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Comparing the cumulative live birth rate of cleavage-stage versus blastocyst-stage embryo transfers between IVF cycles: a study protocol for a multicentre randomised controlled superiority trial (the ToF trial)

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ABSTRACT

Introduction In vitro fertilisation (IVF) has evolved as an intervention of choice to help couples with infertility to conceive. In the last decade, a strategy change in the day of embryo transfer has been developed. Many IVF centres choose nowadays to transfer at later stages of embryo development, for example, transferring embryos at blastocyst stage instead of cleavage stage. However, it still is not known which embryo transfer policy in IVF is more efficient in terms of cumulative live birth rate (cLBR), following a fresh and the subsequent frozen–thawed transfers after one oocyte retrieval. Furthermore, studies reporting on obstetric and neonatal outcomes from both transfer policies are limited.

Methods and analysis We have set up a multicentre randomised superiority trial in the Netherlands, named the Three or Fivetrial. We plan to include 1200 women with an indication for IVF with at least four embryos available on day 2 after the oocyte retrieval. Women are randomly allocated to either (1) control group: embryo transfer on day 3 and cryopreservation of supernumerary good-quality embryos on day 3 or 4, or (2) intervention group: embryo transfer on day 5 and cryopreservation of supernumerary good-quality embryos on day 5 or 6. The primary outcome is the cLBR per oocyte retrieval. Secondary outcomes include LBR following fresh transfer, multiple pregnancy rate and time until pregnancy leading a live birth. We will also assess the obstetric and neonatal outcomes, costs and patients' treatment burden.

Ethics and dissemination The study protocol has been approved by the Central Committee on Research involving Human Subjects in the Netherlands in June 2018 (CCMO NL 64060.000.18). The results of this trial will be submitted for publication in international peer-reviewed and in open access journals.

Strengths and limitations of this study

- The foremost strength of the study is use of 'cumulative live birth rate' as primary outcome, as this is an important clinical outcome for patients for which it is yet unclear what day of transfer is preferred.
- This multicentre randomised superiority trial is one of the largest studies comparing cleavage-stage with blastocyst-stage embryo transfers in women with at least four available at day 2 after oocyte retrieval.
- The multicentre setting, the broad inclusion criteria of patients and the use of local protocols according to each individual in vitro fertilisation centre (clinical and laboratory routine) allow application of these results to different clinical settings and will contribute to the generalisability of the outcomes.
- A broad range of secondary outcomes, including follow-up of obstetric and neonatal outcomes, patient's treatment burden and costs, will contribute in implementing study outcomes in definitive policy.
- The study is limited by the exclusion of women with a low number of embryos suitable at day 2.

Trial registration number Netherlands Trial Register (NL 6857).

INTRODUCTION

As many as one in six couples experience subfertility, defined as the failure to conceive after 1 year of unprotected intercourse, at least once during their reproductive lifetime.[1]

In vitro fertilisation (IVF) with or without intracytoplasmic sperm injection has evolved as an intervention to help these couples. Selection of the morphologically best embryo(s) for transfer into the uterine cavity and cryopreservation of surplus good-quality embryo(s) for future use is the current practice in most centres.

The chance of a live birth per oocyte retrieval defined as the cumulative live birth rate (cLBR) (ie, live births from both the fresh and the frozen–thawed embryo transfers) is now generally considered as the most valuable key performance indicator to evaluate the performance of the treatment offered.[2 3]

Over the last few years, there has been an ongoing debate regarding the most efficient embryo transfer policy in IVF cycles: cleavage-stage or blastocyst-stage embryo transfer. A blastocyst-stage embryo transfer is considered to improve the embryo selection process, since only the viable embryos are expected to develop into blastocysts. However, before the introduction of vitrification as a routinely laboratory procedure, cryopreservation of blastocysts with the use of the slow-freezing techniques appeared arduous and less successful. Since the introduction of the vitrification cryopreservation techniques, the survival rate of blastocysts after thawing is now comparable with that of cleavage stage embryos.[4 5] Fresh and frozen blastocyst-stage embryo transfer has become a true alternative to cleavage-stage embryo transfer. However, extended culture in the laboratory implies other culture challenges and risks. In general, the number of embryos available for transfer or cryopreservation is lower at day 5 than on day 3, as some embryos will arrest in their development in vitro. The higher number of embryos available in cleavage-stage transfer leads to more embryo transfers per oocyte retrieval and thus, potentially, to a higher cLBR.

A recent Cochrane review comparing cleavage-stage versus blastocyst-stage embryo transfer concluded that the LBR after fresh blastocyst transfer is 3%–13% higher.[6] Conversely, cleavage-stage transfer is associated with a higher number of embryos available for fresh or frozen–thawed embryo transfer than blastocyst stage transfer.[6] However, it is important to indicate that a higher LBR, after fresh blastocyst-stage embryo transfer, does not automatically implicate a higher cLBR. This same Cochrane systematic review concluded that current available evidence is inconclusive for the outcome cLBR.[6]

Concerns about blastocyst-stage embryo transfer have been raised regarding impaired obstetric and neonatal outcomes. Studies have shown higher rates of preterm birth after blastocyst-stage transfer compared with cleavage-stage transfer.[7–12] Also, higher risks of monozygotic twins[7 13 14] and placental complications[7 8] have been reported after blastocyst-stage transfer. The choice for extended culture also seems to alter the male/female ratio.[7 15 16]

In short, there is insufficient evidence on which transfer policy, that is, cleavage-stage or blastocyst-stage embryo transfers, is more effective and safe regarding the cLBR.[6 14 17 18] Furthermore, prospective studies concerning obstetric and neonatal outcome of the cleavage stage versus blastocyst stage transfer policies are limited and should be addressed as well.[7–12 19–21] Based on the lack of available evidence, we have designed a multicentre randomised study that will assess the efficiency as well as the safety of the transfer strategy.

METHODS AND ANALYSIS

Study design

We have set up a multicentre superiority trial to be carried out in the Netherlands. The flow chart of this study is shown in figure 1.

Study period

This study is planned to be conducted in 5 years (first participant recruited: 28 August 2018; estimated primary completion date: October 2023). At the time of the manuscript preparation, we have recruited about 470 women. As a result of the limiting orders surrounding the current COVID-19 pandemic, the recruitment process was temporarily on hold from 1st of April 2020 until 10th of June 2020. Afterwards a restart with the recruitment was planned over a time period of 3 months (June until September), with different new start dates depending on local limiting orders of the centre. For this reason, on average the time period for calculation of the cumulative results will be extended for 3 months for those women who started the treatment before the lock down.

Interventions

Couples are randomly allocated to either (1) the control group, with embryo transfer on day 3 after oocyte retrieval and with cryopreservation of supernumerary good-quality embryos on day 3 or 4 according to the local protocol and criteria, or (2) the intervention group, with embryo transfer on day 5 after oocyte retrieval with cryopreservation of supernumerary good-quality embryos on day 5 or 6. Cryopreserved embryos on day 6 will only be transferred after all frozen–thawed embryo transfer(s) on day 5 have been transferred without an ongoing pregnancy.

Study population

Women between 18 and 43 years of age, aiming to start an IVF treatment, are being selected for inclusion in this study. For inclusion and randomisation, at least four embryos should be available on culture day 2 (an embryo is defined as an oocyte with cell division on day 2 after insemination; ≥three pronucleus embryos are excluded). A woman can participate in the study in her first, second or third IVF treatment, and can participate in only one treatment cycle.

Women are excluded if they meet any of the following criteria: use of preimplantation genetic diagnosis or use of vitrified oocytes. No cycles with preimplantation genetic testing for aneuploidy will be part of this study as this procedure is not allowed in the Netherlands.

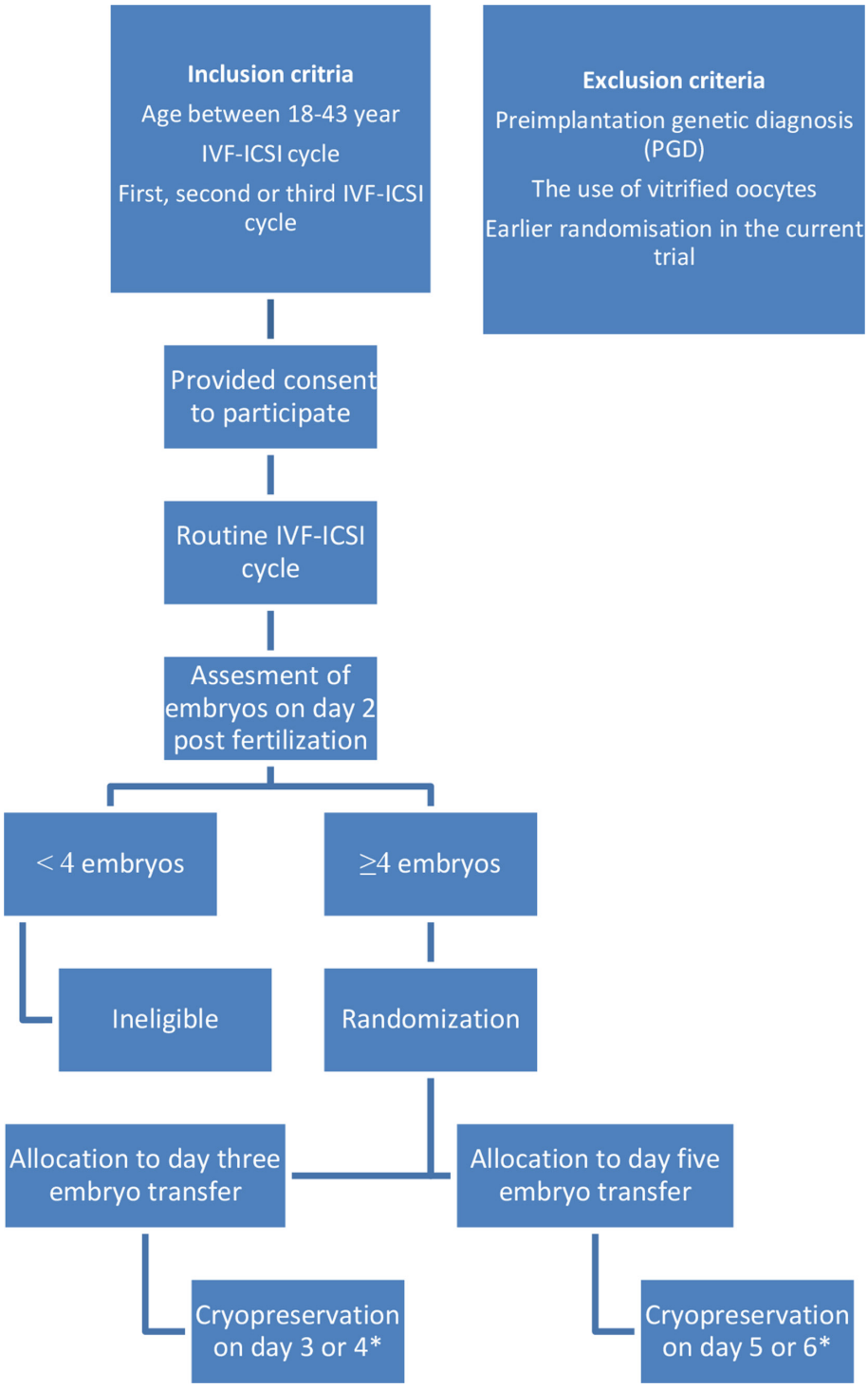

Figure 1 Study flow diagram.

Settings

Participating centres are academic and non-academic hospitals and fertility clinics, all located in the Netherlands (a list of participating centres is available at: http://zorgevaluatienederland.nl/tof). At this moment, there are 11 participating centres. Standard for most Dutch centres is embryo transfer on day 3 and cryopreservation of supernumerary good-quality embryos on day 3 or 4. The Three or Five trial is affiliated with the Dutch Consortium for Healthcare Evaluation and Research in Obstetrics and Gynaecology, which provides national attention and therefore ensures the right amount of participating hospitals to achieve adequate patient enrolment.

Informed consent procedure

Eligible couples are counselled by trained fertility doctors or research nurses by means of both oral and written information to ensure that they are fully informed about the content of the study. Those couples who agree to participate are asked to sign a written informed consent

by both partners. In case of a single woman, the informed consent form is only signed by herself. Patients are given at least 1-hour time to make their decision on participation in the study. The rules of Good Clinical Practice (GCP) are applied. All participants must provide their signed informed consent forms before start of oocyte retrieval. Participants can withdraw from the trial at any time. Eligible participants do not need to state a reason for withdrawal.

Randomisation

For randomisation, all cases that subsequently meet all inclusion criteria will be randomised by the local laboratory staff, on the second day after fertilisation using the online software program Castor (V.2018.3.11, Castor Electronic Data Capture, Amsterdam, the Netherlands). Laboratory staff can access the online randomisation program using a unique password for this study. The laboratory staff is unable to access forthcoming random assignments prior to randomisation.

Allocation to the cleavage-stage embryo transfer arm or the blastocyst-stage embryo transfer arm transfer will be based on a 1:1 randomisation with randomly selected block sizes of 2, 4 and 6 and stratification for age (≥36 years or <36 years). Laboratory staff, clinicians and the participants cannot be blinded, due to the nature of the intervention. Participating clinicians, laboratory staff and investigators will not be able to access the randomisation sequence.

Patient and public involvement

This study protocol has been designed with active input and feedback of experts and patient representatives from the Dutch patient organisation Freya (www.freya.nl).

OUTCOME MEASURES

Primary outcome measure

The primary outcome is the cLBR per oocyte retrieval, which includes the results of the fresh and frozen–thawed embryo transfers. Endpoints of the study are live birth, no pregnancy leading to live birth after transfer of all available embryos or after a follow-up time of 12 months after the oocyte retrieval.

There is an exception due to the current COVID-19 crisis for the patients with an oocyte retrieval date after the 16th of March 2019. Due to restrictive measures of the COVID-19 crisis, treatments were interrupted or postponed. Therefore, the maximum follow-up period for this group is extended by 5–17 months. For participants with an oocyte retrieval after the 1st of September 2020, the maximum follow-up time will be 12 months again.

Secondary outcome measures

Secondary outcome measures are LBR after fresh embryo transfer, ongoing pregnancy rate, clinical pregnancy rate, multiple pregnancy rate, miscarriage rate following the fresh and frozen–thawed embryo transfers, failure to transfer embryos, embryo utilisation rate, obstetric and neonatal outcomes (ie, gender, gestation age, birth weight, small for gestational age, large for gestational age, birth defects, stillbirth, perinatal death, neonatal death, hypertensive disorders in pregnancy, gestational diabetes mellitus, placental abruption, placenta previa, induction of labour, mode of delivery, postpartum haemorrhage), patient treatment burden, costs and time to pregnancy leading to live birth. For this last outcome, the time of randomisation as start point and the time of term live birth as an endpoint will be used, measured in weeks and number of treatment cycles.

The patient treatment burden is determined using questionnaires; we intend to measure the impact on the quality of life (QoL) and the decision regret of the patient choice to participate in the study. The results are given on a scale with a range from 0 to 100. Higher scores indicate a better QoL and high regrets, respectively. The evaluation of the questionnaires is reported in a separate paper.

Sample size calculation

The study is designed as a superiority trial. Previous studies demonstrated a 3%–13% increase in LBR after a fresh blastocyst transfer.[6] We expect an estimated cLBR of 31% per oocyte retrieval using the cleavage-stage embryo transfer policy[22 23] and at least a cLBR of 39% in the investigator arm (superiority design). To evaluate the increase of the LBR of 8% in the blastocyst-stage embryo transfer, with a power of 80% and an alpha error of 0.05, a total of 1.176 women needs to be included. Anticipating a 2% loss between randomisation and follow-up, we plan to include 1.200 women.

Data collection

All data will be systematically recorded in an electronic Case Report Form in Castor. All data will be kept anonymously where possible. All participants will be assigned an identification code based on the number of the hospital and number of inclusion. A list linking the code to the subject will be kept safe by the local investigators. Personal data will be stored for a maximum of 15 years in participating centres. Apart from the collection of clinical data, each woman will complete questionnaires. These are validated questionnaires about QoL (EQ5D-5L: EuroQol-5D-5L)[24] and the specific fertility QoL (FertiQoL) tool.[25] Four months after oocyte retrieval, patients receive the EQ5D-5L, FertiQoL and a questionnaire containing information about decision regret.[26] This last mentioned questionnaire is to measure satisfaction with the allocated transfer policy. When the subject reaches a study endpoint (ie, delivery, end of the IVF cycle without ongoing pregnancy, or 12/17 months after the oocyte retrieval date), patients receive again the EQ5D-5L, FertiQoL and the decisional regret questionnaire. In case of an ongoing pregnancy, participants receive an extra questionnaire about the pregnancy course (delivery birth date, gender, weight and other medical information) (table 1).

Table 1 Schematic overview of questionnaire follow-up

Measurement	Follow-up				
	Point 1	Point 2	Point 3	Point 4	Point 5
	Randomisation	4 months	No ongoing pregnancy*	Ongoing pregnancy†	12 months after oocyte retrieval‡
EQ5D-5L	x	x	x	x	x
FertiQol	x	x	x	x	x
Decision regret scale		x	x	x	x
Pregnancy, delivery and child characteristics				x	

*In case the end of the treatment cycle was reached within 4 months, the measurements of point 3 were not requested again from the patient.
†Questionnaires sent after due date.
‡Only sent if point 3 or 4 is not reached.
EQ5D-5L, EuroQol-5D-5L; FertiQoL, fertility quality of life.

Data analysis

All statistical analyses will be performed according to the intention-to-treat principle. Descriptive analysis will be used to describe the outcome variables. Pregnancy outcomes will be compared by calculating relative risks with corresponding 95% boundaries. A logistic regression analysis will be performed comparing the cumulative live birth among both treatment arms stratified for age (≥36 and <36 years) and risk plus ORs with corresponding 95% CI will be provided for each age group. We will assess time-to-pregnancy leading to a live birth by calculating hazard rates with 95% CI overall and for the age-stratified groups using Cox proportional hazards regression analysis.

For issues such as loss to follow-up, missing data and protocol violations, we attempt sensitivity ('worst-case scenario') analyses to explore the effect of these factors on the trial findings.

Treatment burden in terms of impact on QoL and decisional regret will be studied using linear mixed-model analysis. We will use IBM SPSS Statistics for Windows, version 25.0.0.2, released 2017, IBM corp., Armonk, NY, USA, to perform the statistical analysis.

Economic evaluation

A cost-effectiveness analysis will be performed from a healthcare perspective according to Dutch guidelines[6] with a time horizon of 12 months. A cost-utility analysis will be performed to relate the burden of intervention to the transfer strategy. Bivariate regression analyses will be used to estimate cost-and-effect differences between transfer in cleavage stage and transfer in blastocyst stage, while adjusting for confounders if necessary. Incremental cost-effectiveness ratios (ICERs) will be calculated by dividing the difference in the mean total costs between the treatment groups by the difference in mean effect between the treatment groups. Bias-corrected and accelerated bootstrapping with 5000 replications will be used to estimate 95% CIs around the cost differences and statistical uncertainty surrounding the ICERs. Uncertainty surrounding ICERs will be graphically presented on cost-effectiveness planes. The economic evaluation will be reported in a separate paper.

DISCUSSION

This protocol describes a multicentre randomised superiority trial where different efficacy and safety, social and economic aspects regarding cleavage-stage versus blastocyst-stage embryo transfer policy are analysed. To our knowledge, this will be the first large randomised study using cLBR as primary outcome. For a well-adjusted decision between a cleavage-stage or blastocyst-stage embryo transfer, professionals and couples should consider multiple variables, such as the chance of pregnancy, the time to pregnancy, the safety of the treatment, its burden and the costs involved. Prior to this study, it has been already recognised that for fresh transfers, a blastocyst-stage embryo is associated with a higher LBR per transferred embryo.[6] Conversely, a cleavage-stage embryo transfer policy is associated with a higher number of embryos that can be chosen for fresh or frozen–thawed embryo transfer.[6] The argumentation is that, in contrast to the higher LBR after fresh embryo transfer in the blastocyst-stage strategy, this strategy does not automatically translate into a higher cLBR, that is, the chance of a live birth per oocyte retrieval. However, the time to pregnancy, as valued by patients, could be shorter with the blastocyst transfer policy and in that sense, could be more effective from a patient's viewpoint. Higher cumulative LBRs will probably lead to less burden, less costs and less treatment cycles. This multicentre randomised superiority trial will reveal whether there is a difference in terms of effectiveness, safety, patient treatment burden and costs between a cleavage-stage embryo transfer and blastocyst-stage embryo transfer policy. We expect the outcomes of this study to contribute in the decision-making for best practice at the moment a couple requires a fertility treatment.

ETHICS AND DISSEMINATION

This study protocol was designed with input and feedback of patient representatives and experts. Ethical

approval by the Dutch Central Committee on Research Involving Human Subjects was obtained in 2018 (CCMO NL 64060.000.18) and is in accordance with the Declaration of Helsinki, the Medical Research Involving Human Subjects Act (WMO), the Guideline for GCP, and all other applicable regulatory requirements. All amendments will be notified and need to be approved by the CCMO. Results will be disseminated through peer-reviewed publications and presentations at international scientific meetings.

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Contributors SC, KF, SM and MvW designed the trial. KF, SM and MvW were responsible for the development of the protocol applied for the grant. LR and SM are the coordinating investigators. MvW is in charge of statistical analysis. SC, LR, BA, JJBvdV, JWPdB, MHJNC, JD, AvD, JvE-A, ERG, JWMM, QP, EJPvS, ES, MT, JV, CGV, HRV, LAJvdW, YW, MvdZ, DB, SM and KF are responsible for implementation of the study and inclusion of the eligible women. SC is responsible for the overall logistical aspects of the trial and drafted the paper. All authors reviewed and contributed to the manuscript.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were involved in the design, or conduct, or reporting, or dissemination plans of this research. Refer to the Methods section for further details.

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