Freeze-all versus fresh blastocyst transfer strategy during in vitro fertilisation in women with regular menstrual cycles: multicentre randomised controlled trial

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ABSTRACT

OBJECTIVE

To compare the ongoing pregnancy rate between a freeze-all strategy and a fresh transfer strategy in assisted reproductive technology treatment.

DESIGN

Multicentre, randomised controlled superiority trial.

SETTING

Outpatient fertility clinics at eight public hospitals in Denmark, Sweden, and Spain.

PARTICIPANTS

460 women aged 18-39 years with regular menstrual cycles starting their first, second, or third treatment cycle of in vitro fertilisation or intracytoplasmic sperm injection.

INTERVENTIONS

Women were randomised at baseline on cycle day 2 or 3 to one of two treatment groups: the freeze-all group (elective freezing of all embryos) who received gonadotropin releasing hormone agonist triggering and single frozen-thawed blastocyst transfer in a subsequent modified natural cycle; or the fresh transfer group who received human chorionic gonadotropin triggering and single blastocyst transfer in the fresh cycle. Women in the fresh transfer group with more than 18 follicles larger than 11 mm on the day of triggering had elective freezing of all embryos and postponement of transfer as a safety measure.

MAIN OUTCOME MEASURES

The primary outcome was the ongoing pregnancy rate defined as a detectable fetal heart beat after eight weeks of gestation. Secondary outcomes were live birth rate, positive human chorionic gonadotropin rate, time to pregnancy, and pregnancy related, obstetric, and neonatal complications. The primary analysis was performed according to the intention-to-treat principle.

RESULTS

Ongoing pregnancy rate did not differ significantly between the freeze-all and fresh transfer groups (27.8% (62/223) *v* 29.6% (68/230); risk ratio 0.98, 95% confidence interval 0.87 to 1.10, P=0.76). Additionally, no significant difference was found in the live birth rate (27.4% (61/223) for the freeze-all group and 28.7% (66/230) for the fresh transfer group; risk ratio 0.98, 95% confidence interval 0.87 to 1.10, P=0.83). No significant differences between groups were observed for positive human chorionic gonadotropin rate or pregnancy loss, and none of the women had severe ovarian hyperstimulation syndrome; only one hospital admission related to this condition occurred in the fresh transfer group. The risks of pregnancy related, obstetric, and neonatal complications did not differ between the two groups except for a higher mean birth weight after frozen blastocyst transfer and an increased risk of prematurity after fresh blastocyst transfer. Time to pregnancy was longer in the freeze-all group.

CONCLUSIONS

In women with regular menstrual cycles, a freeze-all strategy with gonadotropin releasing hormone agonist triggering for final oocyte maturation did not result in higher ongoing pregnancy and live birth rates than a fresh transfer strategy. The findings warrant caution in the indiscriminate application of a freeze-all strategy when no apparent risk of ovarian hyperstimulation syndrome is present.

TRIAL REGISTRATION

Clinicaltrials.gov NCT02746562.

WHAT IS ALREADY KNOWN ON THIS TOPIC

Increasing pregnancy rates after frozen-thawed embryo transfer have encouraged wider implementation of a freeze-all (elective freezing of all embryos) strategy in assisted reproductive technology treatment

A freeze-all strategy that uses gonadotropin releasing hormone agonist triggering can minimise the risk of ovarian hyperstimulation syndrome

Existing studies are lacking that compare a freeze-all strategy using gonadotropin releasing hormone agonist triggering with a fresh transfer strategy using human chorionic gonadotropin triggering

WHAT THIS STUDY ADDS

In women with regular menstrual cycles, a freeze-all strategy with gonadotropin releasing hormone agonist triggering did not result in higher ongoing pregnancy and live birth rates than a fresh transfer strategy with human chorionic gonadotropin triggering

A safe fresh embryo transfer strategy can be applied to women with regular menstrual cycles with strict cancellation criteria for the fresh transfer if an excess number of mature follicles are present

The findings warrant caution in applying a freeze-all strategy in the broad in vitro fertilisation population when no apparent risk of ovarian hyperstimulation syndrome is present

Introduction

In recent years, the use of frozen embryo transfers has gradually increased owing to improvements in laboratory techniques such as vitrification and blastocyst culture. Additionally, the practice of elective freezing of all embryos (freeze-all) is becoming more frequent because pregnancy rates after frozen transfers are approaching those of fresh transfer cycles.[1-3] Supraphysiological levels of oestradiol and progesterone after ovarian stimulation have been hypothesised to accelerate endometrial advancement and impair endometrial receptivity, reducing the implantation rate in the fresh transfer cycles.[4-8] Furthermore, the freeze-all strategy offers the advantage of allowing the application of a gonadotropin releasing hormone agonist for final oocyte maturation to minimise the risk of early and late ovarian hyperstimulation syndrome.[9 10]

The clinical evidence in support of the freeze-all strategy was initially based on a meta-analysis that included three randomised controlled trials, primarily in women with a high response or women with a polycystic ovarian like morphology. The findings of these trials suggested that the risk of ovarian hyperstimulation syndrome could be reduced and reproductive outcomes improved.[6] However, one of the included studies was subsequently retracted because of methodological flaws.[11 12] In 2016, a large Chinese randomised controlled trial in 1508 women with polycystic ovary syndrome reported a higher live birth rate after freeze-all compared with fresh transfer (49.3% *v* 42.0%, respectively).[7]

Currently, research is focusing on whether the overall in vitro fertilisation population could benefit from a freeze-all strategy, which yields improved reproductive outcomes in women with regular menstrual cycles and not only those at increased risk of ovarian hyperstimulation syndrome.[9] New randomised controlled trials have reported conflicting results in reproductive outcomes after the freeze-all strategy compared with fresh transfer in women who have regular menstrual cycles.[13-17] However, the concept of a freeze-all strategy that uses gonadotropin releasing hormone agonist triggering has not been tested.

In this study, we aimed to assess the ongoing pregnancy rate by using a freeze-all strategy with gonadotropin releasing hormone agonist triggering and single frozen-thawed blastocyst transfer in a subsequent modified natural cycle, and a fresh strategy with conventional human chorionic gonadotropin triggering and fresh blastocyst transfer. To avoid ovarian hyperstimulation syndrome in both groups the possibility of transferring to gonadotropin releasing hormone agonist triggering, segmentation, and delayed transfer was allowed in the fresh transfer group if a predefined risk of the condition was present. By minimising the risk of ovarian hyperstimulation syndrome, we tested whether the ongoing pregnancy rate was superior in the freeze-all strategy group compared with the fresh transfer strategy group.

Methods

The study design was a multicentre randomised controlled trial with a 1:1 allocation to either the freeze-all strategy group with gonadotropin releasing hormone agonist triggering and single frozen-thawed blastocyst transfer in a subsequent modified natural cycle; or the fresh transfer strategy group with human chorionic gonadotropin triggering and single blastocyst transfer in the fresh cycle. The primary outcome was ongoing pregnancy rate. Women were recruited from eight clinical sites in Denmark, Sweden, and Spain from May 2016 to September 2018. Follow-up of pregnancies from the first embryo transfer was completed in July 2019. The study was approved by the ethics committees of the participating countries and all couples provided written informed consent. The study rationale and a detailed trial protocol have been published previously.[18]

Study population

Our trial included women aged 18-39 years with a regular menstrual cycle (≥24 and ≤35 days) who were starting their first, second, or third treatment cycle of in vitro fertilisation or intracytoplasmic sperm injection because of male, tubal, uterine, or unexplained infertility. Only women with predicted normal or high responds were included as defined by serum anti-müllerian hormone levels greater than 6.28 pmol/L (Roche Elecsys assay), corresponding to the poor responder anti-müllerian hormone threshold level in the Bologna criteria.[19] Women with a diagnosis of endometriosis (stage III or IV), uterine abnormalities and submucosal fibroids, or dysregulated thyroid disease were excluded from the study. Additionally, we excluded women with any severe comorbidity potentially associated with adverse pregnancy outcomes, such as insulin dependent or non-insulin dependent diabetes mellitus, gastrointestinal, cardiovascular, pulmonary, liver, or kidney disease. Couples that required testicular sperm aspiration or oocyte donation were also excluded from participation. Women were only allowed to participate once.

Randomisation and masking

Randomisation was done on inclusion day (cycle day 2 or 3) before initiation of ovarian stimulation, and was blinded until the day of ovulation triggering so that ovarian stimulation was not influenced by the result. A study nurse or non-treating physician performed randomisation by using a computerised randomisation programme running a minimisation algorithm, initially seeded using a random block sequence for the first patients. The random concealed allocation sequence was generated by statisticians from Statistika Konsultgruppen (Gothenburg, Sweden). To ensure equal distribution between the two groups the minimisation algorithm balanced the following variables: female age (mean and frequency ≥37 years), number of previously performed cycles (frequency of none, one, or two cycles), nulliparous (frequency of yes or no), fertilisation method (in vitro fertilisation or

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intracytoplasmic sperm injection), smoking (frequency of yes or no), anti-müllerian hormone level (≤12, 13-28, or >28 pmol/L), study site, and mean body mass index. Starting dose of gonadotropin hormone was entered in the programme before randomisation.

Treatment procedures

Ovarian stimulation was started on cycle day 2 or 3 in the gonadotropin releasing hormone antagonist protocol regimen with recombinant follicle stimulating hormone (follitropin alpha: Gonal-F, Merck; Bemfola, Gedeon Richter; Pergoveris, Merck; follitropin beta: Puregon, MSD; follitropin delta: Rekovelle, Ferring; corifollitropin alpha: Elonva, MSD) or urinary derived follicle stimulating hormone (Menopur, Ferring). An individual starting dose based on anti-müllerian hormone, weight, and previous response to ovarian stimulation was administered with a maximum allowed daily dose of 300 IU. The dose could be adjusted according to the general practice of the participating clinics. A gonadotropin releasing hormone antagonist (cetrorelix: Cetrotide, Merck; ganirelix: Orgalutran, MSD) at a dose of 0.25 mg was added on stimulation day 5 or 6 and continued throughout the remaining stimulation. The randomisation result was disclosed when three or more follicles of at least 17 mm mean diameter were present. Final oocyte maturation was induced by administering 0.5 mg of a gonadotropin releasing hormone agonist (buserelin: Suprefact, Sanofi) in the freeze-all strategy group or 250 µg of human chorionic gonadotropin (choriongonadotropin alpha: Ovitrelle, Merck) in the fresh transfer strategy group. Women randomised to the fresh transfer strategy group received gonadotropin releasing hormone agonist triggering if more than 18 follicles with a mean diameter larger than 11 mm were present on the day of ovulation triggering to prevent ovarian hyperstimulation syndrome as predefined in the protocol. Consequently, the first single blastocyst transfer was postponed to a subsequent modified natural frozen transfer cycle.[20]

Oocyte retrieval was performed 36 hours after gonadotropin releasing hormone agonist or human chorionic gonadotropin had been administered. All fertilised oocytes were cultured to the blastocyst stage and assessed according to the classification system by Gardner and Schoolcraft.[21] Day 5 blastocysts with a Gardner score of 3BB or higher were considered to be good quality and suitable for transfer or vitrification. Additionally, day 6 blastocysts with a Gardner score of 4BB or higher were considered suitable for vitrification. If only suitable day 6 blastocysts were present in the fresh transfer group, the first single blastocyst transfer was postponed until a subsequent modified natural frozen transfer cycle. The blastocysts were ranked in order so that the blastocyst with the highest implantation potential was used first. Ranking was based on morphological evaluation on day 2 and day 5 or 6. Surplus good quality blastocysts were vitrified on day 5 or 6.

In the fresh transfer strategy group, the blastocyst with the highest ranking was transferred on day 5 of embryo culture by using transabdominal ultrasonographic guidance. Luteal phase support was administered from day 2 after oocyte retrieval with vaginal progesterone (90 mg/day Crinone, Merck, or 300 mg/day Lutinus, Ferring) and continued until a human chorionic gonadotropin test was performed 11 days after blastocyst transfer.

In the freeze-all strategy group, blastocyst vitrification was done on day 5 or 6, depending on embryo development. The highest ranking blastocyst was graded and marked before vitrification using the same criteria as in the fresh transfer group. In case the first thawed blastocyst did not survive the freezing-thawing process, the blastocyst with the second highest ranking was thawed. We required a wash-out period of at least one completed menstrual cycle between stimulation and blastocyst transfer. Endometrial preparation was done in a modified natural cycle regimen, which meant that a single injection of 250 µg of human chorionic gonadotropin was administered as soon as the leading follicle was larger than 17 mm in the natural cycle. A single frozen thawed blastocyst was transferred using transabdominal ultrasonographic guidance six or seven days after human chorionic gonadotropin was injected. No luteal phase support was given.

A serum human chorionic gonadotropin test was conducted 11 days after blastocyst transfer. Providing the test was positive, transvaginal ultrasound was performed three to four weeks later to confirm ongoing pregnancy.

Outcomes

The primary outcome was ongoing pregnancy rate per randomised patient, which also included natural conceptions. We defined ongoing pregnancy as a detectable fetal heart beat after eight weeks of gestation. Ongoing pregnancy rate was recorded per randomised patient, per started stimulation, per oocyte retrieval, and per embryo transfer. Secondary outcomes were positive human chorionic gonadotropin rates (biochemical pregnancies), live birth rates, pregnancy related complications, obstetric complications, and prevalence of ovarian hyperstimulation syndrome, which included women who had ascites puncture and those admitted to hospital with the condition. For pregnancies that continued beyond 22 weeks, pregnancy related, obstetrical, and neonatal outcomes were recorded, including infants born small for gestational age or large for gestational age. Small for gestational age and large for gestational age were calculated from growth curves for Scandinavian children adjusted for sex and gestational age.[22] Cumulative live birth rates including time to delivery in the cumulative cycles, detailed embryo data, and evaluation of cost effectiveness will be accounted for in separate publications.

Post hoc analysis was performed for selected obstetric outcomes (pregnancy induced hypertension, gestational diabetes, chorioamnionitis, postpartum

haemorrhage, induction of birth, mode of birth (vaginal delivery or caesarean section), twin rates, and duration of hospital stay). Supplementary table S1 provides definitions of secondary outcomes.

Statistical analysis

We designed the trial as a superiority study. Sample size calculation indicated a requirement of at least 212 patients in each group to have a power of 80% at a significance level of 0.05 to detect an absolute difference of 13% in the ongoing pregnancy rate in favour of frozen embryo transfer, with an estimated rate of 30% after fresh transfer. The effect size of 13% was based on the existing scarce literature on freeze-all trials. In 2011, Shapiro and colleagues found a difference of 15% in clinical pregnancy rate in favour of frozen embryo transfer compared with fresh transfer.[5] Additionally, a small meta-analysis by Roque and colleagues published in 2013, which was based on three randomised controlled trials, showed an absolute difference of 12% between frozen and fresh embryo transfers.[6] Therefore, the effect size of 13% was based on these limited numbers from the existing literature.

We used the intention-to-treat principle for the primary statistical analysis. Primary and secondary outcomes were assessed by comparing the outcome after the first single blastocyst transfer. We included all women who were randomised except those who withdrew consent in the intention-to-treat analysis. All women were accounted for in the group to which they were randomised, regardless of the treatment they received. We included all women who adhered strictly to the study protocol in a per protocol analysis. The as-treated analysis included women randomised to the fresh transfer group who had fresh embryo transfer, and those randomised to the frozen transfer group who received frozen transfer. We determined the rate of ongoing pregnancy and a risk ratio was used to describe the difference. Per protocol analyses were also performed for selected outcomes. We compared continuous data by using the Student t test and the results are given as mean (standard deviation) or median (interquartile range). Categorical data were assessed by using χ^2 analysis and Fisher's exact test for expected frequencies less than five. A two sided P value of less than 0.05 was considered to indicate statistical significance. We performed analyses by using SPSS version 22.0 and R statistical package version 3.3.1.

Patient and public involvement

Before the initiation of the trial we performed a questionnaire based study exploring patients' attitudes and perceptions towards a freeze-all strategy.[23] In addition, quality of life questionnaires were included in the present trial to evaluate possible strain and distress in both treatment strategy groups; these will be reported in a separate publication. No patients were involved in the design of the study, nor were any patients involved in the implementation, recruitment, or interpretation of the results.

Results

Between 5 May 2016 and 7 September 2018, 460 women were enrolled in the trial; 230 were randomised to the freeze-all strategy group and 230 to the fresh transfer strategy group. Figure 1 presents a flow chart of the trial. Of all the women randomised to the two groups, seven withdrew consent and were excluded from the analysis. Therefore, 453 women started controlled ovarian stimulation: 223 in the freeze-all group and 230 in the fresh transfer group. Of these women, 448 had oocytes retrieved: 221 (99.1%) in the freeze-all group and 227 (98.7%) in fresh transfer group. We found baseline demographics and clinical characteristics (table 1), and ovarian stimulation and embryo transfer characteristics (table 2) to be comparable between the two groups.

After oocyte retrieval, nine women (4.1%) in the freeze-all group and 14 (6.2%) in the fresh transfer group had total fertilisation failure (table 2). Additionally, 39 women (17.6%) in the freeze-all group and 32 (14.1%) in the fresh transfer group did not have an embryo reaching blastocyst stage and embryo transfer was cancelled (table 2). Three (1.4%) women in the freeze-all group did not have a blastocyst for transfer after thawing all existing blastocysts (table 2).

In accordance with the trial protocol, 30 women allocated to the fresh transfer group had the first single blastocyst transfer postponed until a subsequent natural frozen transfer cycle because of risk of ovarian hyperstimulation syndrome (24 women) or lack of blastocyst development on day five (six women). Additionally, seven women in the fresh transfer group had frozen blastocyst transfer for reasons such as fluid filled uterine cavity on day of transfer or dysregulated thyroid disease discovered at baseline. Only one woman randomised to the freeze-all group had fresh embryo transfer. In total, 209 blastocysts were thawed to aim for the first single blastocyst transfer. Of these blastocysts, 11 did not survive the freezing-thawing process, which resulted in a blastocyst survival rate of 94.7%. Moreover, all randomised women had single blastocyst transfer apart from three women randomised to the fresh transfer group, of which two (0.9%) had single cleavage stage transfer and one (0.4%) had double cleavage stage transfer. The proportion of protocol deviations was similar in the two groups: 21 of 223 (9.4%) in the freeze-all group and 31 of 230 (13.5%) in the fresh transfer group (P=0.18; fig 1).

In the freeze-all group, 62 of 223 women (27.8%) had an ongoing pregnancy compared with 68 of 230 (29.6%) in the fresh transfer group, with a difference between groups of −1.8 (95% confidence interval −10.5 to 7.0; risk ratio 0.98, 95% confidence interval 0.87 to 1.10, P=0.76; table 3). No significant difference was found in the live birth rate between the freeze-all and fresh transfer group (61 of 223 (27.4%) and 66 of 230 (28.7%), respectively; risk ratio 0.98, 95% confidence interval 0.87 to 1.10, P=0.83; table 3). Five women (2.3%) in the freeze-all group conceived

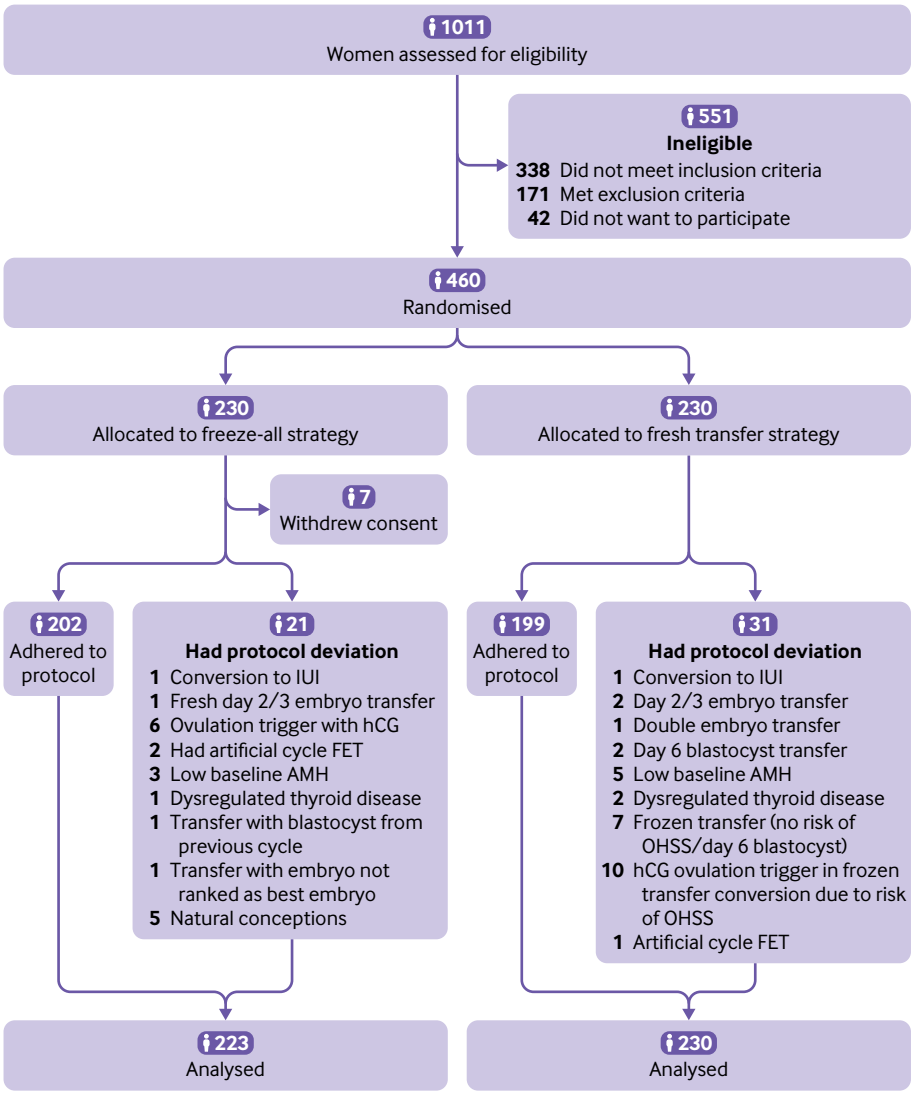

Fig 1 | Flow chart showing women randomised to freeze-all and fresh transfer strategy groups, exclusions, and protocol deviations. AMH=anti-müllerian hormone; FET=frozen embryo transfer; hCG=human chorionic gonadotropin; IUI=intrauterine insemination; OHSS=ovarian hyperstimulation syndrome

naturally during the pause between oocyte retrieval and blastocyst transfer (table 2).

The frequency of β human chorionic gonadotropin, ongoing pregnancy, and live birth per embryo transfer did not differ significantly between the freeze-all group and the fresh transfer group. Live birth rate per embryo transfer was 34.6% versus 36.5% in the freeze-all and fresh transfer groups, respectively (risk ratio 0.97, 95% confidence interval 0.83 to 1.14, P=0.80; table 3). Sensitivity analyses were performed in women undergoing their first cycle only and for women with more than three aspirated oocytes, and the results showed no significant differences in ongoing pregnancy between the two groups. Primary analysis was performed according to the intention-to-treat principle. The results of as-treated and per protocol analyses were consistent with the results of the intention-to-treat analysis. Supplementary table S2 gives details of these results.

Only one woman in the fresh transfer group had ovarian hyperstimulation syndrome and was admitted to hospital; no women in the freeze-all group had the condition (table 2). Rates of pregnancy related, obstetric, and neonatal complications were similar in both groups, apart from a significantly increased risk of premature birth after fresh embryo transfer (P=0.01; table 4) and a significantly higher mean birth weight after frozen embryo transfer compared with fresh transfer (P<0.001; table 4). However, no significant differences were found in the risk of being small for gestational age or large for gestational age (table 4). No twin pregnancies occurred in either of the groups (table 4). Median time to pregnancy was significantly longer in the freeze-all strategy group (86 days, interquartile range 77-107) compared with the fresh transfer strategy group (28 days, interquartile range 27-30; P<0.001).

Table 1 | Baseline characteristics of participants on menstrual cycle days 2-3. Data are numbers (%) unless stated otherwise

Characteristics	Freeze-all group (n=223)	Fresh transfer group (n=230)
Age at inclusion, years (mean (SD)):	32.4 (3.9)	32.3 (4.2)
Age ≥35	78 (35.0)	75 (32.6)
Age ≥37	45 (20.2)	46 (20.0)
Body mass index (mean (SD))*	24.1 (4.0)	24.1 (3.9)
Smoking, daily	10 (4.5)	11 (4.8)
AMH (pmol/L; median (IQR))†	19.1 (13.0-31.0)	19.0 (13.0-31.0)
AMH categories:		
≤12	52 (23.4)	54 (23.6)
13-28	103 (46.4)	104 (45.4)
>28	67 (30.2)	71 (31.0)
FSH (IU/L; mean (SD))‡	7.3 (2.1)	7.2 (2.2)
Menstrual cycle length (days; mean (SD))	28.5 (1.9)	28.6 (2.1)
Duration of infertility (months; mean (SD))	30.5 (16.4)	31.5 (20.5)
Primary cause of infertility:		
Tubal factor	20 (9.0)	22 (9.6)
Male	102 (46.6)	115 (50.0)
Unexplained	75 (33.6)	59 (25.7)
Other	24 (10.8)	34 (14.8)
Endometriosis	2 (0.9)	4 (1.7)
Polycystic ovary syndrome	2 (0.9)	4 (1.7)
Antral follicle count (mean (SD))	21.8 (11.7)	22.4 (11.3)
Previous fertility treatment:	158 (70.9)	159 (69.1)
Previous IUI	131 (82.9)	120 (75.5)
Previous IVF or ICSI	63 (39.8)	67 (42.2)
First IVF cycle	160 (71.7)	163 (70.9)
Second IVF cycle	41 (18.4)	44 (19.1)
Third IVF cycle	22 (9.9)	23 (10.0)
Previously given birth	11 (4.9)	5 (2.2)
Nulliparous	212 (95.1)	225 (97.8)

Not including women who withdrew their consent. No significant differences between groups (P<0.05) were present in any of the baseline characteristics.
AMH=anti-müllerian hormone; FSH=follicle stimulating hormone; ICSI=intracytoplasmic sperm injection; IQR=interquartile range; IUI=intrauterine insemination; IVF=in vitro fertilisation; SD=standard deviation.
*Body mass index is weight (kg) divided by height squared (m^2).
†AMH was missing for one woman in freeze-all group and for one woman in fresh transfer group.
‡FSH was missing for two women in freeze-all group and for one woman in fresh transfer group. Length of infertility was missing for one woman in fresh transfer group.

Discussion

Principal findings

In this multicentre, randomised controlled trial in women with regular menstrual cycles, we found no significant differences in the ongoing pregnancy and live birth rates between a freeze-all strategy with gonadotropin releasing hormone agonist triggering and a fresh transfer strategy with human chorionic gonadotropin triggering. Time to pregnancy was longer in the freeze-all strategy group. Frozen single blastocyst transfer resulted in a significantly higher mean birth weight compared with fresh single blastocyst transfer, however the difference disappeared when we adjusted for child sex and gestational age. Fresh single blastocyst transfer led to an increased risk of preterm birth, while no differences were observed in any other of the pregnancy related, obstetric, and perinatal outcomes analysed in the trial. None of the women had moderate or severe ovarian hyperstimulation syndrome and only one woman was admitted to hospital with ovarian hyperstimulation syndrome in the fresh transfer group.

Comparison to other studies

Exogenous gonadotropin application during ovarian stimulation, which leads to supraphysiological levels of oestrogen and progesterone, has been hypothesised to negatively affect endometrial receptivity and to compromise implantation in fresh transfer cycles.[4] In the frozen-thawed cycles, when the embryo is transferred in an unstimulated environment that mirrors the conditions in a natural menstrual cycle, this negative impact on the endometrium is bypassed. Additionally, extended culture to the blastocyst stage could allow better selection of embryos and possibly improved developmental embryo endometrial synchrony in the frozen cycles have been suggested to enhance the chances of successful implantation.[15]

Previously, four large randomised trials have been conducted evaluating freeze-all versus fresh embryo transfer. Although a notable advantage in live birth rates was reported for women with polycystic ovary syndrome after a freeze-all strategy,[7] results from trials in women with regular menstrual cycles are conflicting. Two trials from China and Vietnam conducted in women who ovulate failed to show any differences in pregnancy and live birth rates between the freeze-all and fresh embryo transfer groups after primarily cleavage stage double embryo transfers.[14 16] However, the latest trial by Wei and colleagues in 2019 studied women who ovulate and have a good prognosis, and showed higher live birth rates after single frozen blastocyst transfer compared with single

Table 2 | Ovarian stimulation and embryo transfer characteristics in study population. Data are number/total number or number (%) unless stated otherwise

Treatment characteristics	Freeze all group (n=223)	Fresh transfer group (n=230)
No of days of ovarian stimulation (mean (SD))	8.7 (1.6)	8.8 (1.8)
Total drug dose administered (IU; mean (SD))*	1681 (643)	1660 (617)
Gonadotrophin starting dose (IU; mean (SD))*	188 (59)	186 (58)
Ovarian stimulation drug:		
rFSH	153 (68.6)	149 (64.8)
hMG	64 (28.7)	76 (33.0)
Elonva (+rFSH/hMG)	6 (2.7)	5 (2.2)
Method of fertilisation:		
IVF	115/221 (52.0)	119/227 (52.4)
ICSI	106/221 (48.0)	108/227 (47.6)
No of oocytes retrieved (median (IQR))	9.0 (6.0-12.0)	8.0 (6.0-12.0)
No of 2 PN oocytes (fertilised; median (IQR))†	5.00 (2.0-7.0)	4.0 (2.0-7.0)
No of high quality blastocysts (median (IQR))‡	2.0 (1.0-3.0)	2.0 (1.0-3.0)
No of embryos transferred (mean):		
Single embryo transfer	162/162 (100)	180/181 (99.4)
Double embryo transfer	0/162 (0.0)	1/181 (0.6)
Embryo transfer stage:		
Cleavage stage transfer	1 (0.6)	2 (0.9)
Blastocyst transfer	161 (99.4)	179 (98.9)
Endometrial preparation regimen:		
Modified natural cycle	160/162 (98.8)	—
Programmed cycle§	2/162 (1.2)	—
Clinical signs of OHSS:		
Ascites puncture	0	0
Hospital admission owing to OHSS	0	1 (0.4)
Fresh transfer group converted to eFET:	—	37/230 (16.1)
Risk of OHSS	—	24/230 (10.4)
Day 6 blastocyst	—	6/230 (2.6)
Other reasons	—	7/230 (3.0)
Freeze-all randomised receiving fresh transfer	1/223 (0.4)	—
No of women with no embryo transfer after aspiration:	59/221 (26.7)	46/227 (20.3)
No fertilisation	9/221 (4.1)	14/227 (6.2)
No blastocyst development	39/221 (17.6)	32/227 (14.1)
No embryos to transfer after thawing all blastocysts	3/221 (1.4)	—
Natural conception after oocyte retrieval	5/221 (2.3)	0/227 (0.0)
Other	3/221 (1.4)	—
No of women with frozen blastocysts after the first transfer	117/162 (72.2)	128/181 (70.7)

Not including women who withdrew their consent. eFET=elective frozen embryo transfer; hMG=human menopausal gonadotropin; ICSI=intracytoplasmic sperm injection; IQR=interquartile range; IVF=in vitro fertilisation; OHSS=ovarian hyperstimulation syndrome; rFSH=recombinant follicle stimulating hormone; SD=standard deviation.
*Total drug dose and gonadotropin start dose were missing for one woman in freeze-all group and three women in fresh transfer group owing to the administration of Rekovelle (rFSH). Remaining blastocyst data were missing for one woman in fresh transfer group.
†Two distinct pronuclei defined by four cells, a maximum of 10% fragmentation, and no multinucleation.
‡Defined as Gardner score 3BB or higher.
§Programmed cycle defined by administration of both oestradiol and progesterone.

fresh blastocyst transfer (50% *v* 40%, risk ratio 1.26, 95% confidence interval 1.14 to 1.41).[15] Importantly, the trial by Wei and colleagues included women with a mean age of 28.8 years and a mean number of aspirated oocytes of 14. These characteristics are not representative of a general in vitro fertilisation population in Europe, where the mean age of women referred for treatment is higher. Additionally, the mean number of aspirated oocytes is lower in the average in vitro fertilisation population. Therefore, the population in the Wei study represents patients with a good prognosis.

None of the previous trials have applied gonadotropin releasing hormone agonist triggering in the freeze-all group. Our study explored the freeze-all concept with a maximally reduced risk of ovarian hyperstimulation syndrome. For safety reasons, we included an upper cut-off level defined by more than 18 follicles larger than 11 mm on the day of ovulation triggering; when this cut-off level was exceeded, women in the fresh transfer group had elective freezing of all embryos.[20] In our trial, 24 women allocated to the fresh transfer group had freeze-all treatment according to the predefined cut-off level. By applying this policy, only one woman in the fresh transfer group was admitted to hospital with ovarian hyperstimulation syndrome, but without the need for ascites or pleural drainage. Therefore, ovarian hyperstimulation syndrome was practically eliminated in both groups with the strict criteria of our study design. In comparison, in previous trials that used human chorionic gonadotropin triggering in the freeze-all group, the risk of ovarian hyperstimulation syndrome, although lower compared with the fresh transfer group, varied between 0.5% and 1.3% in the freeze-all group.[7 14-16]

Table 3 | Reproductive outcomes for women in freeze-all and fresh transfer groups (intention-to-treat analysis). Data are number/total number (%) of women

Outcomes	Freeze-all group (n=223)	Fresh embryo transfer (n=230)	Difference between groups (percentage points (95% CI))	Risk ratio (95% CI)	P value
Primary outcome: ongoing pregnancy*					
Ongoing pregnancy rate/No of randomised women	62/223 (27.8)	68/230 (29.6)	−1.8 (−10.5 to 7.0)	0.98 (0.87 to 1.10)	0.76
Ongoing pregnancy rate/No of women who started stimulation	62/223 (27.8)	68/230 (29.6)	−1.8 (−10.5 to 7.0)	0.98 (0.87 to 1.10)	0.76
Ongoing pregnancy rate/No of oocyte retrievals	62/221 (28.1)	68/227 (30.0)	−1.9 (−10.8 to 6.9)	0.97 (0.86 to 1.10)	0.73
Ongoing pregnancy rate/No of embryo transfers	57/162 (35.2)	68/181 (37.6)	−2.4 (−13.2 to 8.4)	0.96 (0.82 to 1.13)	0.73
Secondary outcome: live birth†					
Live birth rate/No of randomised women	61/223 (27.4)	66/230 (28.7)	−1.3 (−10.1 to 7.4)	0.98 (0.87 to 1.10)	0.83
Live birth rate/No of women started stimulation	61/223 (27.4)	66/230 (28.7)	−1.3 (−10.1 to 7.4)	0.98 (0.87 to 1.10)	0.83
Live birth rate/No of oocyte retrievals	61/221 (27.6)	66/227 (29.1)	−1.5 (−10.3 to 7.3)	0.98 (0.87 to 1.10)	0.81
Live birth rate/No of embryo transfers	56/162 (34.6)	66/181 (36.5)	−1.9 (−12.6 to 8.8)	0.97 (0.83 to 1.14)	0.80

Not including women who withdrew their consent. All analyses by intention to treat.
*Ongoing pregnancy was defined as a detectable fetal heart beat after eight weeks of gestation.
†Live birth was defined as any sign of life following birth after 22 weeks of gestation.

In our study, we found no increased risk of pre-eclampsia after frozen single blastocyst transfer compared with fresh single blastocyst transfer. Recent studies have indicated that the corpus luteum plays an important part in maternal cardiovascular adaptation during pregnancy. Therefore, embryo transfer in a programmed endometrial preparation frozen transfer cycle is associated with an increased risk of hypertensive disorders and macrosomia, possibly owing to the absence of a corpus luteum.[24 25] In this trial, all but two women randomised to the freeze-all group had frozen-thawed blastocyst transfer in a modified natural endometrial preparation cycle. In contrast, we found a higher mean birth weight after frozen blastocyst transfer compared with fresh blastocyst transfer, and an increased risk of preterm birth in the fresh transfer cycles, which is in accordance with previous trials, and was also shown in large observational studies.[26 27] However, when we adjusted for sex of the child and gestational age at birth, we showed no increased risk of being large for gestational age after frozen single blastocyst transfer.

Table 4 | Pregnancy outcomes and complications after the first embryo transfer (as-treated analysis). Values are number/total number (%) unless stated otherwise

Outcomes and complications	Freeze-all group	Fresh transfer group	P value
Ectopic pregnancies*	0/79 (0.0)	1/91 (1.1)	1.00
Pregnancy loss, total*:	17/79 (21.5)	23/91 (25.3)	0.57
Pregnancy loss ≤12 weeks of gestation*	17/79 (21.5)	23/91 (25.3)	0.57
Pregnancy loss >12 weeks of gestation*	0/79 (0.0)	2/91 (2.2)	0.50
Induced abortion	0/79 (0.0)	1/91 (1.1)	1.00
Maternal complications†:			
Pregnancy induced hypertension	2/57 (3.5)	2/55 (3.6)	1.00
Pre-eclampsia	4/57 (7.0)	4/55 (7.3)	1.00
Gestational diabetes	1/57 (1.8)	0/55 (0.0)	1.00
Placental abruption	1/57 (1.8)	0/55 (0.0)	1.00
Chorioamnionitis	0/57 (0.0)	2/55 (3.6)	0.24
Postpartum haemorrhage	3/57 (5.3)	3/55 (5.5)	1.00
Caesarean section	12/57 (21.1)	20/55 (36.4)	0.07
Assisted birth	10/57 (17.5)	6/55 (10.9)	0.32
Induction of birth	13/57 (22.8)	13/55 (23.6)	0.92
Duration hospital stay (mean (SD))	2.6 (1.8)	3.0 (2.6)	0.40
Twin births	0/57 (0.0)	0/55 (0.0)	1.00
Perinatal complications:			
Preterm birth‡	0/56 (0.0)	6/54 (11.1)	0.01
Weight at birth (mean (SD))	3586 (610)	3117 (641)	<0.001
Low birth weight§	3/56 (5.4)	8/53 (15.1)	0.12
Small for gestational age¶	4/56 (7.1)	9/52 (17.3)	0.14
Large for gestational age¶	4/56 (7.1)	0/52 (0.0)	0.12
Perinatal death†**	1/57 (1.8)	1/55 (1.8)	1.00

SD=standard deviation.
*Denominator defined as number of positive human chorionic gonadotropin values (≥10 IU/mL) in each group.
†Denominator defined as number of deliveries including stillbirths and live births, only including pregnancies and births after frozen embryo transfers in freeze-all group and fresh transfers in fresh transfer group. Data on stillbirth in freeze-all group in gestational week 22+3 not included in analysis of preterm birth, birth weight, low birth weight, and small for gestational age or large for gestational age. Birth weight was missing for two children in fresh transfer group.
‡Preterm birth was defined as live birth before 37 weeks of gestation. Data on gestational age at birth missing for one woman in fresh transfer group.
§Low birth weight was defined as birth weight of less than 2500 g.
¶Small and large for gestational age defined as less than or more than two standard deviation units from expected birth weight.
**Defined as stillbirth and live birth from day 0 to 6, starting from at least 22 completed gestational weeks.

Strengths and limitations

Our trial was a multicentre randomised trial exploring the freeze-all strategy using a gonadotropin releasing hormone trigger approach. Additionally, we included women with a relatively low ovarian reserve, testing the strategy in a broad population of women undergoing in vitro fertilisation and not only those with a good prognosis. Apart from the latest trial by Wei and colleagues, previous trials have primarily used cleavage stage embryos and most transfers were double embryo transfers; European guidelines no longer recommend using double embryo transfers as the primary choice of treatment.[28] Furthermore, we performed randomisation at baseline on menstrual cycle day 2 or 3. This approach ensured minimal selection bias in the women included in our study and not only those with high response and good prognosis after ovarian stimulation.

A limitation of our study is that the trial design did not allow us to distinguish between the effects of using gonadotropin releasing hormone agonist for ovulation triggering and the freeze-all transfer strategy. However, gonadotropin releasing hormone agonist triggering is not considered inferior to human chorionic gonadotropin triggering when considering oocyte quality.[29 30] Our aim was to test the freeze-all concept with the lowest possible risk of ovarian hyperstimulation syndrome development in women with regular menstrual cycles who are undergoing assisted reproductive technology treatment. In both treatment groups, the individualised starting dose of gonadotropin was based on female age, weight, antral follicle count, anti-müllerian hormone, and previous response to stimulation. Randomisation was performed at baseline, and patients and doctors were blinded to the randomisation group until the day of ovulation triggering to avoid influence on ovarian stimulation dosing depending on allocation. We could argue that a possible benefit of the freeze-all strategy including gonadotropin releasing hormone agonist triggering could be missed in our study because of similar dosing in the two groups. Moreover, the superiority study design had the power to detect a 13% difference in ongoing pregnancy rate between the two groups, therefore smaller but clinically important differences might be overlooked. Additionally, the study was not designed or powered to show differences in pregnancy related, obstetric, and perinatal outcomes. Although there were no major differences in most outcomes between the two groups, the median time to pregnancy was longer in the freeze-all group. Time to pregnancy has been identified as an important factor among couples undergoing fertility treatment in several studies[23] and should be considered if no apparent treatment advantage or immediate risk of ovarian hyperstimulation syndrome is present. Finally, the postponement of embryo transfer in the freeze-all strategy group might result in more naturally conceived pregnancies than the fresh transfer group owing to the introduced waiting time for this strategy.

Unanswered questions and future research

This trial compared a freeze-all strategy with a fresh transfer strategy in a broad population of women with regular menstrual cycles who were undergoing assisted reproductive technology treatment. We found no significant difference in the ongoing pregnancy rates between the two groups. Whether a freeze-all strategy that includes single blastocyst transfer is superior to fresh embryo transfer in women with anovulation in European patient populations is still an open question. Additionally, the optimal criteria for cancellation of fresh embryo transfer should be explored in future research. Studies on optimisation of freezing-thawing programmes, timing of embryo transfer, and optimal endometrial preparation of luteal phase support in frozen cycles are also warranted to improve outcomes after frozen embryo transfers.

Conclusions and implications for clinicians and policy makers

In women with regular menstrual cycles, a freeze-all strategy with gonadotropin releasing hormone agonist triggering for final oocyte maturation did not result in higher ongoing pregnancy and live birth rates than a fresh transfer strategy.

By using gonadotropin releasing hormone agonist triggering in the freeze-all group and a predefined cut-off level for ovarian hyperstimulation syndrome risk in the fresh transfer group, ovarian hyperstimulation syndrome was practically eliminated in both groups. Time to pregnancy was longer in the freeze-all group, therefore fresh embryo transfer should be used as the gold standard if no apparent treatment advantage or immediate risk of ovarian hyperstimulation syndrome is present because it is vital for patients not to postpone pregnancy.

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Contributors: ANA, AP, KL, and SS designed the trial and wrote the study protocol. HSN and CB contributed to the revision and editing of the study protocol. AP and SS supervised the study. AP, SS, KL, JB, LP, ANA, NS, NCF, HSN, CB, AK, PH, NPP, MLK, ALM, and SOS were involved in the recruitment of patients and the acquisition of data. AZ and SZ were involved in developing the laboratory criteria for the study. AZ and JVJ performed the laboratory evaluation of the blastocysts. ALS and SS analysed the data. SS wrote the first draft of this manuscript. AP, SS, KL, JB, LP, ANA, NS, NCF, HSN, JVJ, SZ, CB, AK, AZ, ALS, PH, NPP, MLK, ALM, and SOS were all involved in critical revision of the manuscript. All authors approved the final version of the manuscript to be submitted. SS is guarantor. The corresponding author attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted.

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Data sharing: Data collected for the study, including deidentified individual participant data and a data dictionary defining each field in the set will be made available after publication. Requests on data sharing can be made by contacting the corresponding author. Data will be shared after review and approval by the trial scientific board and terms of collaboration will be reached together with a signed data access agreement.

The lead author (SS) affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned have been explained.

Dissemination to participants and related patient and public communities: The results will be disseminated to study participants upon request and to the general public.

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Web appendix 1: Supplementary table 1
Web appendix 2: Supplementary table 2