Perinatal outcomes of children born after frozen-thawed embryo transfer: a Nordic cohort study from the CoNARtAs group

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STUDY QUESTIONS: What are the risks of adverse outcomes in singletons born after frozen-thawed embryo transfer (FET)?

SUMMARY ANSWER: Singletons born after FET have a better perinatal outcome compared with singletons born after fresh IVF and ICSI as regards low birthweight (LBW) and preterm birth (PTB), but a worse perinatal outcome compared with singletons born after spontaneous conception.

WHAT IS KNOWN ALREADY: Previous studies have shown a worse perinatal outcome in children born after IVF in general compared with children born after spontaneous conception. In singletons born after FET, a lower rate of PTB and LBW and a higher rate of large for gestational age (LGA) compared with singletons born after fresh IVF have been shown.

STUDY DESIGN: A retrospective Nordic population-based cohort study of all singletons conceived after FET in Denmark, Norway and Sweden until December 2007 was performed.

PARTICIPANTS/MATERIALS, SETTING AND METHODS: Singletons born after FET (n = 6647) were compared with a control group of singletons born after fresh IVF and ICSI (n = 42 242) and singletons born after spontaneous conception (n = 288 542). Data on perinatal outcomes were obtained by linkage to the national Medical Birth Registries. Odds ratios were calculated for several perinatal outcomes and adjustments were made for maternal age, parity, year of birth, offspring sex and country of origin.

MAIN RESULTS AND THE ROLE OF CHANCE: Singletons born after FET had a lower risk of LBW (adjusted odds ratio (aOR) 0.81, 95% confidence interval (CI) 0.71–0.91), PTB (aOR 0.84, 95% CI 0.76–0.92), very PTB (VPTB; aOR 0.79, 95% CI 0.66–0.95) and small for gestational age (SGA; aOR 0.72, 95% CI 0.62–0.83), but a higher risk of post-term birth (aOR 1.40, 95% CI 1.27–1.55), LGA (aOR 1.45, 95% CI 1.27–1.64), macrosomia (aOR 1.58, 95% CI 1.39–1.80) and perinatal mortality (aOR 1.49, 95% CI 1.07–2.07) compared with singletons born after fresh IVF and ICSI. Compared with children born after spontaneous conception, singletons born after FET had a higher risk of LBW (aOR 1.27, 95% CI 1.13–1.43), very LBW (aOR 1.69, 95% CI 1.33–2.15), PTB (aOR 1.49, 95% CI 1.35–1.63), VPTB (aOR 2.68, 95% CI 2.24–3.22), SGA (aOR 1.18, 95% CI 1.03–1.35), LGA (aOR 1.29, 95% CI 1.15–1.45), macrosomia (aOR 1.29, 95% CI 1.15–1.45) and perinatal (aOR 1.39, 95% CI 1.03–1.87) neonatal (aOR 1.87, 95% CI 1.23–2.84) and infant mortality (aOR 1.92, 95% CI 1.36–2.72). When analyzing trends over time, the risk of being born LGA increased over time for singletons born after FET compared with singletons born after fresh IVF and ICSI (P = 0.04).
Cryopreservation of embryos has for many years been a well-established part of assisted reproduction technology (ART) with increasing delivery rates as reported by European and US registries (Ferraretti et al., 2012; Sunderam et al., 2012). The pregnancy and live birth rates after cryopreservation have increased and are now described to be close to or even higher (Roque et al., 2013) compared with fresh cycles. Results from randomized trials (Aflatoonian et al., 2010; Shapiro et al., 2011, 2012) and a meta-analysis (Roque et al., 2013) have indicated a significantly higher clinical pregnancy rate after cryopreservation compared with fresh cycles. In addition, both the increasing use of single-embryo transfer and the introduction of more effective cryopreservation techniques, such as vitrification, have made more embryos available for freezing and increased the use of cryopreservation of human embryos.

Safety aspects of different ART techniques are of profound importance. Several large cohort studies (Pelkonen et al., 2010; Pinborg et al., 2010; Sazonova et al., 2012), systematic reviews (Wennerholm et al., 2009) and meta-analyses (Maheshwari et al., 2012; Pinborg et al., 2012) have shown a similar or even better outcome for singletons after cryopreservation compared with singletons conceived after fresh cycles, while compared with singletons from spontaneous conception, the outcome has been less good. However, recently higher rates of newborns being large for gestational age (LGA) and macrosomic (birthweight ≥ 4500 g) compared with both singletons from fresh cycles and singletons from spontaneous conception have been described (Pelkonen et al., 2010; Pinborg et al., 2010; Sazonova et al., 2012). The consequences and explanations for being born large remain unclear.

The aim of this study was, in a large Nordic collaboration study, to investigate the risks for adverse outcomes, assessed as low birthweight (LBW), preterm birth (PTB), small for gestational age (SGA), LGA and perinatal mortality in singletons born after cryopreservation compared with singletons born after fresh embryo transfer and singletons born after spontaneous conception. A further aim was to analyze trends in neonatal outcomes after cryopreservation over time.

Data sources
We used a Nordic population-based cohort of all singletons conceived after frozen embryo transfer (FET) in Denmark, Norway and Sweden from when cryopreservation of embryos was initiated in the different countries until December 2007. Only children with a gestational age of 22 + 0 weeks or more were included. Data on the embryo freezing method and culture time were not available, but during the study period, the vast majority of all FETs were slow-freeze day-2-transfers. Singletons born after FET were compared with: (i) all singletons conceived after fresh IVF and ICSI treatment in the three Nordic countries and (ii) a group of singletons conceived after spontaneous conception. The size of the control group of spontaneously conceived children was four-fold the size of the total group of ART children (children born after FET + children born after fresh IVF and ICSI) and matched on parity (0 versus ≥ 1) and year of birth (Henningsson et al., 2011). LBW was defined as birthweight < 2500 g and very low birthweight (VLBW) as < 1500 g. Macrosomia was defined as birthweight ≥ 4500 g. PTB was birth < 37 weeks of gestation and very preterm birth (VPTB) < 32 weeks of gestation. Postterm birth was birth ≥ 42 + 0 weeks of gestation. Since 2004, > 90% of all Danish women have had the gestational age of their pregnancy determined by ultrasound when participating in the national prenatal screening programme. Before 2004, approximately half of all pregnant women underwent screening for malformations with ultrasoundography during the second trimester. Whenever the gestational age was determined by ultrasound, this overruled the gestational age calculated by either the last menstrual cycle or day of embryo transfer in case of ART. In Sweden and Norway, gestational age was mainly calculated from the second trimester ultrasonography for both ART and spontaneously conceived pregnancies.

Marsal’s formula was used to calculate SGA and LGA, which was defined as a birthweight of less than −2 standard deviations (SD) or greater than +2SD, respectively, according to the reference value with adjustment for gestational age and sex (Marsal et al., 1996). In the analyses of perinatal outcomes, such as LBW and PTB, we excluded stillbirth (n = 1313) and children with missing values of birthweight (n = 1547) and gestational age (n = 3083) as well as children with a gestational age of > 315 days (n = 139) (Fig. 1). During the study period, Sweden has defined stillbirth from 28 + 0 weeks of gestation, Denmark from gestational week 28 + 0 in the beginning of the study period and from gestational week 22 + 0 since April 2004, and Norway even earlier than gestational week 22 + 0. Early neonatal death was defined as death within the first week of life (0–6 days); perinatal mortality covered stillbirth and early neonatal death and neonatal death was death within 0–27 days, whereas infant mortality was death of a live born child within the first year of life (0–365 days).

The study was approved by the Data Protection Agency and register keeping authorities in each participating country. Permission from the ethical committees was given in Norway (REK 2010/1909-11) and Sweden (the Regional Ethical Committee in Sweden, at the University of Gothenburg Onr 023-09, T431-09), but not required in Denmark for register research.

Statistical analysis
When comparing children born after FET with children born after fresh IVF and ICSI treatment, crude analyses were presented together with
multivariate analyses adjusting for: maternal age (< 30; 30–34; 35–39; ≥ 40 years) mother’s parity (0 versus ≥ 1); year of birth, offspring sex and country. In the analyses where children born after FET were compared with spontaneously conceived children, the two groups have, to some extent, been matched on mother’s parity and year of birth (children born after FET with a four-fold the size of a spontaneously conceived control group). The rest of the spontaneously conceived singletons were originally matched controls to the fresh IVF and ICSI singletons four-fold. Maternal ages, birthweights and gestational ages were compared in general linear models. The risks of adverse perinatal outcomes were analyzed using logistic regression. Chi-square tests evaluated the distribution of parity, child sex, mode of delivery and year of birth in the different groups. Analysis was performed comparing trends over time for adverse perinatal outcomes during the time periods: 1982–1991; 1992–1996; 1997–2001; 2002–2004 and 2005–2007. Statistical tests were declared significant for a two-sided \( P \)-value not exceeding 0.05. All analyses were performed using the SAS statistical software, version 9.1 (SAS Institute).

**Results**

A flow-chart of the study groups is shown in Fig. 1. The annual number of children born after FET has increased during the study period. Women pregnant after FET were older and more likely to be multiparous compared with women who had conceived after fresh IVF and ICSI. More Caesarean sections were performed in the FET
group (26.3%) compared with the fresh IVF and ICSI group (22.6%) and
the spontaneously conceived group (15.4%). There was no statistically
significant difference in the sex ratio between the three different
groups (Table I).

Neonatal outcomes in singletons born after
FET compared with singletons born after fresh
IVF and ICSI

The mean birthweight and mean gestational age were higher in singletons
born after FET compared with singletons born after fresh IVF and ICSI
(Table I). Distribution of birthweight in 500 g intervals for singletons
born after FET and fresh IVF and ICSI is shown in Fig. 2.

Singletons born after FET had a lower rate of LBW [adjusted odds ratio
(aOR) 0.81; 95% confidence interval (CI) 0.71–0.91] and PTB (aOR
0.84; 95% CI 0.76–0.92) compared with singletons born after fresh
IVF and ICSI. The rate of post-term birth was higher in the FET group
(aOR 1.40; 95% CI 1.27–1.55) (Table II, Fig. 3). The rate of SGA
(<−2 SD) was lower (aOR 0.72; 95% CI 0.62–0.83) and the rate of
LGA (> +2 SD) was higher (aOR 1.45; 95% CI 1.27–1.64). More
infants were macrosomic in the FET group (aOR 1.58; 95% CI 1.39–
1.80). When stratifying for sex, we found that although both boys and
girls conceived after FET had a higher risk of LGA compared with children
conceived after fresh embryo transfer, the risk for boys was significantly
higher (aOR 1.66; 95% CI 1.40–1.98 versus aOR 1.25; 95% CI 1.03–
1.51, P = 0.049).

The perinatal mortality rate (≥28 weeks) was significantly higher in the
FET group, 0.71 versus 0.64% (aOR 1.49; 95% CI 1.07–2.07) (Table II,
Fig. 4). There was no significant difference in regard to the risk of
stillbirth ≥28 weeks (aOR 1.38; 95% CI 0.87–2.19), neonatal mortality
(aOR 1.36; 95% CI 0.86–2.12) or death within the first year of life (aOR
1.39; 95% CI 0.96–2.03) when comparing singletons born after FET with
Singletons born after replacement of a fresh embryo. Since fewer FET
children were born preterm compared with children conceived after
fresh embryo transfer, the risk of neonatal mortality and infant death
increased when further adjusting for gestational age in the multivariate
analyses (aOR 1.84; 95% CI 1.12–3.01) and (aOR 1.78; 95% CI 1.18–
2.69), respectively.

Table I Characteristics of singletons born after FET and their mothers compared with singletons born after fresh embryo
transfer (IVF, ICSI or IVF/ICSI) and their mothers and singletons born after spontaneous conception (SC) and their mothers.

<table>
<thead>
<tr>
<th></th>
<th>Singletons born after FET (n = 6647)</th>
<th>Singletons born after fresh IVF, ICSI, IVF/ICSI (n = 42 242)</th>
<th>Singletons born after SC (n = 288 542)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Mean (years) ± SD</td>
<td>33.7 ± 3.9</td>
<td>33.3 ± 4.0</td>
<td>28.5 ± 5.0</td>
</tr>
<tr>
<td></td>
<td>&lt;30</td>
<td>1014 (15.3%)</td>
<td>7403 (17.5%)</td>
<td>170 629 (59.1%)</td>
</tr>
<tr>
<td></td>
<td>30–34</td>
<td>2888 (43.5%)</td>
<td>18 218 (43.1%)</td>
<td>83 437 (28.9%)</td>
</tr>
<tr>
<td></td>
<td>35–39</td>
<td>2320 (34.9%)</td>
<td>14 329 (33.9%)</td>
<td>29 406 (10.2%)</td>
</tr>
<tr>
<td></td>
<td>≥40</td>
<td>425 (6.4%)</td>
<td>2292 (5.4%)</td>
<td>5070 (1.8%)</td>
</tr>
<tr>
<td>Parity</td>
<td>0</td>
<td>3306 (49.7%)</td>
<td>28 824 (68.2%)</td>
<td>204 656 (70.9%)</td>
</tr>
<tr>
<td></td>
<td>≥1</td>
<td>3329 (50.1%)</td>
<td>13 393 (31.7%)</td>
<td>83 024 (28.8%)</td>
</tr>
<tr>
<td>Baby’s sex</td>
<td>Male</td>
<td>3360 (50.6%)</td>
<td>21 846 (51.7%)</td>
<td>147 592 (51.2%)</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>3286 (49.4%)</td>
<td>20 372 (48.2%)</td>
<td>140 768 (48.8%)</td>
</tr>
<tr>
<td>Year of birth</td>
<td>≤1987</td>
<td>0</td>
<td>106 (0.3%)</td>
<td>583 (0.2%)</td>
</tr>
<tr>
<td></td>
<td>1988–1992</td>
<td>64 (1.0%)</td>
<td>1636 (3.9%)</td>
<td>13 591 (4.7%)</td>
</tr>
<tr>
<td></td>
<td>1993–1997</td>
<td>706 (10.6%)</td>
<td>6999 (16.6%)</td>
<td>51 893 (18.0%)</td>
</tr>
<tr>
<td></td>
<td>1998–2002</td>
<td>2064 (31.1%)</td>
<td>13 010 (30.8%)</td>
<td>95 450 (33.1%)</td>
</tr>
<tr>
<td></td>
<td>2003–2007</td>
<td>3813 (57.4%)</td>
<td>20 491 (48.3%)</td>
<td>127 025 (44.0%)</td>
</tr>
<tr>
<td>Mode of delivery</td>
<td>Vaginal</td>
<td>4898 (73.7%)</td>
<td>32 707 (77.4%)</td>
<td>244 096 (84.6%)</td>
</tr>
<tr>
<td></td>
<td>Caesarean</td>
<td>1749 (26.3%)</td>
<td>9535 (22.6%)</td>
<td>44 446 (15.4%)</td>
</tr>
<tr>
<td>Birthweight</td>
<td>Mean (grams) ± SD</td>
<td>3552 ± 635</td>
<td>3419 ± 636</td>
<td>3505 ± 569</td>
</tr>
<tr>
<td>Gestational age</td>
<td>Mean (days) ± SD</td>
<td>277.7 ± 15.3</td>
<td>276.4 ± 16.2</td>
<td>278.8 ± 13.4</td>
</tr>
</tbody>
</table>

FET, frozen embryo transfer; SC, spontaneous conception.

*aComparison of FET versus fresh.

*bComparison of FET versus SC.
Neonatal outcomes in singletons born after FET compared with singletons born after spontaneous conception

The mean birthweight was higher in singletons born after FET compared with spontaneously conceived singletons but the mean gestational age was lower (Table I).

Singletons born after FET had higher risk of LBW (aOR 1.27; 95% CI 1.13–1.43), VLBW (aOR 1.69; 95% CI 1.33–2.15), PTB (aOR 1.49; 95% CI 1.35–1.63) and VPTB (aOR 2.68; 2.24–3.22) (Table III, Fig. 3). The rate of SGA was slightly increased in FET children (aOR 1.18, 95% CI 1.03–1.35). But also more singletons in the FET group were LGA (>/=2 SD) and macrosomic (aOR 1.29; 95% CI 1.15–1.45 for both) compared with spontaneously conceived children. When stratifying the risk of being LGA for sex, the risk of being born LGA tended to be higher in boys conceived after FET (aOR 1.41; 95% CI 1.21–1.66) than in girls conceived after FET (aOR 1.17; 95% CI 0.98–1.39), albeit this difference between genders is not significant (P=0.12).

Perinatal mortality (>/=22 weeks) and perinatal mortality (>/=28 weeks) were increased in the FET group, 0.81% versus 0.55% (aOR 1.41; 95% CI 1.06–1.86) and 0.71 versus 0.49% (aOR 1.39; 95% CI 1.03–1.87), respectively (Table III, Fig. 4). Neonatal and infant death were increased (0.36 versus 0.22%; aOR 1.87; 95% CI 1.23–2.84 and 0.53% versus 0.31%; aOR 1.92; 95% CI 1.36–2.72, respectively). Since more FET children were born preterm compared with spontaneously conceived children, the risk of both neonatal and infant death decreased when further adjusting for gestational age (aOR 1.49; 95% CI 0.96–2.33) and (aOR 1.61; 95% CI 1.11–2.32). The rates of stillbirth (>/=22 weeks and >/=28 weeks) did not differ between children born after FET and spontaneous conception.

Discussion

The main finding of this large Nordic cohort study was that children born after FET have an improved outcome regarding PTB, LBW and SGA compared with children born after fresh IVF and ICSI. However, compared with spontaneously conceived controls, the risk of almost all investigated adverse perinatal outcomes was increased in children conceived after FET.

We confirm the higher mean birthweight and the higher proportion of both macrosomic newborns and children being LGA in singletons born after FET compared with both singletons conceived after fresh embryo transfer and spontaneous conception, respectively. This agrees with other studies (Pelkonen et al., 2010; Pinborg et al., 2010; Sazonova et al., 2012). The strength of our study is the large sample size, which was achieved by accessing the population-based data in the national registers, thereby giving accurate risk estimates of several perinatal outcomes (Henningsen et al., 2011). The limitations of this study are that some important maternal background variables are missing. For example, data on smoking and BMI were not available in all Nordic registers. These are variables that could influence the distribution in birthweight. Women who conceive after IVF smoke less and have a higher

Trends over time in neonatal outcomes

Analyses were performed comparing trends over time for adverse perinatal outcomes (LBW, VLBW, birthweight >4500 g, PTB, VPTB, SGA, LGA and perinatal mortality) during five time periods for singletons born after FET versus both singletons born after fresh IVF and ICSI and versus spontaneously conceived singletons.

When comparing singletons born after FET with singletons born after fresh IVF and ICSI, children born after FET were at a significantly increased risk of being born with a birthweight of >4500 g. In the trend analyses, children born after FET were only at a significantly increased risk of macrosomia from 1997 and onwards, but the overall difference in risk of macrosomia did not change over time, P=0.77. The small sample sizes in the early time periods are probably the explanation of why we do not find children born after FET to be at a significantly increased risk of birthweight >4500 g before 1997. When analyzing the risk of being born LGA in the total group of children, it was significantly increased among children born after FET. However, in the trend analyses, the risk of LGA was only significantly increased for children born after FET in the last period from 2005 to 2007 and we confirmed that the difference in risk of LGA actually increased over time, P=0.04. For other outcomes, the sample sizes, in particular in the early time periods, were too small for us to interpret the trends over time.

In the trend analyses, when comparing singletons born after FET with singletons born after spontaneous conception, children born after FET were at a significantly increased risk of being born preterm from 1997 and onwards and at a significantly increased risk of being born with a LBW from 1997 to 2001 and from 2002 to 2004 compared with spontaneously conceived children. Children born after FET were at a significantly increased risk of both outcomes compared with the total group of spontaneously conceived children, and since the overall difference in risk of PTB and LBW did not change between the groups over time (P=0.28 and P=0.36, respectively), it is most likely because of the small sample sizes that we do not find an increased risk of PTB and LBW in the early time periods.
Table II  Children born after FET versus a fresh embryo (IVF, ICSI or IVF/ICSI).

<table>
<thead>
<tr>
<th>Birthweight</th>
<th>Singleton born after FET (n = 6647)</th>
<th>Singleton born after fresh IVF, ICSI, IVF/ICSI (n = 42242)</th>
<th>OR [95%CI]</th>
<th>P-value</th>
<th>aOR* [95%CI]</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LBW (&lt;2500 g)*</td>
<td>311 (4.71%)</td>
<td>2671 (6.38%)</td>
<td>0.73 [0.64–0.82]</td>
<td>&lt;0.0001</td>
<td>0.81 [0.71–0.91]</td>
<td>0.0007</td>
</tr>
<tr>
<td>VLBW (&lt;1500 g)*</td>
<td>73 (1.11%)</td>
<td>610 (1.46%)</td>
<td>0.76 [0.59–0.97]</td>
<td>0.03</td>
<td>0.87 [0.68–1.12]</td>
<td>0.29</td>
</tr>
<tr>
<td>PTB (&lt;37 weeks)*</td>
<td>521 (7.90%)</td>
<td>4091 (9.77%)</td>
<td>0.79 [0.72–0.87]</td>
<td>&lt;0.0001</td>
<td>0.84 [0.76–0.92]</td>
<td>0.0003</td>
</tr>
<tr>
<td>VPTB (&lt;32 weeks)*</td>
<td>136 (2.06%)</td>
<td>1225 (2.93%)</td>
<td>0.70 [0.58–0.84]</td>
<td>&lt;0.0001</td>
<td>0.79 [0.66–0.95]</td>
<td>0.01</td>
</tr>
<tr>
<td>Post-term birth (≥42 weeks)*</td>
<td>534 (8.09%)</td>
<td>2751 (6.57%)</td>
<td>1.25 [1.14–1.38]</td>
<td>&lt;0.0001</td>
<td>1.40 [1.27–1.55]</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>SGA (&lt;–2SD)*</td>
<td>229 (3.48%)</td>
<td>2246 (5.38%)</td>
<td>0.63 [0.55–0.73]</td>
<td>&lt;0.0001</td>
<td>0.72 [0.62–0.83]</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LGA (&gt;2 SD)*</td>
<td>325 (4.94%)</td>
<td>1288 (3.09%)</td>
<td>1.63 [1.44–1.85]</td>
<td>&lt;0.0001</td>
<td>1.45 [1.27–1.64]</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Very large for gestational age (&gt;3 SD)*</td>
<td>60 (0.91%)</td>
<td>275 (0.66%)</td>
<td>1.39 [1.05–1.84]</td>
<td>0.02</td>
<td>1.23 [0.92–1.64]</td>
<td>0.16</td>
</tr>
<tr>
<td>Birthweight ≥4500 g</td>
<td>336 (5.09%)</td>
<td>1293 (3.09%)</td>
<td>1.68 [1.49–1.90]</td>
<td>&lt;0.0001</td>
<td>1.58 [1.39–1.80]</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Birthweight ≥5500 g</td>
<td>2 (0.03%)</td>
<td>18 (0.04%)</td>
<td>0.70 [0.16–3.04]</td>
<td>0.64</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Stillbirth (≥22 weeks)*</td>
<td>2 (0.04%)</td>
<td>211 (0.50%)</td>
<td>0.93 [0.64–1.36]</td>
<td>0.71</td>
<td>1.24 [0.82–1.91]</td>
<td>0.22</td>
</tr>
<tr>
<td>Stillbirth (≥28 weeks)*</td>
<td>24 (0.36%)</td>
<td>145 (0.34%)</td>
<td>1.05 [0.68–1.62]</td>
<td>0.83</td>
<td>1.38 [0.87–2.19]</td>
<td>0.17</td>
</tr>
<tr>
<td>Perinatal mortality (≥22 weeks)*</td>
<td>54 (0.81%)</td>
<td>338 (0.80%)</td>
<td>1.02 [0.76–1.35]</td>
<td>0.92</td>
<td>1.38 [1.02–1.88]</td>
<td>0.04</td>
</tr>
<tr>
<td>Perinatal mortality (≥28 weeks)*</td>
<td>47 (0.71%)</td>
<td>272 (0.64%)</td>
<td>1.10 [0.81–1.50]</td>
<td>0.55</td>
<td>1.49 [1.07–2.07]</td>
<td>0.02</td>
</tr>
<tr>
<td>Neonatal mortality*</td>
<td>24 (0.36%)</td>
<td>153 (0.36%)</td>
<td>1.00 [0.65–1.53]</td>
<td>0.99</td>
<td>1.36 [0.86–2.12]</td>
<td>0.19</td>
</tr>
<tr>
<td>Infant mortality*</td>
<td>35 (0.53%)</td>
<td>201 (0.48%)</td>
<td>1.11 [0.77–1.59]</td>
<td>0.58</td>
<td>1.39 [0.96–2.03]</td>
<td>0.08</td>
</tr>
</tbody>
</table>

OR, odds ratio; CI, confidence interval; aOR, adjusted OR; LBW, low birthweight; VLBW, very low birthweight; PTB, pre-term birth; VPTM, very pre-term birth; SGA, small for gestational age; LGA, large for gestational age; FET, frozen embryo transfer. For percentages, the denominator varies according to outcome: see footnotes which refer to the populations described in Fig. 1. *Adjusted for parity (0 versus >1); year of birth; maternal age (<30; 30–34; 35–39; ≥40 years); child’s sex and country.

BMI than women who conceive spontaneously (Källen et al., 2005). However, in the study by Sazonova et al. (2012), adjustment was made for these variables and still singletons born after FET had a higher rate of LGA and macrosomia compared with the general. Further, the control group of singletons from spontaneous conception was matched to the original ART population including children both from fresh and frozen cycles instead of using the complete birth registries from all countries as controls. However, in a large register study like this, we do not think our way of selecting controls has affected the results, although analyzing the crude estimates might have become a little more difficult.

The current knowledge of infant outcomes after FET is reassuring. A systematic review of perinatal outcomes of children born after FET revealed that the data seemed reassuring, with even higher birthweights and lower rates of PTB and LBW than among children born after fresh IVF and ICSI (Wennerholm et al., 2009). Large registry-based studies from Finland, Denmark and Sweden confirmed this conclusion (Pelkonen et al., 2010; Pinborg et al., 2010; Sazonova et al., 2012). Unfortunately, we could not include data from Finland in the present study as the Finnish Medical Birth Register data used in this combined Nordic database does not collect information on different in vitro techniques (HenningSEN et al., 2011).

The first paper reporting the increased risk of LGA newborns in FET compared with fresh IVF and ICSI children born during 1995–2006 was from Finland (Pelkonen et al., 2010). They compared the two groups in terms of maternal BMI and abnormal oral glucose tolerance test in a subpopulation (born 2004–2006) and found no differences between the groups. Furthermore, the information on insulin-treated diabetes during pregnancy revealed comparable prevalence (Pelkonen et al., 2010). Earlier it was shown that the aetiology of infertility and treatment type do not seem to play important roles in neonatal outcomes and birthweight according to the majority of studies (Wennerholm et al., 1997; Schieve et al., 2004; Poikkeus et al., 2007; Romundstad et al., 2008) concluded that the adverse outcomes of ART compared with those in the general population could be attributable to the factors leading to infertility, rather than to factors related to the in vitro technology. During the time period of our study, the methods for cryopreservation have been quite stable: slow freezing, using dimethyl sulphoxide or propandiol as cryoprotectant, day 2 freezing and transfer in a natural cycle, if possible.
Perinatal outcomes after cryopreservation

The term LGA is not always well defined (Langer, 2000). The classic definition for LGA is a fetal birthweight more than or equal to the 90th percentile for a given gestational age. This type of threshold for abnormality includes a significant proportion of the normal population. We preferred to use the limit of ≥4500 g. The range of birthweight determined genetically is large. Cellular process characteristics of fetal growth are not yet well understood (Langer, 2000). There is significant maternal influence on fetal size, estimated to be ~20%. The difference in fetal size becomes apparent in the third trimester. One of the growth factors having a central role in controlling growth is insulin and the insulin-like growth factor (IGF) system, including their binding proteins and receptors. It has been suggested that IGF-1 is an important regulator of fetal growth in infants with macrosomia of pregnant women without diabetes (Wiznitzer et al., 1998). This autocrine-paracrine mechanism may be affected by epigenetic mechanisms. The epigenetic regulation of the placenta evolves during preimplantation development (Nelissen et al., 2011).

The finding of a significantly increased risk of LGA and increased risk of macrosomia in the FET group compared with the fresh IVF and ICSI group and the spontaneously conceived control group needs further attention. As shown in animal studies, reproductive technology might induce phenotypic effects. For example, in vitro culture methods and composition of culture media may induce large offspring syndrome in ruminants (Young et al., 1998). The mechanism for this effect is unknown and most of the data until now come from animal studies. Culture media have been compared in a mouse model; showing a varying ability to maintain genomic imprinting in comparison with in vitro derived mouse embryos. These effects can be maintained, or even be more pronounced, after freezing and thawing of the embryos. It was also shown that both ovulation induction treatment and embryo culture increased the perturbation of genomic imprinting (Market-Velker et al., 2010a) and according to the authors, this might indicate that multiple ART procedures further disrupt genomic imprinting. Their further study revealed that maternal as well as paternal H19 methylation was perturbed by ovulation induction (Market-Velker et al., 2010b). Recently, a randomized study showed that different media used for culturing of human IVF embryos affect the birthweight of the newborns (Dumoulin et al., 2010; Nelissen et al., 2012). The same group followed these patients during consecutive embryo transfer cycles, including the use of frozen-thawed embryos, and they could show a trend towards a different birthweight also in cryopreservation cycles (Nelissen et al., 2012).

Gestational age was not affected by culture medium. Nelissen et al. (2011) discussed whether this effect was through epigenetic disturbances of imprinted genes in fetal or placental tissues. All culture media systems contain growth factors, antioxidants, cytokines and vitamins, etc., and their effect on human embryos is not known. A Dutch study, analyzing two culture media, noticed no difference in birthweight between the culture methods but they showed higher birthweight after freezing compared with fresh cycles (Vergouw et al., 2012). They discussed that one possible explanation might be in the interaction of the cryoprotectants used with the main enzyme involved in epigenetic programming (De Geyter et al., 2006). Furthermore, a Finnish study has shown that a longer period of in vitro culture might promote LGA babies, as they found that babies born after day 5–6 transfer had significantly higher ORs for LGA compared with babies born after day 2 transfer (Mäkinen et al., 2012). In our study, we found that boys born after FET had a significantly higher risk of LGA than girls born after FET compared with children born after fresh embryo transfer. We do not have an explanation for this. It is interesting to note that a recent Japanese study showed that the risk of LBW after both fresh embryo transfer and FET was higher in female than that in male neonates (Nakashima et al., 2012).

Previous studies have revealed divergent results concerning perinatal mortality. In a Swedish registry study (Sazonova et al., 2012), a higher perinatal mortality was found for singletons born after FET compared with singletons born after fresh cycles, while in other studies no differences have been found, either compared with singletons born after...
The confirmation of an increased rate of LGA after cryopreservation as well as the increased rate of macrosomia and perinatal mortality warrants further research. Our study strengthens the importance of large follow-up studies of children after ART.

**Authors’ roles**

All the authors contributed equally to conception and design of the study, acquisition of data and analysis and interpretation of data. AKH and JF performed the statistical analyses. UBW, AKH, AT and CB drafted the manuscript and revised it. All the authors approved the final version.

**Funding**

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**Table III Children born after FET versus SC.**

<table>
<thead>
<tr>
<th>Birthweight</th>
<th>Singleton born after FET (n = 6647)</th>
<th>Singleton born after SC (n = 288542)</th>
<th>OR [95%CI]</th>
<th>P-value</th>
<th>aORa [95%CI]</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LBW (&lt;2500 g)b</td>
<td>311 (4.71%)</td>
<td>10829 (3.78%)</td>
<td>1.26</td>
<td>&lt;0.0001</td>
<td>1.27</td>
<td>[1.13–1.43]</td>
</tr>
<tr>
<td>VLBW (&lt;1500 g)b</td>
<td>73 (1.11%)</td>
<td>1831 (0.64%)</td>
<td>1.74</td>
<td>&lt;0.0001</td>
<td>1.69</td>
<td>[1.33–2.15]</td>
</tr>
<tr>
<td>PTB (&lt;37 weeks)c</td>
<td>521 (7.90%)</td>
<td>15750 (5.54%)</td>
<td>1.46</td>
<td>&lt;0.0001</td>
<td>1.49</td>
<td>[1.35–1.63]</td>
</tr>
<tr>
<td>VPTB (&lt;32 weeks)c</td>
<td>136 (2.06%)</td>
<td>2181 (0.77%)</td>
<td>2.72</td>
<td>&lt;0.0001</td>
<td>2.68</td>
<td>[2.24–3.22]</td>
</tr>
<tr>
<td>Postterm birth (≥42 weeks)f</td>
<td>534 (8.09%)</td>
<td>24596 (8.65%)</td>
<td>0.93</td>
<td>[0.85–1.02]</td>
<td>0.11</td>
<td>0.95</td>
</tr>
<tr>
<td>SGA (&lt;−2 SD)d</td>
<td>229 (3.48%)</td>
<td>8920 (3.15%)</td>
<td>1.11</td>
<td>[0.97–1.27]</td>
<td>0.13</td>
<td>1.18</td>
</tr>
<tr>
<td>LGA (≥2 SD)d</td>
<td>325 (4.94%)</td>
<td>8817 (3.11%)</td>
<td>1.62</td>
<td>&lt;0.0001</td>
<td>1.29</td>
<td>[1.15–1.45]</td>
</tr>
<tr>
<td>Very large for gestational age (≥3 SD)d</td>
<td>60 (0.91%)</td>
<td>1793 (0.63%)</td>
<td>1.45</td>
<td>[1.12–1.87]</td>
<td>0.005</td>
<td>1.18</td>
</tr>
<tr>
<td>Birthweight ≥4500 gh</td>
<td>336 (5.09%)</td>
<td>9571 (3.35%)</td>
<td>1.55</td>
<td>[1.39–1.73]</td>
<td>&lt;0.0001</td>
<td>1.29</td>
</tr>
<tr>
<td>Birthweight ≥5500 gh</td>
<td>2 (0.03%)</td>
<td>117 (0.04%)</td>
<td>0.74</td>
<td>[0.18–3.00]</td>
<td>0.67</td>
<td>0.77</td>
</tr>
<tr>
<td>Stillbirth (≥22 weeks)j</td>
<td>31 (0.47%)</td>
<td>1071 (0.37%)</td>
<td>1.26</td>
<td>[0.88–1.80]</td>
<td>0.21</td>
<td>1.09</td>
</tr>
<tr>
<td>Stillbirth (≥28 weeks)j</td>
<td>24 (0.36%)</td>
<td>901 (0.31%)</td>
<td>1.16</td>
<td>[0.77–1.74]</td>
<td>0.48</td>
<td>1.01</td>
</tr>
<tr>
<td>Perinatal mortality (≥22 weeks)k</td>
<td>54 (0.81%)</td>
<td>1573 (0.55%)</td>
<td>1.49</td>
<td>[1.14–1.96]</td>
<td>0.004</td>
<td>1.41</td>
</tr>
<tr>
<td>Perinatal mortality (≥28 weeks)k</td>
<td>47 (0.71%)</td>
<td>1403 (0.49%)</td>
<td>1.46</td>
<td>[1.09–1.95]</td>
<td>0.01</td>
<td>1.39</td>
</tr>
<tr>
<td>Neonatal mortalityl</td>
<td>24 (0.36%)</td>
<td>624 (0.22%)</td>
<td>1.67</td>
<td>[1.11–2.52]</td>
<td>0.01</td>
<td>1.87</td>
</tr>
<tr>
<td>Infant mortalityl</td>
<td>35 (0.53%)</td>
<td>888 (0.31%)</td>
<td>1.71</td>
<td>[1.22–2.41]</td>
<td>0.002</td>
<td>1.92</td>
</tr>
</tbody>
</table>

For percentages, the denominator varies according to the outcome: see footnotes which refer to the populations described in Fig. 1. FET, frozen embryo transfer; SC, spontaneous conception; LBW, low birthweight; VLBW, very low birthweight; PTB, pre-term birth; VPTM, very pre-term birth; SGA, small for gestational age; LGA, large for gestational age.

aAdjusted for parity (0 versus ≥1), year of birth, maternal age (≤30, 30-34, 35-39; ≥40 years), child’s sex and country.

bPopulation C (see flow-chart; Fig. 1). Stillbirth and missing data on birthweight excluded.

cPopulation D (flow-chart). Stillbirth, missing data on gestational age and gestational age ≥30 days excluded.

dPopulation E (flow-chart). Stillbirth, missing data on birthweight and gestational age and gestational age ≥315 days excluded.

ePopulation A (flow-chart). Stillbirth from gestational age 22 + 0 weeks. Data from Norway and Denmark from April 2004.

fPopulation A (flow-chart). Stillbirth from gestational age 28 + 0 weeks. Data from Norway, Sweden and Denmark.

ghPopulation A (flow-chart). Stillbirth from gestational age 22 + 0 weeks; early neonatal death (day 0–6).

iPopulation A (flow-chart). Stillbirth from gestational age 28 + 0 weeks; early neonatal death (day 0–6).

jPopulation B (flow-chart). Live born, dead day 0–28.

kPopulation B (flow-chart). Live born, dead day 0–365.

For percentages, the denominator varies according to the outcome: see footnotes which refer to the populations described in Fig. 1. FET, frozen embryo transfer; SC, spontaneous conception; LBW, low birthweight; VLBW, very low birthweight; PTB, pre-term birth; VPTM, very pre-term birth; SGA, small for gestational age; LGA, large for gestational age.

For percentages, the denominator varies according to the outcome: see footnotes which refer to the populations described in Fig. 1. FET, frozen embryo transfer; SC, spontaneous conception; LBW, low birthweight; VLBW, very low birthweight; PTB, pre-term birth; VPTM, very pre-term birth; SGA, small for gestational age; LGA, large for gestational age.

Fresh cycles or compared with singletons in the general population (Pekkonen et al., 2010; Pinborg et al., 2010; Kato et al., 2012). In the present study, the slightly increased risk of perinatal mortality in FET singletons compared with singletons from fresh cycles might be a chance finding due to multiplex comparisons, but when comparing the FET singletons with singletons from spontaneous conception, it seems to be a consistent finding that the risk of perinatal and infant mortality is increased among FET children. We tried to analyze whether perinatal mortality was related to LGA or macrosomia, since more large children were observed among singletons born after FET, but only very few perinatal deaths occurred among the FET singletons that were either LGA or macrosomic.

Several comparisons have been performed in the present study and some differences might of course be due to chance. Adjustment for multiple comparisons was not made in order to not miss important differences between groups. We leave it to the reader to judge whether the stated differences are true differences.

We conclude that embryo freezing does not adversely affect perinatal outcomes in terms of PTB and SGA compared with fresh IVF and ICSI. The confirmation of an increased rate of LGA after cryopreservation as well as the increased rate of macrosomia and perinatal mortality warrants further research. Our study strengthens the importance of large follow-up studies of children after ART.
Conflict of interest

There are no conflicts of interest to declare.

References


