BMJ Open

Comparison of the effect of immediate versus delayed transfer following a stimulated IVF cycle on the ongoing pregnancy rate of frozen-thawed embryo transfer cycles: a study protocol for a randomised trial.

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2017-020507
Article Type:	Protocol
Date Submitted by the Author:	07-Nov-2017
Complete List of Authors:	LI, HE LI, LU; Shanghai Ji Ai Genetics and IVF Institute, Obstetrics and Gynecology Hospital, Fudan University, Shanghai, China LU, XIANG; Shanghai Ji Ai Genetics and IVF Institute, Obstetrics and Gynecology Hospital, Fudan University, Shanghai, China SUN, XIAOXI; Shanghai Ji Ai Genetics and IVF Institute, Obstetrics and Gynecology Hospital, Fudan University, Shanghai, China; Key Laboratory of Female Reproductive Endocrine Related Diseases, Obstetrics and Gynecology Hospital, Fudan University, Shanghai, China Ng, Ernest; The University of Hong Kong, Department of Obstetrics and Gynecology
Keywords:	Reproductive medicine < GYNAECOLOGY, Assisted reproductive technology, Frozen embryo transfer

Comparison of the effect of immediate versus delayed transfer following a stimulated IVF cycle on the ongoing pregnancy rate of frozen-thawed embryo transfer cycles: a study protocol for a randomised trial.

Authors and affiliations

He Li[1], Lu Li[1], Xiang Lu[1], Xiaoxi Sun[1,2*] , Ernest Hung Yu Ng[3*]

1. Shanghai Ji Ai Genetics and IVF Institute, Obstetrics and Gynecology Hospital, Fudan University, Shanghai, China
2. Key Laboratory of Female Reproductive Endocrine Related Diseases, Obstetrics and Gynecology Hospital, Fudan University, Shanghai, China
3. Department of Obstetrics and Gynaecology, The University of Hong Kong, Hong Kong Special Administrative Region, Hong Kong, Hong Kong

* Corresponding authors: Email: nghye@hku.hk (EHY Ng) or: Email: xiaoxi_sun@aliyun.com (X Sun)

ABSTRACT

Introduction: Frozen-thawed embryo transfer (FET) has become an increasingly important part of in vitro fertilization (IVF) treatment. Currently, there is still no good scientific evidence to support when to perform FET following a stimulated IVF cycle. Since all published studies are retrospective and the findings are contradictory, a randomized study is needed to provide Level 1 evidence to guide the clinical practice.

Methods/analysis: This is a randomized trial. A total of 724 women undergoing the first FET following ovarian stimulation in IVF will be enrolled and randomized according to a computer-generated randomization list to either (1) the immediate group in which FET will be performed in the first cycle following the stimulated IVF cycle or (2) the delayed group in which FET will be performed at least in the second cycle following the stimulated IVF cycle. The primary outcome is the ongoing pregnancy defined as a viable pregnancy beyond 12 weeks gestation.

Ethics/dissemination: An ethical approval has been granted from the ethics committee of assisted reproductive medicine in Shanghai JiAi Genetics and IVF institute (JIAI E2017-12). A written informed consent will be obtained from each woman before any study procedure is performed, according to good clinical practice. The results of this trial will be disseminated in a peer-reviewed journal.

Trial registration numbers: NCT03201783

Strengths and limitations of this study

1. This is the first randomised controlled trial comparing the ongoing pregnancy rate of immediate versus delayed FET following a stimulated IVF cycle.
2. The researchers, doctors and the participants cannot be blinded to treatment allocation.

BACKGROUND

Frozen-thawed embryo transfer (FET) has become an increasingly important part of in-vitro fertilisation (IVF) treatment.[1] When women fail to get pregnant after replacing embryos in the stimulated IVF cycle, many of those who have frozen embryos would like to proceed FET as soon as possible in order to get pregnant as soon as possible.

Ovarian stimulation exerts a detrimental effect on endometrial receptivity.[2] Ovarian stimulation leads to supraphysiological hormonal concentrations in blood which may exert negative influence on perinatal and neonatal outcomes.[3-5] The freeze-all strategy has drawn attention in recent literature with the advantages of increased maternal safety, improved pregnancy rates, lower ectopic pregnancy rates and better obstetric and neonatal outcomes.[6] The better outcomes after elective FET in the context of a freeze-all strategy may be at least partially attributed to the lack of endometrial impairment that is observed during ovarian stimulation.

Robust information regarding the optimal timing for FET following a stimulated IVF cycle is still lacking. One option is to perform FET in the first cycle following the stimulated IVF cycle i.e. immediate transfer. Another option is to postpone FET for at least one menstrual cycle i.e. delayed transfer. Delaying FET may add to the stress and anxiety accompanying the IVF treatment. Several retrospective studies showed similar clinical pregnancy rates or live birth rates between immediate and delayed FET performed following fresh embryo transfers or in a frozen-all policy.[7-9] Another retrospective analysis showed that significantly higher implantation, clinical pregnancy and live birth

rates were found in the delayed FET group than in the immediate group after failed fresh ET cycles.[10] Since these studies are all retrospective and the findings are contradictory, a randomized study is needed to provide Level 1 evidence to guide the clinical practice.

We aim in this randomized trial to compare the ongoing pregnancy rate of immediate versus delayed FET following a stimulated IVF cycle. The hypothesis is that the ongoing pregnancy rates of immediate and delayed FET are comparable.

MATERIALS AND METHODS

Study design (figure 1)

This is a single-center randomized study carried out in the Shanghai JiAi Genetics and IVF institute. The trial has been registered at ClinicalTrial.gov (NCT03201783)

Participants

Eligible women will be recruited if they fulfil all of the inclusion criteria and do not meet any of the exclusion criteria. They will be included once for this study. Informed written consent will be obtained.

Inclusion criteria are:

- Women aged <=43 years at the time of IVF treatment
- Undergoing IVF with a standard stimulation
- At least one frozen embryo or blastocyst
- The first FET cycle following ovarian stimulation in IVF

Exclusion criteria include

- Use of mild stimulation or natural cycle for IVF treatment
- Severe ovarian hyperstimulation syndrome during IVF treatment
- Preimplantation genetic diagnosis treatment
- Use of donor oocytes

- Presence of hydrosalpinx which is not surgically treated or endometrial polyp on scanning during ovarian stimulation

Randomization

Women will be randomized according to a computer-generated randomization list prepared by a designated research staff into one of the following two groups:

(1) the immediate group in which FET will be performed in the first cycle following the stimulated IVF cycle and
(2) the delayed group in which FET will be performed at least in the second cycle following the stimulated IVF cycle.

Blinding

No blinding because of the nature of the intervention.

Interventions

Women will undergo IVF treatment in the centre as clinically indicated. Standard ovarian stimulation with gonadotrophins in either a GnRH antagonist protocol or long GnRH agonist will be employed. Oocyte retrieval will be performed under transvaginal ultrasound guidance 34–36h after triggering with hCG or an agonist. Oocytes will be fertilized using either conventional insemination or intracystoplasmic sperm injection depending the semen quality of the husbands in accordance with the standard protocol. Normal fertilization will be assessed and confirmed by the presence of two pronuclei and a second polar body at 16–18 h after insemination or intracystoplasmic sperm injection. On day 3 after egg retrieval, an embryo with at least seven blastomeres and Grades 1 and 2 is defined as good quality. Embryos with at least six blastomeres and fragments<50% will be frozen. All good embryos will be frozen or vitrified using the Crytop method as cleavage stage embryos on Day 3 or as full to expanded blastocysts on Day 5 or Day 6 of embryo culture according to the standard protocol.

Hormone replacement treatment (HRT) will be used for endometrial preparation. On Day 3 of the menstrual cycle, estradiol valerate (E2, Progynova, Schering AG, Berlin, Germany) will be commenced 4mg daily for 10 days. When the thickness of the endometrial layer reaches at least 8 mm on pelvic scanning, vaginal progesterone 90 mg per day (Crinone, Merck-Serono, Switzerland) will be administered. For Day 3 embryos, FET is scheduled on the fourth day of starting vaginal progesterone. For blastocysts, FET is scheduled on the sixth day of starting vaginal progesterone. A maximum of 1–2 embryos or blastocysts with the best morphology will be transferred under ultrasound guidance using a soft embryo transfer catheter. Serum hCG level will be checked 14 days after FET. All hormone therapy will be stopped if the serum hCG level is negative. All pregnant women will continue the hormonal therapy until 12 weeks of gestation.

Follow-up and data collection

If the serum hCG level is positive, transvaginal ultrasound will be performed two weeks later to locate the pregnancy and confirm foetal viability. Subsequent management will be the same as other women with early pregnancy. They will be referred for antenatal care when the ongoing pregnancy is 12 weeks.

Written consent regarding retrieval of pregnancy and delivery data will be sought from the patient at the time of study. The patient will be contacted after delivery by phone to retrieve the information of the pregnancy outcomes. The outcome of the pregnancy (delivery, miscarriage), number of babies born, birth weights and obstetrics complications will be recorded.

Outcome measurements

Primary outcome

The primary outcome is an ongoing pregnancy defined as a viable pregnancy beyond gestation 12 weeks

Secondary outcomes include

- positive hCG level
- clinical pregnancy defined as presence of intrauterine gestational sac on ultrasound
- implantation rate as the number of gestational sacs per embryo transferred
- multiple pregnancy, ectopic pregnancy and miscarriage rates
- live birth rate and
- birth weight of newborns

Data entry and quality control of data

Participates information forms will be developed for data entry, and quality control of the data will be handled at two different levels. The investigators will be required to ensure the accuracy of the data as the first level of control, and the second level will include data monitoring and validation that will be carried out on a regular basis through out the study.

Sample size calculations and statistical analysis

Sample size estimation

According to our data of the Centre, the ongoing pregnancy rate per FET was about 30%. We hypothesize that a difference in the ongoing pregnancy rate of 10% between the immediate versus delayed groups as equivalence, the sample size required for a test of equivalence would be 329 in each arm to give a power of 0.8 and type I error of 0.05. Allowing 10% drop-out, 724 subjects or 362 in each arm will be needed.

Data analysis

Data will be analysed with an intention to treat and per protocol. Demographic features of the two groups will be compared. Comparison of quantitative variables will be performed using Student's t, while categorical variables will be compared using a Chi-square analysis. A multivariable logistic regression analysis will be used to compare the variables between two groups. All statistical analyses of the data will be performed using the SPSS program V.21.0 (SPSS Inc, Chicago, Illinois, USA), and a p value <0.05 will be

considered statistically significant.

ETHICS AND DISSEMINATION

FET in HRT cycles is a standard procedure in IVF centers. The women who agree to participate in the study will sign a consent form after detailed counseling of the study and they are free to withdraw from the study at any time without giving any reasons and having any impact on the medical care they are receiving.

Data will be entered electronically. All data will be stored in locked computer files that are accessible only to the investigators and research staffs involved in the study. Original study forms will be kept locked at the study site and maintained in storage for a period of 3 years after the completion of the study. The principal investigator will be responsible for data management including data coding, monitoring and verification. The investigators have always maintained a strict privacy policy. The investigators permit trial-related monitoring, audits, IRB/IEC review and regulatory inspections, providing direct access to source data/documents. For questions about the study, the participants should contact their physician.

A data and safety monitoring committee will review and interpret the data generated from the study, and its primary objectives will be to ensure the safety of the study participants and the integrity of the research data. The committee consists of two independent researchers with experience in reproductive medicine.

The results of this trial will be disseminated in peer-reviewed journals and presented at international meetings.

DISCUSSION

FET has been a routine procedure in the IVF treatment,[11,12] but the optimal time for FET following ovarian stimulation is still unknown. This randomized study has been designed, therefore, to evaluate ongoing pregnancy rate of

immediate versus delayed FET following a stimulated IVF cycle. It seeks to add significantly to the clinical evidence base and to allow conclusions to be made on the time interval in the FET following a stimulated IVF cycle. The present study is the first RCT to compare immediate versus delayed FET followed stimulated IVF cycle on ongoing pregnancy rate.

In order to increase the generalizability of our results, we include both patients having the first FET cycle after a failed stimulated IVF cycle or undertaking the freeze all strategy.

The study was designed in May 2017, and the first participant was randomised on 9 August 2017. At the time of the manuscript preparation, we have recruited 200 women and the recruitment is ongoing.

Authors' contributions: HL, XXS and EHYN conceived and designed the study. HL and EHYN drafted and critically revised the manuscript for important intellectual content. XXS sought ethical approval. LL and XL participated in the coordination of the study and recruitment of subjects. All the authors contributed to the further writing of the manuscript and approved the final manuscript.

Funding statement: This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests：None declared.

Patient consent: Obtained.

Ethics approval: Approval was obtained from the Ethics Committee of assisted reproductive medicine in Shanghai JiAi Genetics and IVF institute (JIAI E2017-12).

Provenance and peer review: Not commissioned; externally peer reviewed.

References:

1. Doody KJ. Cryopreservation and delayed embryo transfer-assisted reproductive technology registry and reporting implications. *Fertil Steril* 2014;102(1):27-31.

2. Shapiro BS, Daneshmand ST, Garner FC, et al. Evidence of impaired endometrial receptivity after ovarian stimulation for in vitro fertilization: a prospective randomized trial comparing fresh and frozen-thawed embryo transfer in normal responders. *Fertil Steril* 2011;96(2):344-8.

3. Venetis CA, Kolibianakis EM, Bosdou JK, et al. Estimating the net effect of progesterone elevation on the day of hCG on live birth rates after IVF: a cohort analysis of 3296 IVF cycles. *Hum Reprod* 2015;30(3):684-91.

4. Weinerman R, Mainigi M. Why we should transfer frozen instead of fresh embryos: the translational rationale. *Fertil Steril* 2014;102(1):10-8.

5. Roque M, Valle M, Guimaraes F, et al. Freeze-all policy: fresh vs. frozen-thawed embryo transfer. *Fertil Steril* 2015;103(5):1190-3.

6. Blockeel C, Drakopoulos P, Santos-Ribeiro S, et al. A fresh look at the freeze-all protocol: a SWOT analysis. *Hum Reprod* 2016;31(3):491-7.

7. Santos-Ribeiro S, Polyzos NP, Lan VT, et al. The effect of an immediate frozen embryo transfer following a freeze-all protocol: a retrospective analysis from two centres. *Hum Reprod* 2016;31(11):2541-48.

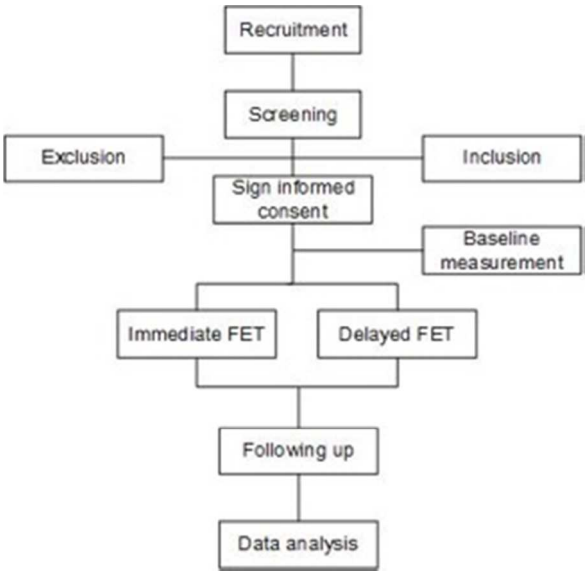
8. Santos-Ribeiro S, Siffain J, Polyzos NP, et al. To delay or not to delay a frozen embryo transfer after a failed fresh embryo transfer attempt? *Fertil Steril* 2016;105(5):1202-07 e1.

9. Lattes K, Checa MA, Vassena R, et al. There is no evidence that the time from egg retrieval to embryo transfer affects live birth rates in a freeze-all strategy. *Hum Reprod* 2017;32(2):368-74.

10. Volodarsky-Perel A, Eldar-Geva T, Holzer HE, et al. Cryopreserved embryo transfer: adjacent or non-adjacent to failed fresh long GnRH-agonist protocol IVF cycle. *Reprod Biomed Online* 2017;34(3):267-73.

11. Cedars MI. Fresh versus frozen: initial transfer or cumulative cycle results: how do we interpret results and design studies? *Fertil Steril* 2016;106(2):251-6.

12. Wong KM, Mastenbroek S, Repping S. Cryopreservation of human embryos and its contribution to in vitro fertilization success rates. *Fertil Steril* 2014;102(1):19-26.



Study design (figure 1)

102x99mm (72 x 72 DPI)

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description
Administrative information		
Title P1	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym
Trial registration P2	2a	Trial identifier and registry name. If not yet registered, name of intended registry
	2b	All items from the World Health Organization Trial Registration Data Set
Protocol version	3	Date and version identifier
Funding P9	4	Sources and types of financial, material, and other support
Roles and responsibilities P9	5a	Names, affiliations, and roles of protocol contributors
	5b	Name and contact information for the trial sponsor
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)
Introduction		
Background and rationale P3-4	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention
	6b	Explanation for choice of comparators
Objectives P4	7	Specific objectives or hypotheses
Trial design P4	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)

Methods: Participants, interventions, and outcomes

Study setting P4	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained
Eligibility criteria P4-5	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)
Interventions P5-6	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial
Outcomes P6-7	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended
Participant timeline P4-6	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)
Sample size P7	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size

Methods: Assignment of interventions (for controlled trials)

Allocation:

Sequence generation P5	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions

Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions
Blinding (masking) P5	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial
Methods: Data collection, management, and analysis		
Data collection methods P6-7	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols
Data management P7-8	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol
Statistical methods P7	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)
Methods: Monitoring		
Data monitoring P8	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed

	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial
Harms P8	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor
Ethics and dissemination		
Research ethics approval P9	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)
Consent or assent P8	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable
Confidentiality P8	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site
Access to data P8	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation
Dissemination policy P8	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions
	31b	Authorship eligibility guidelines and any intended use of professional writers
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code

Appendices

Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

Comparison of the effect of immediate versus delayed transfer following a stimulated IVF cycle on the ongoing pregnancy rate of frozen-thawed embryo transfer cycles: a study protocol for a randomised controlled trial.

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2017-020507.R1
Article Type:	Protocol
Date Submitted by the Author:	11-Jan-2018
Complete List of Authors:	LI, HE LI, LU; Shanghai Ji Ai Genetics and IVF Institute, Obstetrics and Gynecology Hospital, Fudan University, Shanghai, China LU, XIANG; Shanghai Ji Ai Genetics and IVF Institute, Obstetrics and Gynecology Hospital, Fudan University, Shanghai, China SUN, XIAOXI; Shanghai Ji Ai Genetics and IVF Institute, Obstetrics and Gynecology Hospital, Fudan University, Shanghai, China; Key Laboratory of Female Reproductive Endocrine Related Diseases, Obstetrics and Gynecology Hospital, Fudan University, Shanghai, China Ng, Ernest; The University of Hong Kong, Department of Obstetrics and Gynecology
Primary Subject Heading:	Reproductive medicine
Secondary Subject Heading:	Obstetrics and gynaecology
Keywords:	Subfertility < GYNAECOLOGY, IVF, FET, ART

Comparison of the effect of immediate versus delayed transfer following a stimulated IVF cycle on the ongoing pregnancy rate of frozen-thawed embryo transfer cycles: a study protocol for a randomised controlled trial.

Authors and affiliations

He Li[1], Lu Li[1], Xiang Lu[1], Xiaoxi Sun[1,2*] , Ernest Hung Yu Ng[3*]

1. Shanghai Ji Ai Genetics and IVF Institute, Obstetrics and Gynecology Hospital, Fudan University, Shanghai, China
2. Key Laboratory of Female Reproductive Endocrine Related Diseases, Obstetrics and Gynecology Hospital, Fudan University, Shanghai, China
3. Department of Obstetrics and Gynaecology, The University of Hong Kong, Hong Kong Special Administrative Region, Hong Kong, Hong Kong

* Corresponding authors: Email: nghye@hku.hk (EHY Ng) or: Email: xiaoxi_sun@aliyun.com (X Sun)

ABSTRACT

Introduction: Frozen-thawed embryo transfer (FET) has become an increasingly important part of in vitro fertilization (IVF) treatment. Currently, there is still no good scientific evidence to support when to perform FET following a stimulated IVF cycle. Since all published studies are retrospective and the findings are contradictory, a randomized controlled study is needed to provide Level 1 evidence to guide the clinical practice.

Methods/analysis: This is a randomized controlled trial. A total of 724 women undergoing the first FET following ovarian stimulation in IVF will be enrolled and randomized according to a computer-generated randomization list to either (1) the immediate group in which FET will be performed in the first cycle following the stimulated IVF cycle or (2) the delayed group in which FET will be performed at least in the second cycle following the stimulated IVF cycle. The primary outcome is the ongoing pregnancy defined as a viable pregnancy beyond 12 weeks gestation.

Ethics/dissemination: An ethical approval has been granted from the ethics committee of assisted reproductive medicine in Shanghai JiAi Genetics and IVF institute (JIAI E2017-12). A written informed consent will be obtained from each woman before any study procedure is performed, according to good clinical practice. The results of this trial will be disseminated in a peer-reviewed journal.

Trial registration numbers: NCT03201783

Strengths and limitations of this study

1. This is the first randomised controlled trial comparing the ongoing pregnancy rate of immediate versus delayed FET following a stimulated IVF cycle.
2. This is the first trial that seeks to add significantly to the clinical evidence base and to allow conclusions to be made on the time interval in the FET following a stimulated IVF cycle.
3. The study includes women aged 20–43 years undergoing the first FET after GnRH agonist and GnRH antagonist ovarian stimulation in IVF/ICSI; thus, results can be extrapolated to the majority of the infertile population.
4. The researchers, doctors and the participants cannot be blinded to treatment allocation.

BACKGROUND

Frozen-thawed embryo transfer (FET) has become an increasingly important part of in-vitro fertilisation (IVF) treatment.[1] When women fail to get pregnant after replacing embryos in the stimulated IVF cycle, many of those who have frozen embryos would like to proceed FET as soon as possible in order to get pregnant as soon as possible.

Ovarian stimulation exerts a detrimental effect on endometrial receptivity.[2] Ovarian stimulation leads to supraphysiological hormonal concentrations in blood which may exert negative influence on perinatal and neonatal outcomes.[3-5] The freeze-all strategy has drawn attention in recent literature with the advantages of increased maternal safety, improved pregnancy rates, lower ectopic pregnancy rates and better obstetric and neonatal outcomes.[6] The better outcomes after elective FET in the context of a freeze-all strategy may be at least partially attributed to the lack of endometrial impairment that is observed during ovarian stimulation.

Robust information regarding the optimal timing for FET following a stimulated IVF cycle is still lacking. One option is to perform FET in the first cycle following the stimulated IVF cycle i.e. immediate transfer. Another option is to postpone FET for at least one menstrual cycle i.e. delayed transfer. Delaying FET may add to the stress and anxiety accompanying the IVF treatment. Several retrospective studies showed similar clinical pregnancy rates or live birth rates between immediate and delayed FET performed following fresh embryo transfers or in a frozen-all policy.[7-9] Another retrospective analysis showed that significantly higher implantation, clinical pregnancy and live birth rates were found in the delayed FET group than in the immediate group after failed fresh ET cycles.[10] Since these studies are all retrospective and the findings are contradictory, a randomized study is needed to provide Level 1 evidence to guide the clinical practice.

We aim in this randomized trial to compare the ongoing pregnancy rate of immediate versus delayed FET following a stimulated IVF cycle. The hypothesis is that the ongoing pregnancy rates of immediate and delayed FET are comparable.

MATERIALS AND METHODS

Study design

This is a multi-center randomized controlled study carried out in the Shanghai JiAi Genetics and IVF institute and Department of Obstetrics and Gynaecology, the University of Hong Kong. The trial has been registered at ClinicalTrials.gov (NCT03201783). The flow chart of this study is shown in figure 1 and the overview of the study visits is shown in table 1.

Table 1 Overview of study visits

	Screen and Baseline visit	Treatment visit	Pregnancy visit	Follow up visit
Physical examination (weight, height)	√			
Menstrual cycle	√			
Fasting blood samples for E2, P,		√		
Preconception counseling	√			
Questionnaire	√	√		
Transvaginal ultrasound	√	√	√	√
Pregnancy test			√	√
Pregnancy and neonatal records				√

E2, estradiol; P, progesterone.

Participants

The study participants will consist of women and their partners initiating ART treatment at the Shanghai JiAi Genetics and IVF institute in China and Department of Obstetrics and Gynaecology, the University of Hong Kong. Recruitment will be carried out by the doctors at the fertility clinics. Eligible women will be recruited if they fulfil all of the inclusion criteria and do not meet any of the exclusion criteria. They will be included once for this study. Informed written consent will be obtained.

Inclusion criteria are:

- Women aged <=43 years at the time of IVF/ICSI treatment
- Undergoing IVF with a standard stimulation
- At least one frozen embryo or blastocyst
- The first FET cycle following ovarian stimulation in IVF

Exclusion criteria include

- Use of mild stimulation or natural cycle for IVF/ICSI treatment
- Severe ovarian hyperstimulation syndrome during IVF treatment
- Preimplantation genetic diagnosis treatment

- Use of donor oocytes
- Presence of hydrosalpinx which is not surgically treated or endometrial polyp on scanning during ovarian stimulation

Standard and mild stimulation is defined according to the published terminology for ovarian stimulation for IVF.[11] OHSS is diagnosed and classified according to the RCOJ guideline.[12]

Randomization

Women having the first FET cycle after a failed stimulated IVF cycle or undertaking the freeze all strategy will be randomized according to a computer-generated randomization list prepared by a study nurse who will not be involved in the recruitment into one of the following two groups. The exact timing of randomization is on the day of embryo freezing for patients taking the freeze all strategy and on the day of blood HCG test on 14 days after fresh-ET for the failed fresh-ET women. The randomization is carried out by a project nurse who is not involved in the recruitment and clinical management of patients using an online randomization program through the website www.randomization.com. Then the nurse will prepare the randomization arm and put it into opaque envelops for use. On the randomization day, the recruited women will be randomized according to the opaque envelops into one of the two groups

(1) the immediate group in which FET will be performed in the first cycle following the stimulated IVF cycle and
(2) the delayed group in which FET will be performed at least in the second cycle following the stimulated IVF cycle.

Blinding

Both the researchers and the participants cannot be blinded because the nature of the study. The embryologist performing the quality assessment is blinded to the allocated treatment.

Interventions

Women will undergo IVF treatment in the centre as clinically indicated. Standard ovarian stimulation with gonadotrophins in either a GnRH antagonist protocol or long GnRH agonist will be employed.

Oocyte retrieval will be performed under transvaginal ultrasound guidance 34–36h after triggering with hCG or an agonist. Oocytes will be fertilized using either conventional insemination or intracystoplasmic sperm injection depending the semen quality of the husbands in accordance with the standard protocol. Normal fertilization will be assessed and confirmed by the presence of two pronuclei and a second polar body at 16–18 h after insemination or intracystoplasmic sperm injection. On day 3 after egg retrieval, an embryo with at least seven blastomeres and Grades 1 and 2 is defined as good quality. Embryos with at least six blastomeres and fragments<50% will be frozen. All good embryos will be frozen or vitrified using the Crytop method as cleavage stage embryos on Day 3 or as full to expanded blastocysts on Day 5 or Day 6 of embryo culture according to the standard protocol. Patients ≥6 good quality embryos of on day 3 were eligible for a blastocyst culture and transfer.

We will measure the stress and anxiety levels by the standard questionnaire before the randomization and at the time of starting FET. Chinese State-Trait Anxiety Inventory (C-STAI). The C-STAI was used to measure the patient's anxiety level.[13]

Hormone replacement treatment (HRT) will be used for endometrial preparation. On Day 3 of the menstrual cycle, estradiol valerate (E2, Progynova, Schering AG, Berlin, Germany) will be commenced 4mg daily for 10 days. When the thickness of the endometrial layer reaches at least 8 mm on pelvic scanning, vaginal progesterone 90 mg per day (Crinone, Merck-Serono, Switzerland) will be administered. For Day 3 embryos, FET is scheduled on the fourth day of starting vaginal progesterone. For blastocysts, FET is scheduled on the sixth day of starting vaginal progesterone. A maximum of 1–2 embryos or blastocysts with the best morphology will be transferred under ultrasound guidance using a soft embryo transfer catheter. Serum hCG level will be checked 14 days after FET. All hormone therapy will be stopped if the serum hCG level is negative. All pregnant women will continue the hormonal therapy until 12 weeks of gestation.

Follow-up and data collection

If the serum hCG level is positive, transvaginal ultrasound will be performed two weeks later to locate the pregnancy and confirm foetal viability. Subsequent management will be the same as other women with early pregnancy. They will be referred for antenatal care when the ongoing pregnancy is 12 weeks.

Written consent regarding retrieval of pregnancy and delivery data will be sought from the patient at the time of study. The patient will be contacted after delivery by phone to retrieve the information of the pregnancy outcomes. The outcome of the pregnancy (delivery, miscarriage), number of babies born, birth weights and obstetrics complications will be recorded.

Outcome measurements

Primary outcome

The primary outcome is an ongoing pregnancy defined as a viable pregnancy beyond gestation 12 weeks.

Secondary outcomes include

- positive hCG level: Conception is defined with the result of serum β-hCG ≥10 mIU/mL.
- clinical pregnancy defined as presence of intrauterine gestational sac by transvaginal ultrasound at gestational week 6.
- implantation rate as the number of gestational sacs per embryo transferred.
- multiple pregnancy, ectopic pregnancy and miscarriage rates. Miscarriage rate is defined as a clinically recognized pregnancy loss before the 22 weeks of pregnancy. The denominator is the clinical pregnancy.
- live birth rate and: A live birth is defined as the delivery of any number of newborns ≥22 weeks' gestation with heartbeat and breath.
- birth weight of newborns.

Data entry and quality control of data

Treatment-related data including baseline information and COH data are collected at the day of embryo frozen. Data on FET cycle are collected at frozen embryo transfer day. Follow-up data on all pregnancies resulting from FET according to the study protocol will be followed from study inclusion and 1 year onwards. Participants information forms will be developed for data entry, and quality control of the data will be handled at two different levels. The investigators will be required to ensure the accuracy of the data as the first level of control, and the second level will include data monitoring and validation that will be carried out on a regular basis through out the study. Data are backed up daily to another computer in the same physical location as the server.

Sample size calculations and statistical analysis

Sample size estimation

According to our data of the Centre, the ongoing pregnancy rate per FET was about 30%. We hypothesize that a difference in the ongoing pregnancy rate of 10% between the immediate versus delayed groups as equivalence, the sample size required for a test of equivalence would be 329 in each arm to give a power of 0.8 and type I error of 0.05. Allowing 10% drop-out, 724 subjects or 362 in each arm will be needed.

Data analysis

Data will be analysed with an intention to treat and per protocol. Demographic features of the two groups will be compared. Comparison of quantitative variables will be performed using Student's t, while categorical variables will be compared using a Chi-square analysis. A multivariable logistic regression analysis will be used to compare the variables between two groups. We use the multivariable logistic regression to adjust for potentially confounding factors and results, namely female age (as a continuous variable), retrieved oocytes, COH protocol, ovulation trigger, number of good quality embryos produced (as a continuous variable) and number of embryos transferred (one versus two), developmental stage (cleavage versus blastocyst stage) and quality of the embryos transferred (quality of the embryo transferred). All

statistical analyses of the data will be performed using the SPSS program V.21.0 (SPSS Inc, Chicago, Illinois, USA), and a p value <0.05 will be considered statistically significant.

ETHICS AND DISSEMINATION

Since FET in HRT cycles is a standard procedure in IVF centers, and there is no agreement regarding the time interval between the stimulated IVF and the subsequent FET in the literature, there are not predefined criteria for premature termination of the study. There is no interim analysis during the study.

The women who agree to participate in the study will sign a consent form (see online supplementary appendix 1) after detailed counseling of the study and they are free to withdraw from the study at any time without giving any reasons and having any impact on the medical care they are receiving.

Data will be entered electronically. All data will be stored in locked computer files that are accessible only to the investigators and research staffs involved in the study. Original study forms will be kept locked at the study site and maintained in storage for a period of 3 years after the completion of the study. The principal investigator will be responsible for data management including data coding, monitoring and verification. The investigators have always maintained a strict privacy policy. The investigators permit trial-related monitoring, audits, IRB/IEC review and regulatory inspections, providing direct access to source data/documents. For questions about the study, the participants should contact their physician.

A data and safety monitoring committee will review and interpret the data generated from the study, and its primary objectives will be to ensure the safety of the study participants and the integrity of the research data. The committee consists of two independent researchers with experience in reproductive medicine.

An audit trail will be designed as another security measure. Computer-generated and time-stamped audit trails will be implemented for tracking changes in the electronic source documentation. Internal safeguards will be built into the computerised system. Records will be regularly backed up, and record logs will be maintained to prevent data loss and to ensure the data`s quality and integrity.

Amendments of the protocol will be agreed on by the IRB/IEC, data and safety monitoring committee and will be approved by the ethics committee prior to implementation.

The results of this trial will be disseminated in peer-reviewed journals and presented at international meetings.

DISCUSSION

FET has been a routine procedure in the IVF treatment,[14,15] but the optimal time for FET following ovarian stimulation is still unknown. This randomized study has been designed, therefore, to evaluate ongoing pregnancy rate of immediate versus delayed FET following a stimulated IVF cycle. It seeks to add significantly to the clinical evidence base and to allow conclusions to be made on the time interval in the FET following a stimulated IVF cycle. The present study is the first RCT to compare immediate versus delayed FET followed stimulated IVF cycle on ongoing pregnancy rate.

In order to increase the generalizability of our results, we include both patients having the first FET cycle after a failed stimulated IVF cycle or undertaking the freeze all strategy.

The study was designed in May 2017, and the first participant was randomised on 9 August 2017. At the time of the manuscript preparation, we have recruited 200 women and the recruitment is ongoing.

Authors' contributions: HL, XXS and EHYN conceived and designed the study. HL and EHYN drafted and critically revised the manuscript for important intellectual content. XXS sought ethical approval. LL and XL participated in the coordination of the study and recruitment of subjects. All the authors contributed to the further writing of the manuscript and approved the final manuscript.

Funding statement: This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests : None declared.

Patient consent: Obtained.

Ethics approval: Approval was obtained from the Ethics Committee of assisted reproductive medicine in Shanghai JiAi Genetics and IVF institute (JIAI E2017-12).

Provenance and peer review: Not commissioned; externally peer reviewed.

References:

1. Doody KJ. Cryopreservation and delayed embryo transfer-assisted reproductive technology registry and reporting implications. *Fertil Steril* 2014;102(1):27-31.

2. Shapiro BS, Daneshmand ST, Garner FC, et al. Evidence of impaired endometrial receptivity after ovarian stimulation for in vitro fertilization: a prospective randomized trial comparing fresh and frozen-thawed embryo transfer in normal responders. *Fertil Steril* 2011;96(2):344-8.

3. Venetis CA, Kolibianakis EM, Bosdou JK, et al. Estimating the net effect of progesterone elevation on the day of hCG on live birth rates after IVF: a cohort analysis of 3296 IVF cycles. *Hum Reprod* 2015;30(3):684-91.

4. Weinerman R, Mainigi M. Why we should transfer frozen instead of fresh embryos: the translational rationale. *Fertil Steril* 2014;102(1):10-8.

5. Roque M, Valle M, Guimaraes F, et al. Freeze-all policy: fresh vs. frozen-thawed embryo transfer. *Fertil Steril* 2015;103(5):1190-3.

6. Blockeel C, Drakopoulos P, Santos-Ribeiro S, et al. A fresh look at the freeze-all protocol: a SWOT analysis. *Hum Reprod* 2016;31(3):491-7.

7. Santos-Ribeiro S, Polyzos NP, Lan VT, et al. The effect of an immediate frozen embryo transfer following a freeze-all protocol: a retrospective analysis from two centres. *Hum Reprod* 2016;31(11):2541-48.

8. Santos-Ribeiro S, Siffain J, Polyzos NP, et al. To delay or not to delay a frozen embryo transfer after a failed fresh embryo transfer attempt? *Fertil Steril* 2016;105(5):1202-07 e1.

9. Lattes K, Checa MA, Vassena R, et al. There is no evidence that the time from egg retrieval to embryo transfer affects live birth rates in a freeze-all strategy. *Hum Reprod* 2017;32(2):368-74.

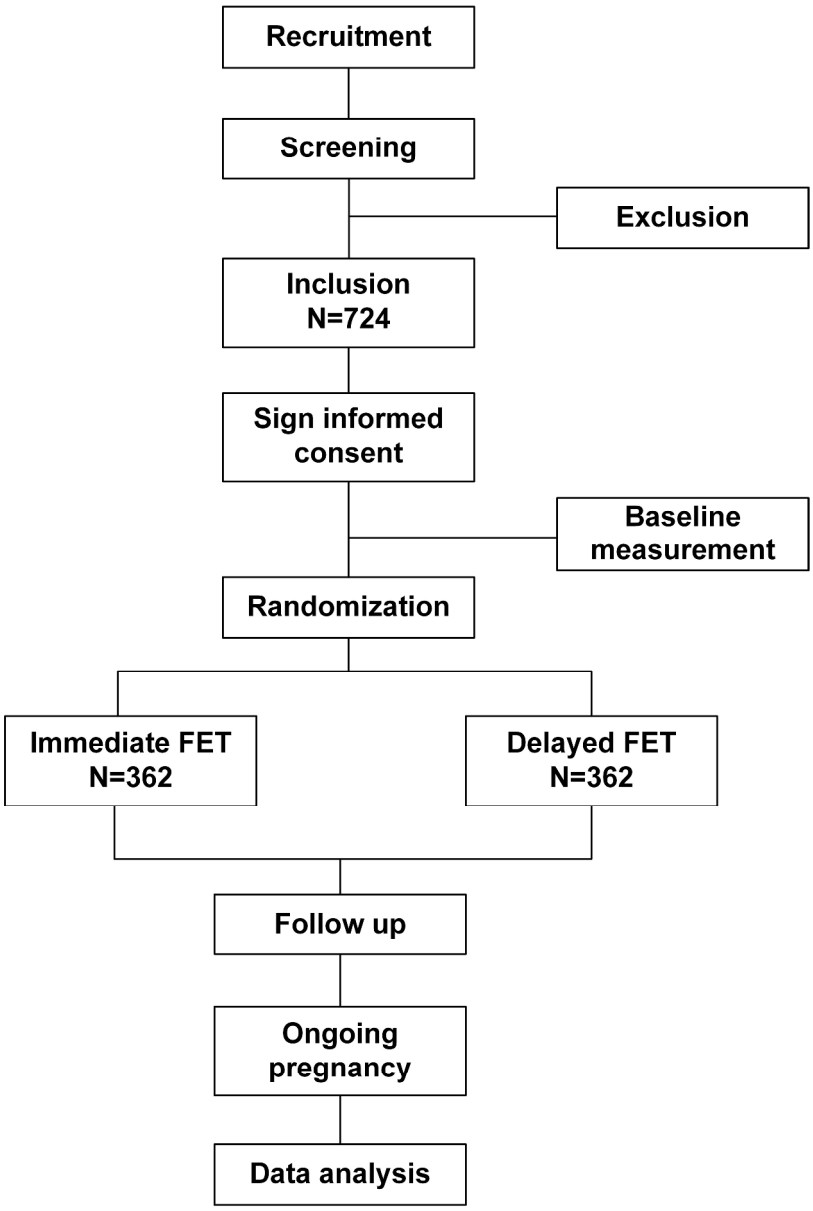
10. Volodarsky-Perel A, Eldar-Geva T, Holzer HE, et al. Cryopreserved embryo transfer: adjacent or non-adjacent to failed fresh long GnRH-agonist protocol IVF cycle. *Reprod Biomed Online* 2017;34(3):267-73.

11.Nargund G, Fauser BC, Macklon NS, et al. The ISMAAR proposal on terminology for ovarian stimulation for IVF. *Hum Reprod* 2007;22(11):2801-4.

12. Green-top guideline No.5: Ovarian Hyperstimulation Syndrome, https://www.rcog.org.uk/en/guidelines-research-services/ guidelines/gtg5/. (26 February 2015, date last accessed).
13. Shek DTL. The Factorial Structure of the Chinese Version of the State-Trait Anxiety Inventory: A Confirmatory Factor Analysis. *Educational & Psychological Measurement* 1991;51(4):985-97.
14. Cedars MI. Fresh versus frozen: initial transfer or cumulative cycle results: how do we interpret results and design studies? *Fertil Steril* 2016;106(2):251-6.
15. Wong KM, Mastenbroek S, Repping S. Cryopreservation of human embryos and its contribution to in vitro fertilization success rates. *Fertil Steril* 2014;102(1):19-26.

Figure 1

The study flow chart. FET, frozen-thawed embryo transfer.



Flow chart of study

178x266mm (600 x 600 DPI)

Shanghai JiAi Genetics & IVF Institute

Study: A randomized trial comparing the effect of immediate versus delayed frozen-thawed embryo transfer following a stimulated IVF cycle

PATIENT INFORMATION AND CONSENT

STUDY TITLE: A randomized trial comparing the effect of immediate versus delayed frozen-thawed embryo transfer following a stimulated IVF cycle

You are being invited to participate in the above named research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

What is the purpose of the study?

Information regarding the optimal timing for frozen-thawed embryo transfer (FET) following a stimulated in vitro fertilization (IVF) is lacking. One option is to perform FET in the first cycle following the stimulated IVF cycle, i.e. immediate transfer. Another option is to postpone FET for at least one menstrual cycle, i.e. delayed transfer.

Several retrospective studies showed similar success for these two options. Another retrospective analysis showed higher clinical pregnancy and live birth rates in the delayed group. Since these studies are all retrospective and the findings are contradictory, a randomized study is needed to provide good evidence to guide the clinical practice.

This randomized study aims to compare the ongoing pregnancy rate of immediate versus delayed FET following a stimulated IVF cycle.

Why have I been chosen?

You are chosen because

- You are <=43 years of age at the time of IVF treatment.
- You underwent IVF with a standard stimulation.
- You have at least one frozen embryo or blastocyst.
- You are undergoing the first FET following ovarian stimulation in IVF.

You will not be included in this study if

- You are using mild stimulation or natural cycle in the IVF treatment.
- You had severe ovarian hyperstimulation syndrome during IVF treatment.
- You had pre-implantation genetic diagnosis treatment.
- You are using donor oocytes.

Shanghai JiAi Genetics & IVF Institute

Study: A randomized trial comparing the effect of immediate versus delayed frozen-thawed embryo transfer following a stimulated IVF cycle

- Presence of hydrosalpinx which is not surgically treated or endometrial polyp on scanning during ovarian stimulation.

Do I have to take part?

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. If you do not join or if you quit the study, you will still receive the standard treatment as other patients in our Department.

What will happen to me if I take part?

If you agree to participate in the study, you will be randomized by a computer-generated list into one of the two groups:

(1) ***Immediate group***: Your thawed embryos will be transferred into your womb in the first cycle following the stimulated IVF cycle.

(2) ***Delayed group*****:** Your thawed embryos will be transferred into your womb at least in the second cycle following the stimulated IVF cycle.

The groups are selected by a computer that has no information about the individual, i.e. by chance. We will compare the outcomes between the two groups at the end of the study.

How many other people will be participating in the study?

We plan to recruit 724 women in this study.

What are the disadvantages and risks of taking part?

There should be no safety concern. No specific risk is expected. No extra charge or visit is required for participating in the study.

What are the benefits of taking part?

No payment will be made to you for this study.

What will happen to the results of the research?

The results of the study will be presented in international meetings and published in a medical journal. You will not be identified in any report or publication.

Shanghai JiAi Genetics & IVF Institute

Study: A randomized trial comparing the effect of immediate versus delayed frozen-thawed embryo transfer following a stimulated IVF cycle

Confidentiality and privacy

The investigators have always maintained a strict privacy policy. We never sell, trade or otherwise share your details with any sources. All correspondence to the department is held confidentially; furthermore, at no time will your personal and/or identifying information be shared outside of our organization, for any reason.

You have the rights of access to personal data and publicly available study results, if and when needed. Under the laws of China, you enjoy or may enjoy rights for the protection of the confidentiality of your personal data, such as those regarding the collection, custody, retention, management, control, use (including analysis or comparison), transfer in or out of China, non-disclosure, erasure and/or in any way dealing with or disposing of any of your personal data in or for this study.

By consenting to participate in this study, you expressly authorize:

- the principal investigator, the research team and the Institutional Review Board responsible for overseeing this study to get access to, to use, and to retain your personal data for the purposes and in the manner described in this informed consent process; and
- the relevant government agencies (e.g. the Shanghai Municipal Commission of Health and Family Planning) to get access to your personal data for the purposes of checking and verifying the integrity of study data and assessing compliance with the study protocol and relevant requirements.

Contact for further information

For questions about the study or reporting of adverse events, you may contact the Principal Investigator, Dr Li He at telephone no.13817223099. The phone number of Shanghai JIAI Genetics & IVF is 021-63459977.

Thank you for your time to read this information sheet and for taking part in the study.

Shanghai JiAi Genetics & IVF Institute

Study: A randomized trial comparing the effect of immediate versus delayed frozen-thawed embryo transfer following a stimulated IVF cycle

PATIENT CONSENT FORM

Patient Identification Number for this trial: _____

Title of Project: A randomized trial comparing the effect of immediate versus delayed frozen-thawed embryo transfer following a stimulated IVF cycle

1. We confirm that we have read and understood the information sheet for the above study and have had the opportunity to ask questions.
2. We understand that our participation is voluntary and that we are free to withdraw at any time, without giving any reason, without our medical care or legal rights being affected.
3. We understand that sections of any of our medical notes may be looked at by responsible individuals from regulatory authorities where it is relevant to our taking part in research. We give permission for these individuals to have access to our records.
4. We agree to take part in the above study.
5. We give permission to the investigators to retrieve pregnancy and delivery data.

Patient's signature	Patient's name	Date
Patient's husband signature	Patient's husband name	Date
Investigator's signature	Investigator's name	Date
Witness's signature	Witness's name	Date

SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	ItemNo	Description
Administrative information		
Title	1 P1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym
Trial registration	2a P4	Trial identifier and registry name. If not yet registered, name of intended registry
	2b n/a	All items from the World Health Organization Trial Registration Data Set
Protocol version	3 n/a	Date and version identifier
Funding	4 P12	Sources and types of financial, material, and other support
Roles and responsibilities	5a P12	Names, affiliations, and roles of protocol contributors
	5b n/a	Name and contact information for the trial sponsor
	5c n/a	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities
	5d P12	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)
Introduction		
Background and rationale	6a P3-4	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention
	6b P3-4	Explanation for choice of comparators
Objectives	7 P4	Specific objectives or hypotheses

Trial design	8 P4	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)
Methods: Participants, interventions, and outcomes		
Study setting	9 P4	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained
Eligibility criteria	10 P5-6	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)
Interventions	11a P6-7	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered
	11b P10	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)
	11c P6-8	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)
	11d P6-8	Relevant concomitant care and interventions that are permitted or prohibited during the trial
Outcomes	12 P8	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended
Participant timeline	13 P4	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)
Sample size	14 P9	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations
Recruitment	15 P5	Strategies for achieving adequate participant enrolment to reach target sample size
Methods: Assignment of interventions (for controlled trials)		
Allocation:		

Sequence generation	16a P6	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions
Allocation concealment mechanism	16b P6	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned
Implementation	16c P6	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions
Blinding (masking)	17a P6	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how
	17b n/a	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial
Methods: Data collection, management, and analysis		
Data collection methods	18a P9-11	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol
	18 P9-11	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols
Data management	19 P9-11	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol
Statistical methods	20a P9-10	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol
	20b P9-10	Methods for any additional analyses (eg, subgroup and adjusted analyses)

	20c n/a	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)
Methods: Monitoring		
Data monitoring	21a P10	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed
	21b P10	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial
Harms	22 P10	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct
Auditing	23 P11	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor
Ethics and dissemination		
Research ethics approval	24 P12	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval
Protocol amendments	25 P11	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)
Consent or assent	26a P10	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)
	26b n/a	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable
Confidentiality	27 P9-11	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial
Declaration of interests	28 P12	Financial and other competing interests for principal investigators for the overall trial and each study site
Access to data	29 P10-11	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators

Ancillary and post-trial care	30 n/a	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation
Dissemination policy	31a P11	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions
	31b n/a	Authorship eligibility guidelines and any intended use of professional writers
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code Please see the data sharing plan in www.ClinicalTrials.gov (NCT03201783)
Appendices		
Informed consent materials	32 P10	Model consent form and other related documentation given to participants and authorised surrogates
Biological specimens	33 n/a	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

Comparison of the effect of immediate versus delayed transfer following a stimulated IVF cycle on the ongoing pregnancy rate of frozen-thawed embryo transfer cycles: a study protocol for a randomised controlled trial.

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2017-020507.R2
Article Type:	Protocol
Date Submitted by the Author:	08-Mar-2018
Complete List of Authors:	LI, HE LI, LU; Shanghai Ji Ai Genetics and IVF Institute, Obstetrics and Gynecology Hospital, Fudan University, Shanghai, China LU, XIANG; Shanghai Ji Ai Genetics and IVF Institute, Obstetrics and Gynecology Hospital, Fudan University, Shanghai, China SUN, XIAOXI; Shanghai Ji Ai Genetics and IVF Institute, Obstetrics and Gynecology Hospital, Fudan University, Shanghai, China; Key Laboratory of Female Reproductive Endocrine Related Diseases, Obstetrics and Gynecology Hospital, Fudan University, Shanghai, China Ng, Ernest; The University of Hong Kong, Department of Obstetrics and Gynecology
Primary Subject Heading:	Reproductive medicine
Secondary Subject Heading:	Obstetrics and gynaecology
Keywords:	Subfertility < GYNAECOLOGY, IVF, FET, ART

Comparison of the effect of immediate versus delayed transfer following a stimulated IVF cycle on the ongoing pregnancy rate of frozen-thawed embryo transfer cycles: a study protocol for a randomised controlled trial.

Authors and affiliations

He Li[1], Lu Li[1], Xiang Lu[1], Xiaoxi Sun[1,2*] , Ernest Hung Yu Ng[3*]

1. Shanghai Ji Ai Genetics and IVF Institute, Obstetrics and Gynecology Hospital, Fudan University, Shanghai, China
2. Key Laboratory of Female Reproductive Endocrine Related Diseases, Obstetrics and Gynecology Hospital, Fudan University, Shanghai, China
3. Department of Obstetrics and Gynaecology, The University of Hong Kong, Hong Kong Special Administrative Region, Hong Kong, Hong Kong

* Corresponding authors: Email: nghye@hku.hk (EHY Ng) or: Email: xiaoxi_sun@aliyun.com (X Sun)

ABSTRACT

Introduction: Frozen-thawed embryo transfer (FET) has become an increasingly important part of in vitro fertilization (IVF) treatment. Currently, there is still no good scientific evidence to support when to perform FET following a stimulated IVF cycle. Since all published studies are retrospective and the findings are contradictory, a randomized controlled study is needed to provide Level 1 evidence to guide the clinical practice.

Methods/analysis: This is a randomized controlled trial. A total of 724 women undergoing the first FET following ovarian stimulation in IVF will be enrolled and randomized according to a computer-generated randomization list to either (1) the immediate group in which FET will be performed in the first cycle following the stimulated IVF cycle or (2) the delayed group in which FET will be performed at least in the second cycle following the stimulated IVF cycle. The primary outcome is the ongoing pregnancy defined as a viable pregnancy beyond 12 weeks gestation.

Ethics/dissemination: Ethical approval has been granted from the ethics committee of assisted reproductive medicine in Shanghai JiAi Genetics and IVF institute (JIAI E2017-12) and from Institutional Review Board of the University of Hong Kong Hospital Authority Hong Kong West Cluster (UW 17-371). A written informed consent will be obtained from each woman before any study procedure is performed, according to good clinical practice. The results of this trial will be disseminated in a peer-reviewed journal.

Trial registration number: NCT03201783

Strengths and limitations of this study

1. This is the first randomised controlled trial comparing the ongoing pregnancy rate of immediate versus delayed FET following a stimulated IVF cycle.
2. This is the first trial that seeks to add significantly to the clinical evidence base and to allow conclusions to be made on the time interval in the FET following a stimulated IVF cycle.
3. The study includes women aged 20–43 years undergoing the first FET after GnRH agonist and GnRH antagonist ovarian stimulation in IVF/ICSI; thus, results can be extrapolated to the majority of the infertile population.
4. The researchers, doctors and the participants cannot be blinded to treatment allocation.
5. The sample size calculation is based on a difference in the ongoing pregnancy rate of 10% between the immediate versus delayed groups as equivalence and may not be able to detect a smaller difference in the ongoing pregnancy rate.

BACKGROUND

Frozen-thawed embryo transfer (FET) has become an increasingly important part of in-vitro fertilisation (IVF) treatment.[1] When women fail to get pregnant after replacing embryos in the stimulated IVF cycle, many of those who have frozen embryos would like to proceed FET as soon as possible in order to get pregnant as soon as possible.

Ovarian stimulation exerts a detrimental effect on endometrial receptivity.[2] Ovarian stimulation leads to supraphysiological hormonal concentrations in blood which may exert negative influence on perinatal and neonatal outcomes.[3-5] The freeze-all strategy has drawn attention in recent literature with the advantages of increased maternal safety, improved pregnancy rates, lower ectopic pregnancy rates and better obstetric and neonatal outcomes.[6] The better outcomes after elective FET in the context of a freeze-all strategy

may be at least partially attributed to the lack of endometrial impairment that is observed during ovarian stimulation.

Robust information regarding the optimal timing for FET following a stimulated IVF cycle is still lacking. One option is to perform FET in the first cycle following the stimulated IVF cycle i.e. immediate transfer. Another option is to postpone FET for at least one menstrual cycle i.e. delayed transfer. Delaying FET may add to the stress and anxiety accompanying the IVF treatment. Several retrospective studies showed similar clinical pregnancy rates or live birth rates between immediate and delayed FET performed following fresh embryo transfers or in a frozen-all policy.[7-9] Another retrospective analysis showed that significantly higher implantation, clinical pregnancy and live birth rates were found in the delayed FET group than in the immediate group after failed fresh ET cycles.[10] Since these studies are all retrospective and the findings are contradictory, a randomized study is needed to provide Level 1 evidence to guide the clinical practice.

We aim in this randomized trial to compare the ongoing pregnancy rate of immediate versus delayed FET following a stimulated IVF cycle. The hypothesis is that the ongoing pregnancy rates of immediate and delayed FET are comparable.

MATERIALS AND METHODS

Study design

This is a two center randomized controlled study carried out in the Shanghai JiAi Genetics and IVF institute and Department of Obstetrics and Gynaecology, the University of Hong Kong. The trial has been registered at ClinicalTrials.gov (NCT03201783). The flow chart of this study is shown in figure 1 and the overview of the study visits is shown in table 1.

Table 1 Overview of study visits

	Screen and Baseline visit	Treatment visit	Pregnancy visit	Follow up visit
Physical examination (weight, height)	√			
Menstrual cycle	√			
Fasting blood samples for E2, P,		√		
Preconception counseling	√			
Questionnaire	√	√		
Transvaginal ultrasound	√	√	√	√
Pregnancy test			√	√
Pregnancy and neonatal records				√

E2, estradiol; P, progesterone.

Participants

The study participants will consist of women and their partners initiating IVF or intracystoplasmic sperm injection (ICSI) treatment at the Shanghai JiAi Genetics and IVF institute in China and Department of Obstetrics and Gynaecology, the University of Hong Kong. Recruitment will be carried out by the doctors at the fertility clinics. Eligible women will be recruited if they fulfil all of the inclusion criteria and do not meet any of the exclusion criteria. They will be included once for this study. After detailed explanation, counselling and signing the informed consent form, the eligible participants will be randomly allocated to either the immediate group or the delayed group.

Inclusion criteria

- Women aged <=43 years at the time of IVF/ICSI treatment
- Undergoing IVF with a standard stimulation
- At least one frozen embryo or blastocyst
- The first FET cycle following ovarian stimulation in IVF/ICSI

Exclusion criteria

- Use of mild stimulation or natural cycle for IVF/ICSI treatment

- Severe ovarian hyperstimulation syndrome during IVF/ICSI treatment
- Preimplantation genetic diagnosis treatment
- Use of donor oocytes
- Presence of hydrosalpinx which is not surgically treated or endometrial polyp on scanning during ovarian stimulation

Standard and mild stimulation is defined according to the published terminology for ovarian stimulation for IVF.[11] OHSS is diagnosed and classified according to the RCOG guideline.[12]

Randomization

Women having the first FET cycle after a failed stimulated IVF cycle or undertaking the freeze all strategy will be randomized according to a computer-generated randomization list into one of the following two groups. The exact timing of randomization is on the day of embryo freezing for patients taking the freeze all strategy and on the day of blood HCG test on 14 days after fresh-ET for the failed fresh-ET women. The randomization is carried out by a project nurse who is not involved in the recruitment and clinical management of patients using an online randomization program through the website www.randomization.com. Then the nurse will prepare the randomization arm and put it into opaque envelops for use. On the randomization day, the recruited women will be randomized according to the opaque envelops into one of the two groups

(1) the immediate group in which FET will be performed in the first cycle following the stimulated IVF cycle and
(2) the delayed group in which FET will be performed at least in the second cycle following the stimulated IVF cycle.

Blinding

Both the researchers and the participants cannot be blinded because the nature of the study. The embryologist performing the quality assessment is blinded to the allocated treatment.

Interventions

Women will undergo IVF/ICSI treatment in the centre as clinically indicated. Standard ovarian stimulation with gonadotrophins in either a GnRH antagonist protocol or long GnRH agonist protocol will be employed. Oocyte retrieval will be performed under transvaginal ultrasound guidance 34–36h after triggering with hCG or an agonist. Oocytes will be fertilized using either conventional insemination or intracystoplasmic sperm injection depending the semen quality of the husbands in accordance with the standard protocol. Normal fertilization will be assessed and confirmed by the presence of two pronuclei and a second polar body at 16–18 h after insemination or intracystoplasmic sperm injection. On day 3 after oocyte retrieval, an embryo with at least seven blastomeres and Grades 1 and 2 is defined as good quality. Embryos with at least six blastomeres and fragments<50% will be frozen. All good embryos will be frozen or vitrified using the Crytop method as cleavage stage embryos on Day 3 or as full to expanded blastocysts on Day 5 or Day 6 of embryo culture according to the standard protocol. Patients who have ≥ 6 good quality embryos on day 3 will be counseled for extended culture and blastocyst transfer.

We will measure the stress and anxiety levels by the standard questionnaire before the randomization and at the time of starting FET. The Chinese State-Trait Anxiety Inventory (C-STAI) was used to measure the patient's anxiety level.[13]

Hormone replacement treatment (HRT) will be used for endometrial preparation. On Day 3 of the menstrual cycle, estradiol valerate (E2, Progynova, Schering AG, Berlin, Germany) will be commenced 4mg daily for 10 days. When the thickness of the endometrial layer reaches at least 8 mm on pelvic scanning, vaginal progesterone 90 mg per day (Crinone, Merck-Serono, Switzerland) will be administered. For Day 3 embryos, FET is scheduled on the fourth day of starting vaginal progesterone. For blastocysts, FET is scheduled on the sixth day of starting vaginal progesterone. A maximum of 1–2 embryos or blastocysts with the best morphology will be transferred under ultrasound guidance using a soft embryo transfer catheter.

Serum hCG level will be checked 14 days after FET. All hormone therapy will be stopped if the serum hCG level is negative. All pregnant women will continue the hormonal therapy until 12 weeks of gestation.

Follow-up and data collection

If the serum hCG level is positive, transvaginal ultrasound will be performed two weeks later to locate the pregnancy and confirm foetal viability. Subsequent management will be the same as other women with early pregnancy. They will be referred for antenatal care when the ongoing pregnancy is 12 weeks.

Written consent regarding retrieval of pregnancy and delivery data will be sought from the patient at the time of study. The patient will be contacted after delivery by phone to retrieve the information of the pregnancy outcomes. The outcomes of the pregnancy (delivery, miscarriage), number of babies born, birth weights and obstetrics complications will be recorded.

Outcome measurements

Primary outcome

The primary outcome is an ongoing pregnancy defined as a viable pregnancy beyond gestation 12 weeks.

Secondary outcomes

- positive hCG level: Conception is defined with the result of serum β-hCG ≥10 mIU/mL.
- clinical pregnancy defined as presence of intrauterine gestational sac by transvaginal ultrasound at 6 gestational weeks.
- implantation rate as the number of gestational sacs per embryo transferred.
- multiple pregnancy, ectopic pregnancy and miscarriage rates. Miscarriage rate is defined as a clinically recognized pregnancy loss before the 22 weeks of pregnancy. The denominator is the clinical pregnancy.
- live birth rate and: A live birth is defined as the delivery of any number of newborns ≥22 weeks gestation with heartbeat and breath.

- birth weight of newborns.

Data entry and quality control of data

Treatment-related data including baseline information and COH data are collected at the day of embryo frozen. Data on FET cycle are collected at frozen embryo transfer day. Follow-up data on all pregnancies resulting from FET according to the study protocol will be followed from study inclusion and 1 year onwards. Participants information forms will be developed for data entry, and quality control of the data will be handled at two different levels. The investigators will be required to ensure the accuracy of the data as the first level of control, and the second level will include data monitoring and validation that will be carried out on a regular basis throughout the study. Data are backed up daily to another computer in the same physical location as the server.

Sample size calculations and statistical analysis

Sample size estimation

According to our data of the Centre, the ongoing pregnancy rate per FET was about 30%. We hypothesize that a difference in the ongoing pregnancy rate of 10% between the immediate versus delayed groups as equivalence, the sample size required for a test of equivalence would be 329 in each arm to give a power of 0.8 and type I error of 0.05. Allowing 10% drop-out, 724 subjects or 362 in each arm will be needed.

Data analysis

Data will be analysed with an intention to treat and per protocol. Demographic features of the two groups will be compared. Comparison of quantitative variables will be performed using Student's t, while categorical variables will be compared using a Chi-square analysis. If randomisation fails to achieve two balanced groups, we will use the multivariable logistic regression to adjust for potentially confounding factors and results, namely female age (as a continuous variable), failed fresh ET or freeze-all, retrieved oocytes, COH protocol, ovulation trigger, number of good quality embryos produced (as a

continuous variable) and number of embryos transferred (one versus two), developmental stage (cleavage versus blastocyst stage) and quality of the embryos transferred (quality of the embryo transferred). If the primary unadjusted analysis and secondary adjusted analysis are discordant, we will give greater weighting to the primary analysis in the interpretation of trial findings.

All statistical analyses of the data will be performed using the SPSS program V.21.0 (SPSS Inc, Chicago, Illinois, USA), and a p value <0.05 will be considered statistically significant.

Patient and public involvement

The research question about the optimal timing for FET following a stimulated IVF cycle was first proposed by patients who failed fresh ET or in freeze-all policy. Patients were not involved in the recruitment and conduct of the study. The study was designed as a randomised trial with participants from the infertility patients attending the clinic. The results will be disseminated to study participants by their physician.

ETHICS AND DISSEMINATION

Since FET in HRT cycles is a standard procedure in IVF centers, and there is no agreement regarding the time interval between the stimulated IVF and the subsequent FET in the literature, there are not predefined criteria for premature termination of the study. There is no interim analysis during the study.

The women who agree to participate in the study will sign a consent form (see online supplementary appendix 1) after detailed counseling of the study and they are free to withdraw from the study at any time without giving any reason and having any impact on the medical care they are receiving.

Data will be entered electronically and all data will be stored in locked computer files that are accessible only to the investigators and research staffs

involved in the study. Original study forms will be kept locked at the study site and maintained in storage for a period of 3 years after the completion of the study. The principal investigator will be responsible for data management including data coding, monitoring and verification. The investigators have always maintained a strict privacy policy. The investigators permit trial-related monitoring, audits, IRB/IEC review and regulatory inspections, providing direct access to source data/documents. For questions about the study, the participants should contact their physician.

A data and safety monitoring committee will review and interpret the data generated from the study, and its primary objectives will be to ensure the safety of the study participants and the integrity of the research data. The committee consists of two independent researchers with experience in reproductive medicine.

An audit trail will be designed as another security measure to preserve the integrity of the trial. Computer-generated and time-stamped audit trails will be implemented for tracking changes in the electronic source documentation. Internal safeguards will be built into the computerised system. Records will be regularly backed up, and record logs will be maintained to prevent data loss and to ensure the data`s quality and integrity.

Amendments of the protocol will be agreed on by the IRB/IEC, data and safety monitoring committee and will be approved by the ethics committee prior to implementation.

The study has been approved by the Ethics Committee of assisted reproductive medicine in Shanghai JiAi Genetics and IVF institute (JIAI E2017-12) and by the Institutional Review Board of the University of Hong Kong Hospital Authority Hong Kong West Cluster (UW 17-371). The results of this trial will be disseminated through peer-reviewed publications and presentations at international scientific meetings.

Trial status

The study was designed in May 2017, and the first participant was randomised on 9 August 2017. At the time of the manuscript preparation, we have recruited 200 women and the recruitment is ongoing.

Authors' contributions: HL, XXS and EHYN conceived and designed the study. HL and EHYN drafted and critically revised the manuscript for important intellectual content. XXS sought ethical approval. LL and XL participated in the coordination of the study and recruitment of subjects. All the authors contributed to the further writing of the manuscript and approved the final manuscript.

Funding statement: This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests : None declared.

Patient consent: Obtained.

Provenance and peer review: Not commissioned; externally peer reviewed.

References:

1. Doody KJ. Cryopreservation and delayed embryo transfer-assisted reproductive technology registry and reporting implications. *Fertil Steril* 2014;102(1):27-31.

2. Shapiro BS, Daneshmand ST, Garner FC, et al. Evidence of impaired endometrial receptivity after ovarian stimulation for in vitro fertilization: a prospective randomized trial comparing fresh and frozen-thawed embryo transfer in normal responders. *Fertil Steril* 2011;96(2):344-8.

3. Venetis CA, Kolibianakis EM, Bosdou JK, et al. Estimating the net effect of progesterone elevation on the day of hCG on live birth rates after IVF: a cohort analysis of 3296 IVF cycles. *Hum Reprod* 2015;30(3):684-91.

4. Weinerman R, Mainigi M. Why we should transfer frozen instead of fresh embryos: the translational rationale. *Fertil Steril* 2014;102(1):10-8.

5. Roque M, Valle M, Guimaraes F, et al. Freeze-all policy: fresh vs. frozen-thawed embryo transfer. *Fertil Steril* 2015;103(5):1190-3.

6. Blockeel C, Drakopoulos P, Santos-Ribeiro S, et al. A fresh look at the freeze-all protocol: a SWOT analysis. *Hum Reprod* 2016;31(3):491-7.

7. Santos-Ribeiro S, Polyzos NP, Lan VT, et al. The effect of an immediate frozen embryo transfer following a freeze-all protocol: a retrospective analysis from two centres. *Hum Reprod* 2016;31(11):2541-48.

8. Santos-Ribeiro S, Siffain J, Polyzos NP, et al. To delay or not to delay a frozen embryo transfer after a failed fresh embryo transfer attempt? *Fertil Steril* 2016;105(5):1202-07 e1.

9. Lattes K, Checa MA, Vassena R, et al. There is no evidence that the time from egg retrieval to embryo transfer affects live birth rates in a freeze-all strategy. *Hum Reprod* 2017;32(2):368-74.

10. Volodarsky-Perel A, Eldar-Geva T, Holzer HE, et al. Cryopreserved embryo transfer: adjacent or non-adjacent to failed fresh long GnRH-agonist protocol IVF cycle. *Reprod Biomed Online* 2017;34(3):267-73.

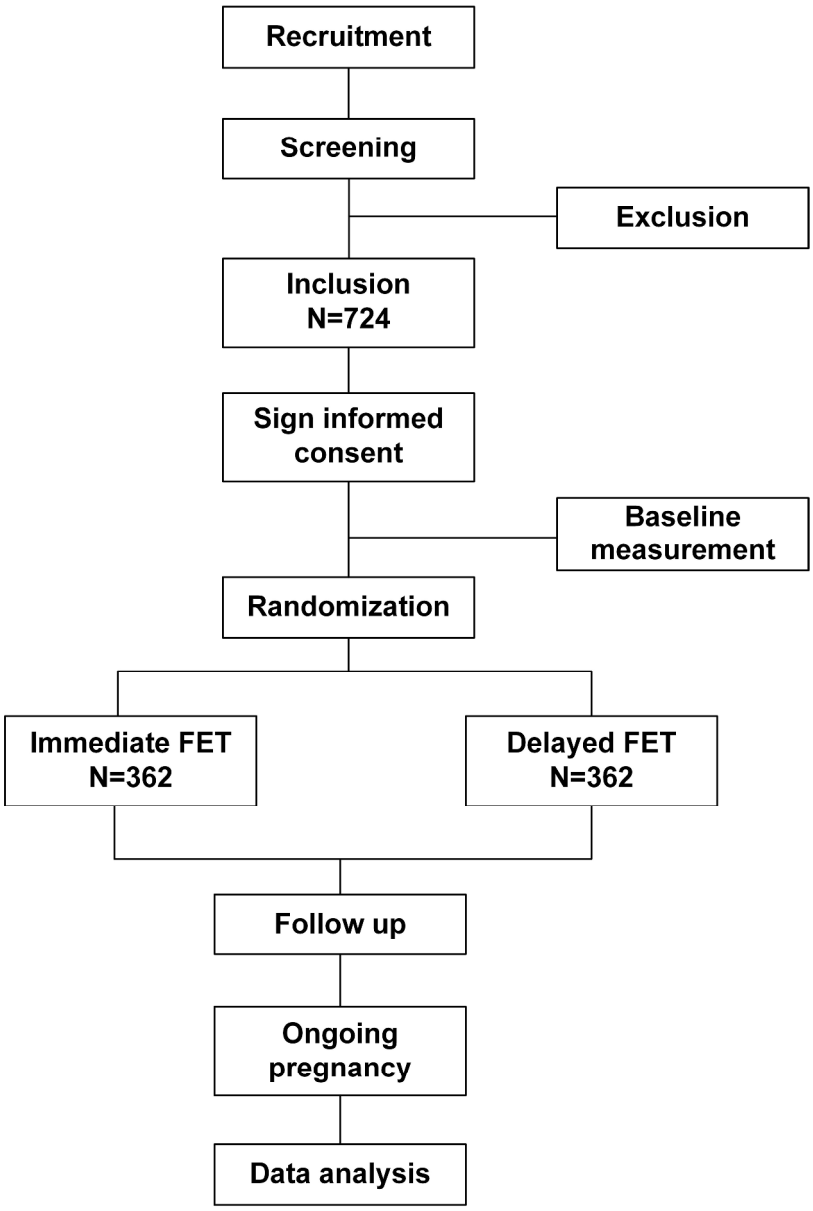
11.Nargund G, Fauser BC, Macklon NS, et al. The ISMAAR proposal on terminology for ovarian stimulation for IVF. *Hum Reprod* 2007;22(11):2801-4.

12. Green-top guideline No.5: Ovarian Hyperstimulation Syndrome, https://www.rcog.org.uk/en/guidelines-research-services/ guidelines/gtg5/. (26 February 2015, date last accessed).

13. Shek DTL. The Factorial Structure of the Chinese Version of the State-Trait Anxiety Inventory: A Confirmatory Factor Analysis. *Educational & Psychological Measurement* 1991;51(4):985-97.

Figure 1

The study flow chart. FET, frozen-thawed embryo transfer.



Flow chart of study

178x266mm (600 x 600 DPI)

Shanghai JiAi Genetics & IVF Institute

Study: A randomized trial comparing the effect of immediate versus delayed frozen-thawed embryo transfer following a stimulated IVF cycle

PATIENT INFORMATION AND CONSENT

STUDY TITLE: A randomized trial comparing the effect of immediate versus delayed frozen-thawed embryo transfer following a stimulated IVF cycle

You are being invited to participate in the above named research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

What is the purpose of the study?

Information regarding the optimal timing for frozen-thawed embryo transfer (FET) following a stimulated in vitro fertilization (IVF) is lacking. One option is to perform FET in the first cycle following the stimulated IVF cycle, i.e. immediate transfer. Another option is to postpone FET for at least one menstrual cycle, i.e. delayed transfer.

Several retrospective studies showed similar success for these two options. Another retrospective analysis showed higher clinical pregnancy and live birth rates in the delayed group. Since these studies are all retrospective and the findings are contradictory, a randomized study is needed to provide good evidence to guide the clinical practice.

This randomized study aims to compare the ongoing pregnancy rate of immediate versus delayed FET following a stimulated IVF cycle.

Why have I been chosen?

You are chosen because

- You are <=43 years of age at the time of IVF treatment.
- You underwent IVF with a standard stimulation.
- You have at least one frozen embryo or blastocyst.
- You are undergoing the first FET following ovarian stimulation in IVF.

You will not be included in this study if

- You are using mild stimulation or natural cycle in the IVF treatment.
- You had severe ovarian hyperstimulation syndrome during IVF treatment.
- You had pre-implantation genetic diagnosis treatment.
- You are using donor oocytes.

Shanghai JiAi Genetics & IVF Institute

Study: A randomized trial comparing the effect of immediate versus delayed frozen-thawed embryo transfer following a stimulated IVF cycle

- Presence of hydrosalpinx which is not surgically treated or endometrial polyp on scanning during ovarian stimulation.

Do I have to take part?

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. If you do not join or if you quit the study, you will still receive the standard treatment as other patients in our Department.

What will happen to me if I take part?

If you agree to participate in the study, you will be randomized by a computer-generated list into one of the two groups:

(1) ***Immediate group***: Your thawed embryos will be transferred into your womb in the first cycle following the stimulated IVF cycle.

(2) ***Delayed group*****:** Your thawed embryos will be transferred into your womb at least in the second cycle following the stimulated IVF cycle.

The groups are selected by a computer that has no information about the individual, i.e. by chance. We will compare the outcomes between the two groups at the end of the study.

How many other people will be participating in the study?

We plan to recruit 724 women in this study.

What are the disadvantages and risks of taking part?

There should be no safety concern. No specific risk is expected. No extra charge or visit is required for participating in the study.

What are the benefits of taking part?

No payment will be made to you for this study.

What will happen to the results of the research?

The results of the study will be presented in international meetings and published in a medical journal. You will not be identified in any report or publication.

Shanghai JiAi Genetics & IVF Institute

Study: A randomized trial comparing the effect of immediate versus delayed frozen-thawed embryo transfer following a stimulated IVF cycle

Confidentiality and privacy

The investigators have always maintained a strict privacy policy. We never sell, trade or otherwise share your details with any sources. All correspondence to the department is held confidentially; furthermore, at no time will your personal and/or identifying information be shared outside of our organization, for any reason.

You have the rights of access to personal data and publicly available study results, if and when needed. Under the laws of China, you enjoy or may enjoy rights for the protection of the confidentiality of your personal data, such as those regarding the collection, custody, retention, management, control, use (including analysis or comparison), transfer in or out of China, non-disclosure, erasure and/or in any way dealing with or disposing of any of your personal data in or for this study.

By consenting to participate in this study, you expressly authorize:

- the principal investigator, the research team and the Institutional Review Board responsible for overseeing this study to get access to, to use, and to retain your personal data for the purposes and in the manner described in this informed consent process; and
- the relevant government agencies (e.g. the Shanghai Municipal Commission of Health and Family Planning) to get access to your personal data for the purposes of checking and verifying the integrity of study data and assessing compliance with the study protocol and relevant requirements.

Contact for further information

For questions about the study or reporting of adverse events, you may contact the Principal Investigator, Dr Li He at telephone no.13817223099. The phone number of Shanghai JIAI Genetics & IVF is 021-63459977.

Thank you for your time to read this information sheet and for taking part in the study.

Shanghai JiAi Genetics & IVF Institute

Study: A randomized trial comparing the effect of immediate versus delayed frozen-thawed embryo transfer following a stimulated IVF cycle

PATIENT CONSENT FORM

Patient Identification Number for this trial: _____

Title of Project: A randomized trial comparing the effect of immediate versus delayed frozen-thawed embryo transfer following a stimulated IVF cycle

1. We confirm that we have read and understood the information sheet for the above study and have had the opportunity to ask questions.
2. We understand that our participation is voluntary and that we are free to withdraw at any time, without giving any reason, without our medical care or legal rights being affected.
3. We understand that sections of any of our medical notes may be looked at by responsible individuals from regulatory authorities where it is relevant to our taking part in research. We give permission for these individuals to have access to our records.
4. We agree to take part in the above study.
5. We give permission to the investigators to retrieve pregnancy and delivery data.

Patient's signature	Patient's name	Date
Patient's husband signature	Patient's husband name	Date
Investigator's signature	Investigator's name	Date
Witness's signature	Witness's name	Date

SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	ItemNo	Description
Administrative information		
Title	1 P1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym
Trial registration	2a P4	Trial identifier and registry name. If not yet registered, name of intended registry
	2b n/a	All items from the World Health Organization Trial Registration Data Set
Protocol version	3 n/a	Date and version identifier
Funding	4 P12	Sources and types of financial, material, and other support
Roles and responsibilities	5a P11-12	Names, affiliations, and roles of protocol contributors
	5b n/a	Name and contact information for the trial sponsor
	5c n/a	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities
	5d P11-12	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)
Introduction		
Background and rationale	6a P3-4	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention
	6b P3-4	Explanation for choice of comparators
Objectives	7 P4	Specific objectives or hypotheses

Trial design	8 P4	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)
Methods: Participants, interventions, and outcomes		
Study setting	9 P4	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained
Eligibility criteria	10 P5-6	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)
Interventions	11a P6-7	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered
	11b P10	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)
	11c P6-8	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)
	11d P6-8	Relevant concomitant care and interventions that are permitted or prohibited during the trial
Outcomes	12 P8-9	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended
Participant timeline	13 P4	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)
Sample size	14 P9	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations
Recruitment	15 P5	Strategies for achieving adequate participant enrolment to reach target sample size
Methods: Assignment of interventions (for controlled trials)		
Allocation:		

Sequence generation	16a P6	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions
Allocation concealment mechanism	16b P6	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned
Implementation	16c P6	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions
Blinding (masking)	17a P6	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how
	17b n/a	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial
Methods: Data collection, management, and analysis		
Data collection methods	18a P9-11	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol
	18 P9-11	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols
Data management	19 P9-11	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol
Statistical methods	20a P9-10	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol
	20b P9-10	Methods for any additional analyses (eg, subgroup and adjusted analyses)

	20c n/a	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)
Methods: Monitoring		
Data monitoring	21a P10-11	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed
	21b P10-11	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial
Harms	22 P10	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct
Auditing	23 P11	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor
Ethics and dissemination		
Research ethics approval	24 P11	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval
Protocol amendments	25 P11	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)
Consent or assent	26a P10	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)
	26b n/a	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable
Confidentiality	27 P9-11	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial
Declaration of interests	28 P12	Financial and other competing interests for principal investigators for the overall trial and each study site
Access to data	29 P10-11	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators

Ancillary and post-trial care	30 n/a	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation
Dissemination policy	31a P11	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions
	31b n/a	Authorship eligibility guidelines and any intended use of professional writers
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code Please see the data sharing plan in www.ClinicalTrials.gov (NCT03201783)
Appendices		
Informed consent materials	32 P10	Model consent form and other related documentation given to participants and authorised surrogates
Biological specimens	33 n/a	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.