singleton control cohort of 4510 naturally conceived (NC) singletons matched on year of birth. The cohort was established by cross-linkage of data from the Danish IVF and Medical Birth Registries, and children were born from 1997 to 2006. This population is a subcohort of FET children, where data on perinatal outcomes have been published earlier (Pinborg et al., 2010). Singletons born from 1994 to 1996 were not included, as specific obstetric outcomes were not available on these children. In the study period, the treatment standard was cryopreservation of embryos with slow freezing of cleavage stage embryos on Day 2 or 3, and >99% of the FET cycles were carried out in this way.

Eighty-six stillborn were excluded [9 (1.0%) FET, 60 (0.6%) fresh and 17 (0.4%) NC singletons]. Furthermore, 106 children without birthweight registration equally distributed between the three cohorts were also excluded.

Sibling FET cohort

The sibling FET cohort is represented by two different groups of siblings: the first group consisted of siblings, where the first sibling was born after Fresh embryo transfer and the second sibling after FET (Fresh/FET, n = 275 + 275 = 550 singletons). The second sibling cohort comprised sibling pairs,
where the first child was born after FET and the second child after Fresh embryo transfer (FET/Fresh, n = 58 + 58 = 116 singletons).

All singletons in the Fresh/FET and FET/Fresh sibling combinations born from 1994 to 2007 were included (Henningsen et al., 2011).

Data on perinatal outcomes were obtained from the Danish Medical Birth Register for both total FET cohort and the sibling FET cohort. 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) 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. For birthweights in the Danish Medical Birth Register, this corresponds to a birthweight of \( \geq 22\% \) above the expected value for the particular sex and gestational age.

Main outcome measures were LGA greater than +2 SD (z-score >2) according to the Marsal standard growth curves and LGA greater than +3 SD (z-score >3) (Marsal et al., 1996). Marsal’s formula describes birthweight according to child’s sex and gestational age at delivery. Macrosomia was defined as birthweight of >4500 g. Secondary outcome was SGA (less than −2 SD below the Marsal standard growth curves, less than −22%).

Meta-analyses

For the meta-analyses, we made a systematic PubMed search from 1982 to June 2013. The main outcome measure was LGA (defined as mean birthweight greater than z-score of 2), which was used in the meta-analyses for calculating summary risk estimates. Only singletons born after slow freezing and transfer of cleavage stage embryos were included in the meta-analyses.

Statistics

Maternal characteristics and perinatal outcomes were summarized using SPSS, version 19.0. Means were compared with Student’s t-test and proportions with the \( \chi^2 \) test. A significance level of \( p < 0.05 \) was considered statistically significant.


In the sibling analyses, where the two groups of Fresh/FET and FET/sibling were combined, additional adjustment for birth order was performed, and a random effect was included to account for correlation between sibling birthweights. Risk differences were reported as crude odds ratios (ORs) and adjusted odds ratios (AORs) with 95% confidence intervals (95% CIs).

Meta-analyses were performed to compare the AORs for the risk of being born LGA or with a birthweight of >4500 g from the two cohort studies (Pelkonen et al., 2010; Sazonova et al., 2012) with our own data. Owing to the clinical heterogeneity from the different study cohorts, a random-effects model was chosen for the analyses. Tests of heterogeneity were not performed, as they are not valid when based on only a few studies (Borenstein et al., 2009). Meta-analyses were performed using the rmeta package in R statistical software, version 2.15.3 (Lumley, 2012; R Core Team, 2013).

Results

Total FET cohort

Maternal characteristics and perinatal outcomes are reported in Table I. In singletons born after cryopreserved/thawed embryos, the maternal age was significantly higher than in singletons conceived by Fresh embryo transfer and NC. More FET singletons had primiparous mothers compared with NC singletons; however, fewer were primiparous compared with the fresh embryo transfer group.

Intracytoplasmic sperm injection (ICSI) as a fertilization method was more frequent in children conceived after Fresh embryo transfer (34.8%) compared with those born after FET (28.1%) (\( P < 0.001 \)). FET children had a lower risk of PT, low birthweight (LBW) and SGA than children from Fresh embryo transfer. The median birthweight of FET singletons was higher compared with that of NC singletons, but the mean gestational age was lower. FET and NC showed no dissimilarities regarding LBW and very low birthweight, although the risk of preterm birth was increased in FET compared with NC. In all, 2.5% of the FET singletons were born SGA versus 4.3% (\( P = 0.008 \)) and 3.0% in children born after Fresh embryo transfer and in NC children, respectively.

The adjusted risk of being SGA in FET versus Fresh was AOR 0.56 (95% CI 0.36–0.88) (Table II). The adjusted risk for placenta praevia (PP) in FET versus NC singletons was AOR 2.80 (95% CI 1.08–7.26) and that for PP after Fresh versus NC was AOR 5.52 (95% CI 3.04–10.01). We found no association between PP and the risk of being SGA in either of the cohorts.

Sibling cohort

In the sibling cohort with the first child conceived after FET and the second conceived after Fresh embryo transfer, the mean birthweight of the FET siblings was significantly higher than that of the siblings conceived after Fresh embryo transfer (Table I). The mean difference in birthweight was greater than expected, as the increase in birthweight from child number one to child number two has been shown to be ~100 g (Romundstad et al., 2008). In contrast with the expected increase in mean birthweight from the first-born child to the second-born child, we found that, in the sibling cohort with first child FET/second child Fresh, there was a decrease in birthweight though not significant from child number one to child number two. In the sibling cohort first child Fresh/second child FET, the prevalence of SGA was significantly higher for the children born after Fresh embryo transfer. For all other perinatal outcomes, there was no difference between the FET sibling and the sibling conceived after Fresh embryo transfer (Table I).

Large for gestational age

Total FET cohort

Significantly more FET singletons were LGA (z-score >2) and more had birthweight of >4500 g compared with both control groups (Table I). The percentage with LGA (z-score >2) was 5.8, 4.0 and 3.9 in FET, fresh and NC singletons, respectively. The percentage of macrosomic babies (birthweight >4500 g) was 5.7% among FET singletons, which was significantly higher compared with both the Fresh (2.8%) and naturally conceived group (3.4%) (Table I). Concerning the fertilization method, we observed no significant differences in the rate of LGA and macrosomia between IVF and ICSI, either after FET or Fresh embryo transfer. The AOR of being LGA (z-score >2) was 1.34 (95% CI 0.98–1.80) in the FET versus the Fresh group, and the AOR for macrosomia was 1.91 (95% CI 1.40–2.62) (Table II). The corresponding AORs for FET versus NC singletons were for macrosomia 1.67 (95% CI 1.18–2.37) and for LGA AOR 1.41 (95% CI 1.01–1.98). In the multivariate analyses, the only significant predictors of LGA were FET transfer and previous childbirth.
**Table I** Maternal characteristics and perinatal outcomes in (A) singletons born after cryopreserved/thawed embryos (FET), Fresh embryo transfer (Fresh) and NC in Denmark 1997–2006 and in (B) singleton sibling pairs, where one sibling is born after FET and the other after Fresh embryo transfer in Denmark 1994–2008

<table>
<thead>
<tr>
<th>(A) Singletons born after cryopreserved/thawed embryos (FET)</th>
<th>(B) Singleton sibling pairs</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Singletons (N)</strong></td>
<td><strong>Fresh–FET siblings</strong></td>
</tr>
<tr>
<td><strong>FET</strong></td>
<td><strong>Fresh</strong></td>
</tr>
<tr>
<td>896</td>
<td>9480</td>
</tr>
<tr>
<td><strong>Maternal age (years), mean (SD)</strong></td>
<td><strong>Fresh singletons</strong></td>
</tr>
<tr>
<td>34.0 (3.8)</td>
<td>33.6 (4.0)</td>
</tr>
<tr>
<td>Primiparous (%)</td>
<td>54.1</td>
</tr>
<tr>
<td>51.6</td>
<td>71.3</td>
</tr>
<tr>
<td>Male sex (%)</td>
<td>51.6</td>
</tr>
<tr>
<td>Perinatal outcome</td>
<td>50.5</td>
</tr>
<tr>
<td>Birthweight (g), mean (SD)</td>
<td>3592 (622)</td>
</tr>
<tr>
<td>Gestational age (days), mean (SD)</td>
<td>277.1 (15)</td>
</tr>
<tr>
<td>&lt;32 weeks (%)</td>
<td>1.2</td>
</tr>
<tr>
<td>&lt;37 weeks (%)</td>
<td>6.5</td>
</tr>
<tr>
<td>&lt;1500 g (%)</td>
<td>1.0</td>
</tr>
<tr>
<td>&lt;2500 g (%)</td>
<td>4.2</td>
</tr>
<tr>
<td>&gt;4500 g (%)</td>
<td>5.7</td>
</tr>
<tr>
<td>SGA (&lt; −2 SD)</td>
<td>2.5</td>
</tr>
<tr>
<td>LGA (&gt;1 SD) (%)</td>
<td>24.4</td>
</tr>
<tr>
<td>LGA (&gt;2 SD) (%)</td>
<td>5.8</td>
</tr>
<tr>
<td>LGA (&gt;3 SD) (%)</td>
<td>1.5</td>
</tr>
<tr>
<td>PP (%)</td>
<td>0.9</td>
</tr>
</tbody>
</table>

FET, frozen embryo transfer; LGA, large for gestational age; NC, naturally conceived, non-significant; SGA, small for gestational age; PP, placenta praevia.
the pre-

Meta-analyses

The previously published cohort studies on LGA in FET singletons are included in Table IV. The AOR of LGA in FET singletons found in the previously published studies together with the results from our study was used in the meta-analyses to estimate the summary effects of LGA and macrosomia in FET versus Fresh singletons and in FET versus NC singletons. Forest plots of the pooled estimates are shown in Figs 1–4. The pooled estimate of LGA (z-score > 2) in FET versus Fresh singletons was AOR 1.54 (95% CI 1.31–1.81), while the pooled estimate for FET versus NC was AOR 1.32 (95% CI 1.07–1.61). For birthweight of >4500 g, the AOR in FET versus Fresh was 1.64 (95% CI 1.26–2.12) and in FET versus NC AOR was 1.41 (95% CI 1.11–1.80).

The Japanese study included only singletons born after vitrified/thawed embryos and LGA was defined according to the 10th percentile; hence, the results of the study were not included in the meta-analyses (Kato et al., 2012).

**Discussion**

FET singletons were at an increased risk of being born LGA and of being macrosomic compared with singletons born after conception with fresh embryos and with naturally conceived singletons. After adjustments, the increased risk remained in all analyses apart from the risk of being LGA (z-score > 2) in FET versus Fresh singletons, whereas the increased risk no longer reached statistical significance AOR 1.34 (95% CI 0.98–1.80). When included in the meta-analyses with the two previously

**Table II** The risk of being born SGA or LGA in singletons born after cryopreserved/thawed embryos (FET) versus Fresh embryo transfer (Fresh) and in singletons from NC in the total population born in 1997–2006.

<table>
<thead>
<tr>
<th>OR (95% CI)</th>
<th>FET versus Fresh</th>
<th>FET versus fresh</th>
<th>FET versus NC</th>
<th>FET versus NC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Crude OR</td>
<td>Adjusted OR</td>
<td>Crude OR</td>
<td>Adjusted OR</td>
</tr>
<tr>
<td>Total FET cohort</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birthweight &gt;4500 g</td>
<td>2.08 (1.53–2.83)</td>
<td>1.91 (1.40–2.62)</td>
<td>1.71 (1.23–2.36)</td>
<td>1.67 (1.18–2.37)</td>
</tr>
<tr>
<td>LGA (&gt;1 SD)</td>
<td>1.80 (1.53–2.12)</td>
<td>1.65 (1.40–1.94)</td>
<td>1.55 (1.30–1.84)</td>
<td>1.54 (1.28–1.85)</td>
</tr>
<tr>
<td>LGA (&gt;2 SD)</td>
<td>1.49 (1.10–2.01)</td>
<td>1.34 (0.98–1.80)</td>
<td>1.53 (1.11–2.10)</td>
<td>1.41 (1.01–1.98)</td>
</tr>
<tr>
<td>LGA (&gt;3 SD)</td>
<td>1.35 (0.76–2.42)</td>
<td>1.18 (0.65–2.11)</td>
<td>1.78 (0.94–3.36)</td>
<td>1.78 (0.90–3.54)</td>
</tr>
<tr>
<td>SGA (&lt;–2 SD)</td>
<td>0.56 (0.36–0.87)</td>
<td>0.56 (0.36–0.88)</td>
<td>0.82 (0.52–1.29)</td>
<td>0.69 (0.42–1.15)</td>
</tr>
</tbody>
</table>

Risks are presented as crude OR and AOR with 95% CI. FET, frozen embryo transfer; Fresh, fresh embryo transfer; OR, odds ratio; SGA, small for gestational age; LGA, large for gestational age; NC, naturally conceived.

**Table III** The risk of being born SGA and LGA in singleton sibling pairs born after cryopreserved/thawed embryos (FET) versus Fresh embryo transfer (Fresh).

<table>
<thead>
<tr>
<th>OR (95% CI)</th>
<th>FET versus Fresh singletons in the first child</th>
<th>FET versus Fresh singletons in the total sibling cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Crude OR Adjusted OR a</td>
<td>Crude OR Adjusted OR b</td>
</tr>
<tr>
<td>Sibling FET cohort</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birthweight &gt;4500 g</td>
<td>1.20 (0.53–2.78)</td>
<td>1.12 (0.45–2.78)</td>
</tr>
<tr>
<td>LGA (&gt;1 SD)</td>
<td>3.03 (2.08–4.55)</td>
<td>3.33 (0.71–16.67)</td>
</tr>
<tr>
<td>LGA (&gt;2 SD)</td>
<td>4.55 (1.85–11.11)</td>
<td>3.45 (1.33–8.33)</td>
</tr>
<tr>
<td>LGA (&gt;3 SD)</td>
<td>2.00 (0.50–8.33)</td>
<td>–</td>
</tr>
<tr>
<td>SGA (&lt;–2 SD)</td>
<td>0.30 (0.09–0.93)</td>
<td>0.69 (0.28–1.67)</td>
</tr>
</tbody>
</table>

Risks are presented as crude OR and AOR with 95% CI. SGA, small for gestational age; LGA, large for gestational age; FET, frozen embryo transfer; OR, odds ratio.

aAdjustments were made for maternal age (<30, 30–34.9, 35–39.9 and >40 years), primiparity (yes/no) and year of birth (1997–1999, 2000–2003 and 2004–2006). The risk of macrosomia (birthweight >4500 g) was further adjusted for sex of the child.

bIn the combined sibling cohorts, analyses were further adjusted for order of birth.

cOnly adjusted for parity and year of birth.

**Sibling FET cohort**

In the sibling cohort (first child Fresh/second child FET), significantly more children conceived after FET (7.6%) were born LGA compared with the fresh group (1.8%). This was confirmed in the multivariate analyses, where FET singletons had an increased OR of 3.45 (95% CI 1.33–8.33) of LGA after adjusting for maternal age, parity and year of birth (Table III). When adding both cohorts of first child Fresh/second child FET and first child FET/second child Fresh siblings and further adjusting for order of birth, the risk of LGA diminished but was still more than double for the FET singletons, AOR 2.50 (95% CI 1.04–5.88).
### Table IV  Existing studies on SGA and LGA in singletons conceived by cryopreserved/thawed embryo transfer (FET) and in those born after Fresh embryo transfer (Fresh) and NC.

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Country</th>
<th>Design, study period</th>
<th>Singleton, N</th>
<th>SGA % (95% CI)</th>
<th>SGA P-values</th>
<th>SGA Crude OR (95% CI)</th>
<th>SGA Adjusted OR (95% CI)</th>
<th>LGA % (95% CI)</th>
<th>LGA P-values</th>
<th>LGA Crude OR (95% CI)</th>
<th>LGA Adjusted OR (95% CI)</th>
<th>Covariates included in the adjusted analyses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pelkonen et al. (2010)</td>
<td>Finland</td>
<td>National register-based cohort study, 1995–2006</td>
<td>Slow freeze Trans D2–3</td>
<td>FET 1830</td>
<td>1.5</td>
<td>FET versus Fresh 0.55 (0.43–0.71)</td>
<td>FET versus Fresh 0.63 (0.49–0.83)</td>
<td>FET versus Fresh 1.86 (1.33–2.61)</td>
<td>FET versus Fresh 1.70 (1.21–2.40)</td>
<td>Maternal age, parity, socioeconomic status and number of fetuses</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sazonova et al. (2012)</td>
<td>Sweden</td>
<td>National register-based cohort study, 2002–2006</td>
<td>Slow freeze Trans D2–3</td>
<td>Cryo SET 1533</td>
<td>2.0</td>
<td>NA</td>
<td>Cryo SET/DET versus fresh SET/DET 0.72 (0.54–0.96)</td>
<td>Cryo SET/DET versus fresh SET/DET 0.78 (0.58–1.04)</td>
<td>Cryo SET/DET versus fresh SET/DET 1.74 (1.39–2.16)</td>
<td>Maternal age, parity, BMI, smoking, years of infertility, no. oocytes, number of transferred embryos/ cryopreserved embryos, vanishing twins</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kato et al. (2012)</td>
<td>Japan</td>
<td>Single-center retrospective cohort study 2006–2008</td>
<td>Vitrification Trans D2 + D5</td>
<td>Vitrified SET 4092</td>
<td>3.6</td>
<td>(3.0–4.2)</td>
<td>Vitrified SET 0.49 (0.39–0.61)</td>
<td>Vitrified SET 0.43 (0.33–0.56)</td>
<td>Vitrified SET 1.51 (1.32–1.74)</td>
<td>Maternal age, BMI, parity, type of stimulation, ICSI, blastocyst culture, infant sex</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Risk estimates are presented as crude OR and AOR with 95% CI. Bolded values are statistically significant values.

Definitions of SGA and LGA: Pelkonen: SGA: birthweight < mean – 2 SD and LGA: birthweight > mean + 2 SD [mean in the Finnish population according to sex (Pihkala et al., 1989)]. Sazonova: SGA: birthweight < mean – 2 SD and LGA: birthweight > mean + 2 SD [mean in the Swedish population according to sex (Marsal et al., 1996)]. Kato: <10th or >90th percentile using Japanese reference tables for singleton newborns stratified by infant sex and paternal parity.

SET, single embryo transfer; DET, double embryo transfer; SD, standard deviation; cryo, cryopreserved/thawed embryos; NA, non-available; NC, naturally conceived/general population; SGA, small for gestational age; LGA, large for gestational age; OR, odds ratio; BMI, body mass index; ICSI, intracytoplasmic sperm injection.
published reports, all summary effects were statistically significant with FET singletons being at risk of LGA and macrosomia, both compared with the Fresh embryo transfer and the naturally conceived group (Pelkonen et al., 2010; Sazonova et al., 2012). We did not include the Japanese cohort study by Kato et al. (2012), as their singleton cohort was born after vitrification of embryos and included a mixture of transfer with cleavage stage embryo and blastocysts. Furthermore, Kato et al. calculated LGA according to the 10th percentile opposing the Nordic studies, where a z-score of >2 SD was used. As both studies included in the meta-analyses were based on Nordic populations, the summary effects can only be extrapolated to Nordic populations.

However, there are indications that higher birthweight in FET singletons is also present in other populations. More than 10 years ago, a French group demonstrated that the prevalence of SGA singletons conceived by FET was half that compared with the rate after fresh embryo transfers (Olivennes et al., 2002). Later, Shih et al. (2008) in an Australian single-center cohort study found that singletons from FET had a higher mean birthweight z-score compared with those conceived by Fresh embryo transfer (+0.17 SD versus −0.131 SD, P < 0.001). Further figures from the Japanese population confirmed a higher risk of being born LGA according to the 10th percentile in singletons from vitrified embryos (Kato et al., 2012). In their study, however, the risk increase disappeared after adjustment for culture length. In the Nordic studies, including our own population, only slow freeze and cleavage stage transfers were performed during the investigated period. A recent Finnish study of 1079 singletons conceived after Fresh embryo transfer revealed that maternal BMI, parity and long in vitro culture had a significant effect on birthweight (Mäkinen et al., 2013). Blastocyst culture significantly increased the risk of being born LGA; however, sample size was small as only 69 children were born after Day 5 or 6 transfer, and of these, 13 were born LGA (Mäkinen et al., 2013). Another large Australian study with 1716 singletons revealed no increased risk of LGA after transfer of fresh blastocysts compared with Days 2–4 transfer (Fernando et al., 2012). If long-term in vitro culture is associated with LGA, this explains why the increased risk of LGA in singletons after vitrification disappeared after adjustment for blastocyst culture (Kato et al., 2012). However, Wikland et al. (2010) found no increased prevalence of LGA in 106 singletons born after transfer of vitrified blastocysts compared with fresh blastocyst transfer and slow freeze cleavage stage transfer (Wikland et al., 2010). Nevertheless, a small study from China revealed that 256 singleton babies born after conception with vitrified Day 3 embryos had significantly higher mean birthweight than in 421 singletons born after Fresh embryo transfer (Shi et al., 2012). These studies were not included in Table IV, as no risk calculations of LGA were performed. With the current knowledge, no firm conclusions can be drawn on the risk of LGA after transfer of vitrified/thawed embryos or after blastocyst transfer with either fresh or frozen embryos. Further residual bias due to incomplete adjustment for maternal age could be present, as FET babies have older mothers.

Our study is the first to explore the risk of being born LGA in a FET/Fresh sibling cohort. In the sibling cohort with first child Fresh/second child FET, the risk of LGA in the FET sibling was significantly increased...
in the pooled risk estimate compared with the sibling conceived after replacement of fresh embryos AOR 3.45 (95% CI 1.33–8.33). This could be related to the birth order as the FET sibling was the second born. However, in the complete sibling cohort where first child FET/second child Fresh sibling pairs were included, the risk of being born LGA in the FET sibling remained significantly increased though lowered [AOR 2.50 (95% CI 1.04–5.88)] compared with Fresh sibling. The sibling analyses indicate that, even in the same mother, the child born after FET is at increased risk of LGA; therefore, this cannot be related only to intrinsic maternal factors but also to factors related to the freezing/thawing procedures themselves. A limitation of our study is that we were not able to adjust for confounding factors such as maternal BMI and gestational diabetes. In the Swedish study, however, adjustments for BMI did not alter the adjusted risk of LGA in singletons conceived after transfer of cryopreserved/thawed embryos (Sazonova et al., 2012). Furthermore, we do not expect that mothers of FET singletons are more obese than those of singletons conceived by Fresh embryo transfer nor have a higher rate of gestational diabetes. In a previous study, our group has shown that BMI in an infertile Danish population do not differ from the background population (Pinborg et al., 2011).

The findings of a higher risk of LGA in singletons born after FET are opposite to the fact that singletons conceived after Fresh embryo transfer have an increased risk of being SGA (Helmerhorst et al., 2004; Jackson et al., 2004; McDonald et al., 2009). The cause of the higher risk of being born LGA in FET singletons remains unknown. It is well known that birth order plays a role, as the second-born child in general is heavier than the first-born (Romundstad et al., 2008). This is in accordance with the results from our sibling cohort, where the highest risk of being born LGA in FET versus Fresh was seen in the sibling cohort with the first child born after Fresh embryo transfer and the second child born after FET. In the cohort with the FET sibling born as the first child, the risk of being born LGA was also increased in the FET sibling however lower. Moreover, a risk increase of being born LGA in FET singletons remained even after adjusting for parity in the complete sibling cohort and after adjusting for birth order. This indicates that the freezing and thawing procedures may play an independent role for the growth potential of the fetus. This may be due to epigenetic alterations at the early embryonic stages during freezing/thawing. There are similarities to the ‘large offspring syndrome’ observed in animals after in vitro culture, though this is also associated with severe organ and placental abnormalities, which are not seen in FET children (Young et al., 1998; Sinclair et al., 2000; Grace and Sinclair, 2009).

As LGA in human singletons from FET transfer is not related to an increased risk of malformations, there is no reason to assume that the higher risk of LGA in FET singletons mirrors the ‘large offspring syndrome’ observed in animals. In a review, Grace and Sinclair (2009), based on animal experiments, claimed that subtle epigenetic modifications to non-imprinted loci in gametes and the pre-implanted embryo may have health consequences which become manifest in adulthood (Grace and Sinclair, 2009). Studies including ART children have yielded that in vitro techniques may alter long-term health risks such as increased blood pressure and fasting glucose in adulthood (Hart and Norman, 2013).
The possible asynchrony between the embryo and the endometrium in FET cycles may also influence fetal growth and development. Another hypothesis is that IVF singletons are prone to intrauterine overgrowth, but this potential is concealed by the ovarian stimulation drug therapy and endocrine profile in the early IVF pregnancy. Hence, the compromised intrauterine environment in fresh cycles after the controlled ovarian stimulation may be compromised rather than a deficiency in the frozen cycles. However, this does not explain the higher risk of being LGA in FET singletons compared with the naturally conceived group. The mechanisms behind epigenetic modification in human embryos and the relation between cryopreservation and thawing of embryos remain to be explored.

Meta-analyses

Meta-analyses with pooling of data on the risk of being born LGA in FET singletons have not been performed prior to this study. Although we found that all summary risk estimates on the risk of being born LGA or with macrosomia for FET singletons versus singletons form Fresh embryo transfer and versus singletons from NC were significantly increased, it should be stressed that only two other studies were included apart from our own study. Owing to the scarcity of studies, tests for heterogeneity are not relevant and we decided to use the random-effect model which allows for heterogeneity. Furthermore, the random-effect model allows extrapolation to similar populations which the fixed-effect model does not allow. Although only three studies were included, the total number of FET singletons in the meta-analyses was n = 5,074; therefore, the summary effects were based on a large pooled population.

Conclusion

FET singletons have an increased risk of being born LGA, which is not explainable by maternal factors or birth order. Two studies have shown that FET singletons have a lower risk of being SGA than singletons conceived by Fresh embryo transfer (Pelkonen et al., 2010; Kato et al., 2012), while one study showed that the risk of SGA was not significantly lower in FET singletons (Sazonova et al., 2012). The improved perinatal outcome in FET singletons was considered reassuring and explained by the mild or even absent ovarian stimulation in FET cycles and/or the positive selection of embryos and patients. How to interpret the lower risk of being born SGA and the higher risk of being born LGA in FET singletons is yet uncertain. Several adverse outcomes such as stillbirth, asphyxia, shoulder dystocia, hypoglycemia, respiratory distress and perinatal mortality are increased in macrosomic babies (Henriksen 2008). Hence, the higher risk of LGA in FET singletons should raise concern, and efforts should be made to outline the causal pathways between freezing and thawing of embryos and growth potential. Future follow-up studies revealing metabolic physiology, including cardiovascular, endocrine and weight status on FET children during child- and adulthood, are needed. The knowledge on epigenetics and in particular metabolomics of FET is very limited; neither do we know the genetic programming. This should be addressed in future animal studies.

Authors’ roles

A.P. participated in study design, execution, analysis, manuscript drafting and critical discussion. A.A.H. participated in study design, execution, analysis, manuscript drafting and critical discussion. A.L. participated in study design, manuscript drafting and critical discussion. S.S.M. participated in study design, manuscript drafting and critical discussion. J.F., participated in study design, analysis and performed the meta-analyses, manuscript drafting and critical discussion. A.N.A. participated in study design, manuscript drafting and critical discussion.

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Conflict of interest

None declared.

References


