**Frozen versus fresh single blastocyst transfer in ovulatory women: a multicentre, randomised controlled trial**

Daimin Wei*, Jia-Yin Liu*, Yun Sun*, Yuhua Shi*, Bo Zhang*, Jian-Qiao Liu, Jichun Tan, Xiaoyan Liang, Yunxia Cao, Ze Wang, Yingying Qin, Han Zhao, Yi Zhou, Haiqin Ren, Guimin Hao, Xiufeng Ling, Junzhao Zhao, Yunshan Zhang, Xiujuan Qi, Lin Zhang, Xiaohui Deng, Xiaoli Chen, Yimin Zhu, Xiaohong Wang, Li-Feng Tian, Qun Lv, Xiang Ma, Heaping Zhang, Richard S Legro, Zi-Jiang Chen

**Summary**

**Background** Elective single embryo transfer (eSET) has been increasingly advocated, but concerns about the lower pregnancy rate after reducing the number of embryos transferred have encouraged transfer of multiple embryos. Extended embryo culture combined with electively freezing all embryos and undertaking a deferred frozen embryo transfer might increase pregnancy rate after eSET. We aimed to establish whether elective frozen single blastocyst transfer improved singleton livebirth rate compared with fresh single blastocyst transfer.

**Methods** This multicentre, non-blinded, randomised controlled trial was undertaken in 21 academic fertility centres in China. 1650 women with regular menstrual cycles undergoing their first cycle of in-vitro fertilisation were enrolled from Aug 1, 2016, to June 3, 2017. Eligible women were randomly assigned to either fresh or frozen single blastocyst transfer. The randomisation sequence was computer generated, with block sizes of two, four, or six, stratified by study site. For those assigned to frozen blastocyst transfer, all blastocysts were cryopreserved and a delayed frozen-thawed single blastocyst transfer was done. The primary outcome was singleton livebirth rate. Analysis was by intention to treat. This trial is registered at the Chinese Clinical Trial Registry. number ChiCTR-IOR-14005405.

**Findings** 825 women were assigned to each group and included in analyses. Frozen single blastocyst transfer resulted in higher rates of singleton livebirth than did fresh single blastocyst transfer (416 [50%] vs 329 [40%]; relative risk [RR] 1·26, 95% CI 1·14–1·41, p<0·0001). The risks of moderate or severe ovarian hyperstimulation syndrome (four of 825 [0·5%] in frozen single blastocyst transfer vs nine of 825 [1·1%] in fresh single blastocyst transfer; p=0·16), pregnancy loss (134 of 583 [23·0%] vs 124 of 481 [25·8%]; p=0·09), other obstetric complications, and neonatal morbidity were similar between the two groups. Frozen single blastocyst transfer was associated with a higher risk of pre-eclampsia (16 of 512 [3·1%] vs four of 401 [1·0%]; RR 3·13, 95% CI 1·06–9·30, p=0·029).

**Interpretation** Frozen single blastocyst transfer resulted in a higher singleton livebirth rate than did fresh single blastocyst transfer in ovulatory women with good prognosis. The increased risk of pre-eclampsia after frozen blastocyst transfer warrants further studies.

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Research in context

Evidence before this study
We searched PubMed and Cochrane Library from database inception to May 1, 2018, with the keywords “frozen embryo” OR “frozen-thawed cycle” OR “cryopreservation” OR “vitrification” OR “freeze all” AND “fresh embryo”. We identified one Cochrane systematic review published in 2017, and five additional randomised trials that found conflicting results. The Cochrane review reported four randomised trials comparing fresh embryo transfer versus elective frozen embryo transfer. The authors concluded that frozen embryo transfer resulted in lower rates of miscarriage and ovarian hyperstimulation syndrome (OHSS), but a higher rate of pregnancy complications. No difference in the cumulative livebirth rate (based on subsequent embryo transfers of embryos cryopreserved from the study cycle of ovarian stimulation) was found. There was great heterogeneity among the trials included in the Cochrane review in terms of study populations, developmental stages of the transferred embryos, freezing methods, and the number of embryos transferred. The result was dominated by the trial undertaken in women with polycystic ovary syndrome (PCOS). Subsequently, two large randomised trials were undertaken in ovulatory women with cleavage-stage embryo transfer with consistent results showing that elective frozen embryo transfer led to similar rates of pregnancy, pregnancy loss, and livebirth, compared with fresh embryo transfer. Another randomised trial compared frozen versus fresh euploid blastocyst transfer after preimplantation genetic screening and found higher rates of pregnancy and livebirth after frozen embryo transfer. The risk of obstetric complications was not reported. There were two other trials that were respectively undertaken in women with gonadotropin releasing hormone ( GnRH) antagonist regimen and GnRH agonist trigger for ovarian stimulation and in women with elevated progesterone on the day of triggering; results showed no significant difference in the rates of pregnancy and livebirth. In all these trials, up to two embryos were transferred in both the fresh and frozen embryo transfer groups, leading to higher rates of multiple pregnancies and their associated perinatal morbidity. Whether frozen single blastocyst transfer could improve singleton livebirth rate compared with fresh single blastocyst transfer remained to be determined.

Added value of this study
In this multicentre randomised trial, 1650 ovulatory women with good prognosis from 21 fertility centres in China were randomly assigned to undergo either a frozen single blastocyst transfer or a fresh single blastocyst transfer. Frozen single blastocyst transfer resulted in a higher rate of singleton livebirth attributed to a higher rate of implantation than did fresh single blastocyst transfer. Frozen single blastocyst transfer also led to a higher singleton birthweight, which was accompanied by a higher risk of pre-eclampsia. The risks of OHSS, pregnancy loss, and other obstetric complications including preterm delivery and congenital anomalies were similar after frozen and fresh single blastocyst transfer.

Implications of all the available evidence
A strategy to transfer a single frozen blastocyst versus two cleavage-stage embryos results in a marked decrease in twin livebirth rates with a comparable overall livebirth rate. The available evidence on so-called freeze-all strategy suggested the risk–benefit ratio of elective frozen embryo transfer was influenced by several factors, including the patient diagnosis and the stage of embryo transferred. Elective frozen embryo transfer seems a better choice to achieve livebirth for women with PCOS, women with a higher risk of OHSS, and women with good prognosis who are planning to undergo single blastocyst transfer. However, its potential for increased maternal pre-eclampsia, as well as the long-term effects on offspring, warrant further studies.

Methods

Study design and participants
This study was a non-blinded, multicentre, randomised controlled trial undertaken in 21 academic fertility centres in China. The trial was approved by the ethics committees of all study sites. All the couples including female and male partners gave written informed consent. In the past 5 years, with the refinement of techniques for blastocyst culture and in compliance with the guidelines for reducing the risk of multiple pregnancies, the application of single blastocyst transfer has become increasingly popular. Frozen single blastocyst transfer could optimise pregnancy rates and maintain perinatal safety compared with fresh single blastocyst transfer.

In this randomised trial, we compared pregnancy outcomes and obstetric and perinatal complications after frozen versus fresh single blastocyst transfer.
A data and safety monitoring board was established to oversee the study. We have previously published the protocol. 16

This trial included women with regular menses who were undergoing the first cycle of IVF with or without intracytoplasmic sperm injection with an indication of tubal, male, or unexplained infertility. Eligible women were aged 20–35 years, and had a menstrual cycle length of 21–35 days indicative of regular ovulation. Women who were planning cycles of preimplantation genetic testing were excluded from this study, as were those with a diagnosis of a congenital or acquired uterine abnormality (such as a uterine malformation, adenomyosis, submucous myoma, or intrauterine adhesion). We also excluded women with medical conditions that are contraindications to IVF procedures or pregnancy, such as uncontrolled hypertension, known symptomatic heart disease, poorly controlled type 1 or type 2 diabetes, undiagnosed liver disease or dysfunction, renal disease, severe anaemia, history of deep venous thrombosis, history of pulmonary embolus, previous cerebrovascular accident, or history of cervical cancer, endometrial cancer, or breast cancer.

Randomisation and masking
The randomisation sequence was computer generated by statisticians in the data coordinating centre in Shandong University. Blocked randomisation was done with dynamic block sizes of two, four, or six and was stratified by study site. This sequence was entered into the central

Figure: Trial profile
IVF=in-vitro fertilisation. IVM=in-vitro maturation. OHSS=ovarian hyperstimulation syndrome.
online database, which was secured by the username and password login.

Randomisation was done on the third day after oocyte retrieval—ie, day 3 of embryo culture. We chose this point in the IVF cycle for randomisation to ensure comparable ovarian stimulation between groups in this non-blinded trial and to minimise exclusions or crossovers after randomisation due to a low number of embryos or poor embryo development. Women who had already planned to undergo frozen embryo transfer before the day of randomisation at the discretion of local physicians because of hydrosalpinx, premature elevation of progesterone, or a high risk of ovarian hyperstimulation syndrome (OHSS) were excluded. Women were randomly assigned to either fresh single blastocyst transfer group or frozen single blastocyst transfer group by 1:1 ratio. Only women who had four or more high-grade embryos on day 3 of embryo culture were randomly assigned.

Procedures
All participants were given gonadotropin releasing hormone (GnRH) antagonist regimen for ovarian stimulation. Recombinant follicle-stimulating hormone (rFSH, PUREGON; MSD Organon, Oss, Netherlands) was started on day 1–3 of menstrual cycle. The dose adjustment of gonadotropin and the initiation of GnRH antagonist regimen for ovarian stimulation between groups in this non-blinded trial and to minimise exclusions or crossovers after randomisation due to a low number of embryos or poor embryo development. Women who had already planned to undergo frozen embryo transfer before the day of randomisation at the discretion of local physicians because of hydrosalpinx, premature elevation of progesterone, or a high risk of ovarian hyperstimulation syndrome (OHSS) were excluded. Women were randomly assigned to either fresh single blastocyst transfer group or frozen single blastocyst transfer group by 1:1 ratio. Only women who had four or more high-grade embryos on day 3 of embryo culture were randomly assigned.

For the fresh blastocyst transfer group, a single blastocyst was selected and transferred on day 5 of embryo culture (details of embryo transfer procedure are provided in the appendix). If two or more blastocysts were of equal grade, their early scores at cleavage stage were referred for the selection of the single blastocyst. Supernumerary embryos were frozen on day 5 or 6 according to embryo development. If pregnancy was achieved after fresh single blastocyst transfer, luteal phase support was continued until 10 weeks’ gestation.

For the frozen blastocyst transfer group, all blastocysts were vitrified on day 5 or day 6 according to embryo development. Luteal phase support was stopped after randomisation. At least 4 weeks later, the endometrium was prepared either with a natural cycle regimen or a programmed cycle regimen, at the discretion of local investigators. For the natural ovulatory cycle regimen, ovulation was determined by ultrasound monitoring. Oral dydrogesterone (Duphaston, Abbott, OLST, Netherlands) 10 mg twice daily.

Table 1: Baseline characteristics of the intention-to-treat population

<table>
<thead>
<tr>
<th></th>
<th>Frozen embryo transfer (n=825)</th>
<th>Fresh embryo transfer (n=825)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>28±3 (3.0)</td>
<td>28±3 (3.0)</td>
</tr>
<tr>
<td>Body-mass index (kg/m²)</td>
<td>24±4 (3.2)</td>
<td>25±5 (3.3)</td>
</tr>
<tr>
<td>Duration of infertility (years)</td>
<td>3.2±2 (1.2)</td>
<td>3.3±2 (2.3)</td>
</tr>
<tr>
<td>Previous conception</td>
<td>363 (44.0%)</td>
<td>338 (41.0%)</td>
</tr>
<tr>
<td>Indications for IVF</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tubal factor</td>
<td>460 (55.8%)</td>
<td>443 (53.7%)</td>
</tr>
<tr>
<td>Male factor</td>
<td>173 (21.0%)</td>
<td>191 (23.2%)</td>
</tr>
<tr>
<td>Unexplained infertility*</td>
<td>43 (5.2%)</td>
<td>33 (4.0%)</td>
</tr>
<tr>
<td>Combined factors</td>
<td>147 (17.2%)</td>
<td>155 (18.8%)</td>
</tr>
<tr>
<td>Others</td>
<td>7 (0.8%)</td>
<td>3 (0.4%)</td>
</tr>
<tr>
<td>Antral follicle count in both ovaries†</td>
<td>16±4 (5.5)</td>
<td>16±0 (5.1)</td>
</tr>
<tr>
<td>Baseline sex hormone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FSH (IU/L)</td>
<td>6±4 (1.5)</td>
<td>6±4 (1.5)</td>
</tr>
<tr>
<td>LH (IU/L)</td>
<td>4±8 (2.6)</td>
<td>4±8 (2.2)</td>
</tr>
<tr>
<td>Oestradiol (pmol/L)</td>
<td>138±6 (56.4)</td>
<td>137±7 (61.3)</td>
</tr>
<tr>
<td>Total testosterone (nmol/L)</td>
<td>0.9±0.5</td>
<td>0.9±0.5</td>
</tr>
</tbody>
</table>

Data are mean (SD) or n (%). IVF—in-vitro fertilisation. FSH=follicle-stimulating hormone. LH=luteinising hormone. *Unexplained infertility is rarely used as a diagnosis indicator for IVF in China because it is a controversial diagnosis. †Antral follicle count was missing for ten patients in the frozen embryo transfer group and for 17 patients in the fresh embryo transfer group. Total testosterone was missing for 28 patients in the frozen embryo transfer group and for 23 patients in the fresh embryo transfer group.

For the natural ovulatory cycle regimen, oral oestradiol valerate (Progynova, Delpharm Lille, Lys-Lez-Lannoy, France) at a dose of 4–8 mg daily was started on day 1–3 of menstrual cycle. Vaginal progesterone gel (Crinone, Merck Serono, Watford, UK) 90 mg per day and oral dydrogesterone (Duphaston, Abbott, OLST, Netherlands) 10 mg three times daily was administered for luteal phase support after ovulation. A single frozen-thawed blastocyst was transferred on the 5th day after ovulation. If pregnancy was achieved after frozen blastocyst transfer, luteal phase support was continued until 10 weeks’ gestation.

For the frozen blastocyst transfer group, all blastocysts were vitrified on day 5 or day 6 according to embryo development. Luteal phase support was stopped after randomisation. At least 4 weeks later, the endometrium was prepared either with a natural cycle regimen or a programmed cycle regimen, at the discretion of local investigators. For the natural ovulatory cycle regimen, ovulation was determined by ultrasound monitoring. Oral dydrogesterone (Duphaston, Abbott, OLST, Netherlands) 10 mg three times daily was administered for luteal phase support after ovulation. A single frozen-thawed blastocyst was transferred on the 5th day after ovulation. If pregnancy was achieved after frozen blastocyst transfer, luteal phase support was continued until 10 weeks’ gestation. For the programmed cycle regimen, oral oestradiol valerate (Progynova, Delpharm Lille, Lys-Lez-Lannoy, France) at a dose of 4–8 mg daily was started on day 1–3 of menstrual cycle. Vaginal progesterone gel (Crinone, Merck Serono, Watford, UK) 90 mg per day and oral dydrogesterone (Duphaston, Abbott, OLST, Netherlands) 10 mg twice daily were added when the endometrial thickness reached 7 mm or more. A single frozen-thawed
Outcomes of ovarian stimulation and embryo culture and transfer

<table>
<thead>
<tr>
<th>Test</th>
<th>Frozen embryo transfer (n=825)</th>
<th>Fresh embryo transfer (n=825)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days of ovarian stimulation</td>
<td>9.3 (1.5)</td>
<td>9.3 (1.5)</td>
<td>0.33</td>
</tr>
<tr>
<td>Total gonadotropin dose (IU)</td>
<td>1604.4 (501.1)</td>
<td>1598.6 (480.0)</td>
<td>0.81</td>
</tr>
<tr>
<td>Oestradiol level on HCG trigger day (pmol/L)*</td>
<td>12419 (6254)</td>
<td>12341 (6467)</td>
<td>0.81</td>
</tr>
<tr>
<td>Progesterone level on HCG trigger day (nmol/L)†</td>
<td>3.7 (1.7)</td>
<td>3.7 (1.7)</td>
<td>0.77</td>
</tr>
<tr>
<td>Endometrial thickness on HCG trigger day (mm)</td>
<td>10.6 (2.0)</td>
<td>10.6 (2.0)</td>
<td>0.63</td>
</tr>
<tr>
<td>Number of oocytes retrieved</td>
<td>14.0 (5.6)</td>
<td>13.8 (5.7)</td>
<td>0.50</td>
</tr>
<tr>
<td>Number of high-score embryos on day 3‡</td>
<td>7.1 (3.3)</td>
<td>6.8 (2.9)</td>
<td>0.08</td>
</tr>
<tr>
<td>Stage of embryo transferred</td>
<td>0.54</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blastocyst transfer</td>
<td>780/796 (98.0%)</td>
<td>799/812 (98.4%)</td>
<td></td>
</tr>
<tr>
<td>Cleavage-stage embryo transfer</td>
<td>16/796 (2.0%)</td>
<td>13/812 (1.6%)</td>
<td></td>
</tr>
<tr>
<td>Mean number of embryos transferred</td>
<td>1.05 (0.22)</td>
<td>1.03 (0.16)</td>
<td>0.0056</td>
</tr>
<tr>
<td>One embryo transferred</td>
<td>754/796 (94.7%)</td>
<td>791/812 (97.4%)</td>
<td>0.0054</td>
</tr>
<tr>
<td>Two embryos transferred</td>
<td>42/796 (5.3%)</td>
<td>21/812 (2.6%)</td>
<td></td>
</tr>
<tr>
<td>Number of patients who did not undergo embryo transfer</td>
<td>29/825 (3.5%)</td>
<td>13/825 (1.6%)</td>
<td>0.012</td>
</tr>
<tr>
<td>No embryo obtained</td>
<td>10 (1.2%)</td>
<td>9 (1.1%)</td>
<td></td>
</tr>
<tr>
<td>Personal issue</td>
<td>10 (1.2%)</td>
<td>3 (0.4%)</td>
<td></td>
</tr>
<tr>
<td>Natural conception after oocyte retrieval</td>
<td>9 (1.1%)</td>
<td>1 (0.1%)</td>
<td></td>
</tr>
</tbody>
</table>

Data are mean (SD), v(N %), or n (%) HCG=human chorionic gonadotropin. *Number of observations was 725 in the frozen embryo transfer group and 811 in the fresh embryo transfer group. †Number of observations was 823 in the frozen embryo transfer group and 735 in the fresh embryo transfer group. ‡Number of observations was 811 in the frozen embryo transfer group and 811 in the fresh embryo transfer group.

Table 2: Outcomes of ovarian stimulation and embryo culture and transfer

The primary outcome was singleton livebirth. Secondary outcomes were rates of conception, clinical pregnancy, ongoing pregnancy, pregnancy loss, livebirth, moderate and severe OHSS, ectopic pregnancy, pregnancy and perinatal complications, neonatal complication and other adverse events, and birthweight (detailed definitions are provided in the appendix). We did a post-hoc analysis for the outcomes of small for gestational age (SGA), large for gestational age (LGA), the rate of cumulative livebirth within 12 months after the first embryo transfer, and the number of embryos remaining, and time to livebirth. The determination of SGA and LGA was based on the birthweight reference for Chinese populations adjusted for sex and gestational age. SGA was defined as birthweight lower than the 10th percentile of referential birthweight. LGA was defined as birthweight higher than the 90th percentile of referential birthweight.

Statistical analysis

The livebirth rate after single fresh blastocyst transfer in women younger than 35 years was about 50% in our retrospective clinical database. We assumed that an absolute difference of 10% in livebirth rate was of clinical significance and thus aimed to test a difference of 10% of livebirth rate between treatment groups at a significance level of 0.01 with statistical power of 90%. The minimal sample size calculated was 735 for each group. In consideration of a dropout rate of 10%, we planned to enrol 817 women in each group.

The primary outcome was analysed according to the intention-to-treat principle. The difference in the primary outcome—ie, singleton livebirth rate—between the two treatment groups was analysed by the Pearson χ² test. The relative risk and 95% CIs were calculated. The between-group differences in secondary outcomes were compared with the Pearson χ² test. The mean birthweight was compared by the Student’s t test. Secondary per-protocol and per-treatment analyses were done among those who adhered to the protocols and according to the actual treatment that participants received, respectively.

Continuous data were expressed as mean (SD), and between-group differences were tested by the Wilcoxon rank sum test. Categorical data were represented as frequency and percentage; differences in these variables between the treatment groups were assessed by χ² analysis, with Fisher’s exact test for expected frequencies less than five.

We performed post-hoc subgroup analyses based on the concentrations of oestradiol and progesterone on the day of hCG administration and according to the cycle regimens of endometrial preparation for frozen embryo transfer. All analyses were done with SAS software (version 9.4).

This trial is registered at the Chinese Clinical Trial Registry, number ChiCTR-IOR-14005405.

Role of the funding source

The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Recruitment was done between Aug 1, 2016, and June 3, 2017. 1650 women were included and randomly assigned to study groups.
assigned to either fresh or frozen single blastocyst transfer groups (figure). The fresh and frozen groups were comparable in baseline demographics and clinical characteristics (table 1) and the outcomes of ovarian stimulation (table 2).

98 (12%) women who were assigned to the fresh embryo transfer group actually underwent a frozen embryo transfer, while 37 (5%) women assigned to the frozen embryo transfer group actually had a fresh embryo transfer (p=0·0001; figure). The main reasons for patients converting to the frozen embryo transfer group were the risk of OHSS (51 of 98) and lack of blastocyst formation at day 5 of embryo culture (14 of 98), while converting to the fresh embryo transfer group was because of patients’ request (32 of 37) and poor embryo quality (five of 37). Additionally, although most women complied with the protocol and underwent single embryo transfer, 42 (5%) of 825 women in the frozen embryo transfer group and 21 (3%) of 812 in the fresh embryo transfer group had a transfer of two embryos (p=0·0054; table 2). More than 98% of women in both groups had the embryo transfer at blastocyst stage according to the protocol, and the other 2% of women in each group had embryo transfer at the cleavage stage (table 2). The overall proportion of deviations from the protocol was similar between the frozen (101 of 825 [12%]) and fresh (122 of 825 [15%]) embryo transfer groups (p=0·13; figure).

Table 2: Livebirth, birthweight, pregnancy, and pregnancy loss

<table>
<thead>
<tr>
<th>Group</th>
<th>Singleton livebirth per woman</th>
<th>Twin livebirth per woman</th>
<th>Total livebirth per woman</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frozen embryo transfer group</td>
<td>416 (50·4%)</td>
<td>23 (2·8%)</td>
<td>439 (53·2%)</td>
</tr>
<tr>
<td>Fresh embryo transfer group</td>
<td>329 (39·9%)</td>
<td>12 (1·5%)</td>
<td>341 (41·3%)</td>
</tr>
<tr>
<td>Relative risk in frozen embryo group (95% CI)</td>
<td>1·26 (1·14–1·41)</td>
<td>1·92 (0·96–3·83)</td>
<td>1·29 (1·16–1·43)</td>
</tr>
</tbody>
</table>

416 (50·4%) of 825 women in the frozen single blastocyst transfer group had a singleton livebirth, which was higher than in the fresh singleton blastocyst transfer group (329 of 825 [39·9%]; relative risk 1·26; 95% CI 1·14–1·41; table 3). The total livebirth rate including singleton and twin was also higher in the frozen single blastocyst transfer group and two assigned to the fresh single blastocyst transfer group but who received a frozen single blastocyst did not have a viable embryo after thawing of the first blastocyst and had a second blastocyst thawed and transferred.

416 (50·4%) of 825 women in the frozen single blastocyst transfer group had a singleton livebirth, which was higher than in the fresh singleton blastocyst transfer group (329 of 825 [39·9%]; relative risk 1·26; 95% CI 1·14–1·41; table 3). The total livebirth rate including singleton and twin was also higher in the frozen single blastocyst transfer group (table 3). Frozen single blastocyst transfer was associated with higher rates of implantation, clinical pregnancy, and ongoing pregnancy than was fresh single blastocyst transfer (table 3). The rate of twin pregnancies was also higher in the frozen embryo transfer group (table 3). However, the rate of pregnancy loss was similar between the two groups (table 3).

Singleton birthweight after frozen single blastocyst transfer was higher than that after fresh single blastocyst transfer (table 3). Risk of moderate or severe OHSS did not differ significantly between the frozen and fresh single blastocyst transfer groups (table 4). Frozen single blastocyst transfer was associated with a higher rate of pre-eclampsia (table 4). The rates of other obstetrical complications including preterm delivery (detailed in the appendix) were similar between the two groups (table 4). The risks of neonatal morbidities including congenital anomalies were also similar between the two groups (appendix).

The results of our per-protocol analyses and per-treatment analyses were consistent with the primary intention-to-treat analysis for the rates of singleton livebirth, livebirth, pregnancy, and implantation (appendix). In these secondary analyses, frozen single blastocyst transfer was associated with lower risks of ectopic pregnancy and SGA, and higher risks of gestational diabetes, pre-eclampsia, and LGA.

Discussion

In ovulatory women with a good prognosis, a frozen single blastocyst transfer resulted in a higher rate of singleton livebirth, which was mainly mediated by a higher rate of implantation, than did fresh single blastocyst transfer. The rate of pregnancy loss after frozen and fresh single blastocyst transfer was similar. Frozen blastocyst transfer led to a higher singleton birthweight...
but also a higher risk of LGA than did fresh single blastocyst transfer. The incidence of pre-eclampsia was higher after frozen single blastocyst transfer than after fresh single blastocyst transfer. The risk of moderate or severe OHSS was similar in both groups.

The higher implantation rate after frozen single blastocyst transfer might involve three factors and their interaction: an embryo with implantation competency, a more receptive endometrium, and improved developmental synchrony between the embryo and the endometrium.10 Ovarian stimulation and possibly the resultant supra-physiological oestrogen concentrations might have a detrimental effect on endometrial development compared with the endometrium in natural cycles.10,21 Frozen embryo transfer allows for the removal of iatrogenically administered gonadotropins and recovery of the stimulated ovaries. The subsequent shedding of the exposed endometrium after ovarian stimulation and a fresh start and regrowth under alternative less intensive endometrial preparation regimens could provide a more favourable uterine environment for embryo implantation with frozen embryo transfer than with fresh transfer. Transfer of a blastocyst into this comparatively more receptive endometrium during a frozen embryo transfer cycle more closely mimics the process of natural implantation compared with a frozen cleavage-stage embryo transfer cycle or a fresh embryo transfer cycle. The implantation rate after frozen or fresh blastocyst transfer in this trial was higher than that after either frozen or fresh cleavage-stage embryo transfer (41% and 42%, respectively) in our previous trial in ovulatory women.12 The better embryo selection by extended culture to blastocyst could also have contributed to the increased implantation rate compared with cleavage-stage embryo transfer.1 Furthermore, the livebirth rate after frozen single blastocyst transfer is very similar to that after the transfer of two cleavage-stage embryos (49% and 50% after two frozen or fresh cleavage-stage embryos transfer, respectively).14 However, the rate of twin livebirth (17% and 16%, respectively) and the risks of preterm birth (16% and 13%, respectively) were significantly reduced in the ovulatory women in this study.

The risk of pre-eclampsia was higher after frozen single blastocyst transfer than after fresh single blastocyst transfer, which was consistent with our previous findings from the randomised trial in women with PCOS.22 In ovulatory women with cleavage-stage embryo transfer, incidence of pre-eclampsia did not differ significantly after frozen embryo transfer (4% vs 3%, p=0.28).19 Frozen blastocyst transfer was also associated with a higher incidence of LGA than was fresh blastocyst transfer. According to the definition of LGA, the incidence of LGA is 10% in the reference population that was mainly composed of natural conception.23 Thus, frozen single blastocyst transfer seems to increase the risk of LGA compared with natural conception and fresh single blastocyst transfer. The underlying mechanism for the increased risk of pre-eclampsia and LGA was unclear; however, embryo cryopreservation was suggested to alter the epigenetics of embryo in in-vitro experiment24 and animal studies,21 and epigenetic dysregulation in turn was associated with abnormal placentation and fetal growth.25 Further study could also show that the decrease in SGA with frozen blastocyst transfer that we noted in our secondary analysis could counterbalance concerns about the increased prevalence of LGA babies. The increase in birthweight predominantly in male babies in the frozen blastocyst transfer group might actually favour survival of preterm males; however, this post-hoc finding requires replication and a sound biological explanation.

The risk of moderate or severe OHSS was similar between the frozen and fresh single blastocyst transfer groups, which contrasted with our previous findings that frozen embryo transfer had a lower risk OHSS.19,21 The absolute incidence of OHSS in both groups was lower in this study than in our previous two trials.21 The randomisation was done 3 days after oocyte retrieval in this study whereas in the previous studies it was done on the day of oocyte retrieval. The longer period of observation allowed for the exclusion of patients who developed or were at risk for developing OHSS before randomisation. The loss of protection against OHSS in the frozen single blastocyst transfer group compared...
with fresh single blastocyst transfer could be due to the selection bias towards women with a low risk of OHSS.

The rate of pregnancy loss was similar after frozen and fresh single blastocyst transfer in this trial. In this environment, the maintenance of pregnancy was not adversely affected by previous ovarian stimulation. This lack of association with pregnancy loss was consistent with the result of our previous trial in ovulatory women with cleavage-stage embryo transfer. However, it contrasts with the results in women with PCOS, in whom frozen embryo transfer was associated with a lower rate of pregnancy loss than was fresh embryo transfer. 

Although the underlying mechanism is still unclear, the effect of supra-physiological oestrogen concentrations on pregnancy loss might vary between ovulatory women and women with PCOS.

A Cochrane meta-analysis including four randomised trials (1892 women) showed that the rates of clinical pregnancy and ongoing pregnancy were similar after frozen versus fresh embryo transfer, whereas frozen embryo transfer was associated with a lower rate of miscarriage and an increased risk of pregnancy complications after the first transfer. However, the result of this Cochrane review was dominated by our previous trial, the largest trial in women with PCOS (1508 women), who could be more susceptible to an increased rate of pregnancy loss after fresh embryo transfer than ovulatory women. Since the publication of the Cochrane review, five new randomised trials comparing frozen with fresh embryo transfer in different populations have been published. The two large trials in ovulatory women with cleavage-stage embryo transfer showed no difference in the rates of implantation, pregnancy, pregnancy loss, or livebirth. The trial comparing frozen versus fresh euploid blastocyst transfer showed higher rates of implantation and livebirth in the frozen-thawed cycle than in the fresh cycle. The mechanism underlying the discrepant result between blastocyst-stage embryo transfer and cleavage-stage embryo transfer is unclear. However, since ovarian stimulation advances the window of implantation, it could decrease endometrial receptivity during a fresh cycle. This notion was supported by a study in which the rates of implantation and pregnancy after fresh single blastocyst transfer were significantly reduced when the normally developing embryo was electively transferred on day 6 compared with on day 5. 

Alternatively, transfer of a cleavage-stage embryo into the uterus might promote the synchronised development between embryo and endometrium at the time of implantation, while extended in-vitro culture might perturb the kinetics of embryonic development and disrupt the synchrony with endometrial development. Furthermore, two embryos were usually transferred at a time when cleavage-stage embryo transfer was done while only one blastocyst was typically transferred in the blastocyst-stage embryo transfer cycle. Two embryos within the uterine cavity may compete with each other for implantation and consequently result in a decreased implantation rate of each embryo. Future studies are needed to elucidate the physiological difference in implantation after blastocyst transfer and cleavage-stage embryo transfer.

The strengths of this study include the large sample size that allows for an accurate estimate of the primary outcome (singleton livebirth), and the multicentre setting and pragmatic design that improves extrapolation of our results. However, there are limitations in this study. First, we included only young women with a good prognosis; more than 25% of the screened women were excluded because of fewer than four high-grade embryos on day 3, poor ovarian response, or a previous failed IVF cycle. We should be cautious to generalise the results to women with an unfavourable or even less favourable prognosis. Second, this study was a pragmatic trial and a reflection of clinical practice. About 5% of women in the frozen embryo transfer group had a transfer of two embryos, which was higher than the proportion in the fresh embryo transfer group. These deviations were mostly because of patients insisting on two embryos being transferred during the wait for a frozen embryo transfer. The rate of twin pregnancies was higher in the frozen blastocyst transfer group probably because of this iatrogenic tendency to transfer more than one embryo in this group. This performance bias probably led to a higher rate of livebirth in the frozen blastocyst transfer group, but alone does not account for the difference in livebirth rate between groups. Results of the per-protocol analysis in women who underwent single blastocyst transfer supported those of the intention-to-treat analysis. Furthermore, because this was a non-blinded study, the question is not whether there was treatment bias, because this influenced treatment crossovers and multiple embryos transfer, but whether we are underestimating its effects. Finally, elements of the pragmatic design such as type of embryo media (we used sequential media, not single-step) or choice of frozen cycle regimen could have affected results. Although this study had a relatively large sample, it was neither designed nor powered to show differences in obstetric and neonatal complications. Future meta-analysis pooling all trials might obtain a consolidated conclusion.

Nonetheless there are practice changing implications to our findings. Our results suggest that frozen single blastocyst transfer is better to achieve singleton livebirth than fresh single blastocyst transfer in women with good prognosis. Compared with our previous studies that allowed multiple cleavage-stage embryo transfers, the practice of single frozen blastocyst transfer reduces multiple pregnancy rates and associated morbidities, while maintaining livebirth rate. Its potential for increased maternal pre-eclampsia and its long-term effects on offspring warrant further studies.

Contributors
DW, YSH, Z-JC, HeZ, and RSL designed the trial. YSH and Z-JC were in charge of the trial conduct. JYL, YSu, BZ, YHS, J-QL, JT, XLia, YC, ZW,
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YaQ, HaZ, YZh, HR, GH, XLin, JZ, YZh, XQ, XD, XC, YZh, XW, L-FT, QL, and XM enrolled participants. DW, ZW, and LZ did the statistical analyses and prepared the tables with oversight by HeZ. DW, HeZ, RSL, and Z-JC drafted the manuscript. Z-JC had a primary responsibility for final content. All authors were involved in data collection, interpreted the data, provided critical input to the manuscript, and approved the final manuscript.

Declaration of interests
HeZ has received grants from the National Institute of Health (NIH) and National Science Foundation during the conduct of the study. RSL reports grants from NIH and Guerbet; grants and consultant’s fees from Ferring; and consultant’s fees from Bayer, AbbVie, Fractyl, and Ogeda, outside the submitted work. All other authors declare no competing interests.

Data sharing
The study protocol and statistical analysis plan will be available online with publication. Data collected for the study, including specified dataset and a data dictionary defining each field in the set, will be made available to others with publication. Investigators can request data sharing by emailing the corresponding author. Our publication committee established for this trial will review and approve the request. An agreement on how to collaborate will be reached based on the overlaps and conflicts between the proposal and our ongoing efforts.

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