

Embryo incubation and assessment by time-lapse system versus conventional incubators in Chinese women with diminished ovarian reserve undergoing IVF/ICSI : a study protocol for a randomized controlled trial

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Study protocol

Keywords: Time-lapse imaging system (TLS), diminished ovarian reserve (DOR), in vitro fertilization (IVF), intracytoplasmic sperm injection (ICSI), Live birth, Randomized controlled trial (RCT)

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Abstract

Background: Time-lapse imaging system (TLS) is a newly developed non-invasive embryo assessment system. Compared with conventional incubators, it provides stable culture condition and consistent observation of embryo development, thereby improving embryo quality and selection. In theory, these benefits could improve clinical outcomes of in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI). Although this system has been routinely used in many IVF centers globally, it remains unclear if the TLS results in higher cumulative live birth rate and high-quality evidence is warranted. The purpose of this study is to compare the effectiveness of the TLS with conventional incubators in infertile diminished ovarian reserve (DOR) patients.

Methods: This study is a double-blind randomized controlled clinical trial (1:1 treatment ratio of TLS vs. conventional incubator). A total of 730 DOR patients undergoing the first or second cycle of IVF or ICSI will be enrolled and randomized into two parallel groups. Participants in group A will undergo embryo culture and selection in the TLS, and participants in group B will undergo embryo culture in the conventional incubators and embryo selection by the morphological characteristics. The primary outcome is the cumulative live birth rate of the trial IVF/ICSI cycle within 12 months after randomization. This study is powered to detect an absolute difference of 10% (35% vs 25%) at the significance level of 0.05 and 80% statistical power based on a two-sided test.

Discussion: The results of this study will provide evidence for the efficacy and safety of time-lapse system compared with conventional incubators in patients with DOR undergoing IVF/ICSI.

Trial registration: Chinese Clinical Trial Registry, ChiCTR1900027746. Registered on 24 Nov 2019.

Plain English Summary

In vitro culture and selection of embryos are a vital step for all assisted reproductive technologies (ART). To date, the morphological characteristics is still the most common method for assessing embryo developmental potential. This method requires embryologist moving embryos outside the conventional incubator for microscopic examination at a specific time point once a day for 3-6 days after insemination to observe the morphology of the embryos and select the embryos. However, the assessments are relatively subjective and not comprehensive enough to observe embryonic development, thus important developmental events might be missed. Although more developmental information can be obtained by removing the embryos multiple times out of the incubator, prolonged exposure to the atmosphere changes the temperature, humidity, and PH value of the embryo culture media, which adversely affects the embryos. Time-lapse imaging system (TLS) is a newly developed non-invasive embryo quality assessment method. The optical system is installed in the TLS, and images can be photographed and stored every 5-20 minutes. Compared with the conventional incubator, the TLS provides stable culture conditions, and consistent observation of embryo development, thereby improving embryo quality and selection. Management of women with diminished ovarian reserve (DOR) is one of the major challenges

in reproductive medicine. Although the TLS has been routinely used in many IVF centers, it remains unclear if the TLS improve ART outcomes in DOR patients. Therefore, we propose a randomized controlled clinical trial to compare the cumulative live birth rate of the TLS with conventional incubators in DOR patients.

Background

Since the first baby was born in 1978 by in vitro fertilization-embryo transfer (IVF-ET), IVF has been used worldwide. More than 7 million babies have been delivered due to assisted reproductive technologies (ART) (1). In the past 40 years, despite continuous efforts in optimizing the ART procedures, the implantation rate of IVF embryos is still low, and the clinical pregnancy rate has not increased significantly in recent years. The latest data from European countries in 2017 and 2018 indicate a clinical pregnancy rate of approximately 35% per transfer cycle (2, 3). More studies are needed to improve the embryo culture environment and the quality of embryos, and choose the best embryos with developmental potential for transfer and freezing to further improve the pregnancy outcomes.

In vitro culture and selection of embryos are a vital step for all ART procedures. At present, the morphological characteristics is still the most common method for assessing embryo developmental potential. The assessments are not objective and comprehensive enough to observe embryonic development, thus important developmental events might be missed. Time-lapse imaging system (TLS) is a newly developed non-invasive embryo quality assessment method. Compared with the conventional culture system, the TLS provides stable culture conditions, and reveals the details of the early stages of human embryo development. Studies have shown that two morphologically similar embryos may have completely different developmental processes if analysed by a TLS (4). Therefore, the TLS can exclude some abnormal cleavage embryos that are considered to have “normal” developmental potential in static assessment by using conventional incubators. Taken together, the TLS may help embryologists to select embryos with developmental potential for transfer, which can help improving implantation rate and clinical pregnancy rate in infertile patients (5).

Although the TLS has been routinely used in many IVF centers globally, it is unclear that if the TLS results in higher live birth rate because high-quality evidence is limited. Retrospective studies and cohort studies have shown that TLS improved embryo quality and selection, and improved clinical outcomes (6, 7). To date, there are only a few randomized controlled trial (RCT) studies assessing the TLS's effectiveness compared with conventional incubators. The data from recent meta-analyses showed conflicting results comparing the TLS with conventional incubators (8-11). These meta-analyses included studies with different populations. Confounding factors such as culture conditions (culture medium, O₂ concentration), day of transfer, number of embryos transferred, fresh or frozen embryo used, oocyte source, patient population, the TLS type and algorithms to predict various clinical outcomes, were inconsistent among the included studies (12). Moreover, these studies were at high risk of bias for randomisation and allocation concealment, thus the results should be interpreted with extreme caution.

Notably, most of the studies investigated patients with normal ovarian response or good prognosis, and only one pilot RCT has been conducted on the effectiveness of the TLS in poor prognosis patients (13).

Diminished ovarian reserve (DOR) generally refers to a quantitative decline of the oocyte pool, shown as an abnormal ovarian reserve test (14, 15). Patients with DOR usually showed less follicles in the ovarian stimulation cycle, lower blood estradiol levels, more gonadotropins (Gn) usage, high cancellation rate, low number of oocyte retrieved and low clinical pregnancy rate (14). Management of women with DOR is one of the major challenges in reproductive medicine.

With the delay of women's reproductive age and the implementation of China's second birth policy, the number of ART cycles in women with DOR has been increased in recent years. Therefore, we propose a randomized controlled clinical trial to compare the cumulative live birth rate of the TLS with conventional incubators in DOR patients.

Methods/design

Study design and setting

This study is a single centre, parallel, double-blind, superiority randomized controlled clinical trial (1:1 treatment ratio). Participants will be recruited at Shanghai First Maternity and Infant Hospital. This protocol has been written in accordance with the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT). The trial design is summarized in Figure 1, whereas the schedule of enrolment, interventions and assessments during the study period is shown in table 1.

Inclusion criteria

- Infertile couples scheduled for their first or second IVF/ICSI cycle
- In line with DOR criteria (AFC<7 follicles or AMH<2ng/ml)
- Informed consent

Exclusion criteria

- Couples undergoing Preimplantation Genetic Testing
- Women over 45 years old
- Women with congenital or secondary uterine abnormalities, such as uterine malformations including single-horned uterus, septate uterus or double uterus, adenomyosis, uterine submucosal fibroids, intrauterine adhesions
- Women with hydrosalpinx
- Women with recurrent miscarriage
- Women with polycystic ovarian syndrome (PCOS)
- Women with endocrine or metabolic abnormalities (pituitary, adrenal, pancreas, liver or kidney)

Recruitment

Infertile couples who come to the outpatient clinic to receive IVF/ICSI will be screened by trained clinical team who are familiar with the eligible criteria. Eligible patients will then be approached by a member of the research team and explained the trial details before the start of IVF/ICSI treatment. Couples will be offered time for consideration to decide to participate the trial. Couples who refuse to participate will be treated according to the conventional protocols at the centre. The decision to refuse or withdraw will not affect their conventional clinical treatments and the relationship with clinical practitioners.

The recruitment in the study centre started in December 2019 and will continue until the needed number of participants is included, anticipated until November 2021.

Randomization and blinding

Eligible women will be randomized to use time-lapse system or conventional incubators. Randomization and allocation of patients to study arms will be performed on the day of the oocyte retrieval. Permuted block randomization is controlled by collaborative investigators who are not involved in the consulting and treatment procedure. When there is an eligible participant to be enrolled into the study, an embryologist will login the trial system (REDCap) to get allocation of patients according to a computer-generated randomization list in a 1:1 ratio, with a variable block size of 2, 4 or 6.

This study will be blinded to participants, clinicians, investigators and nurses who conduct follow-up until the completion of statistical analysis of this study. However, embryologists and laboratory technicians will not be blinded.

Interventions

All participants will receive controlled ovarian hyperstimulation (COH) treatment, which is performed by standard routines at the study centre. The selection of protocol will be done by physicians, who are blinded for group allocation. In the gonadotrophin-releasing hormone antagonist (GnRH-ant) protocol, all participants will be injected gonadotropin (Gonal-F or Puregon or HMG) daily from day 2 or day 3 of menstrual cycle. When at least one follicle has reached a diameter of 12mm or on day 6 of ovarian stimulation, GnRH antagonist (Cetrotide or Ganirelix) 0.25mg daily will be administered subcutaneously until the trigger day (include the trigger day). For long GnRH-a protocol, pituitary down-regulation will be initiated 7-10 days before the menstrual cycle with GnRH agonist (subcutaneous Triptorelin 0.1mg/d or intramuscular Triptorelin 1.25-1.88mg one-time). After 10-14 days or on day 2 of menstrual cycle, gonadotropin treatment will start. For short GnRH-a protocol, mild stimulation protocol or progestin-primed ovarian stimulation (PPOS) protocol, participants will receive Triptorelin (subcutaneous injection 0.1mg/d), oral Clomiphene citrate (50-100mg q.d) or oral Dufston (10mg b.i.d) respectively on day 2 or 3 of menstrual cycle, and gonadotropin will be used on the same time.

For all the protocols, menstrual cycle of patient includes spontaneous menstrual cycle, and irregular menstrual cycle by the use of oral contraceptives (OC) or progestins. Before COH treatment, baseline

pelvic ultrasound, as well as baseline serum hormones such as follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), progesterone (P) and β -hCG) will be measured. The starting dose of gonadotrophins is 150–300IU/day for the first 4 days based on the characteristics of each patient. Transvaginal ultrasound scanning and hormonal measurement will be repeated every 2–3 days to monitor follicle growth. The subsequent dose of gonadotrophin will be adjusted according to the individual response. After two or more follicles reach a diameter ≥ 18 mm, 250ug of hCG will be once injected on trigger day. In women with hyper-response (≥ 15 follicles ≥ 12 mm), 0.2 mg Triptorelin or 4000 IU of hCG will be administered.

Oocyte retrieval is scheduled for 36h (± 2) after hCG injection. Fresh ejaculate semen samples will be obtained on the day of oocyte retrieval, and are prepared by swim-up protocol according to routines (16). For couple who take IVF, the procedure has been previously described (16). All the oocytes will be inseminated with $2\text{--}5 \times 10^6$ per oocyte motile spermatozoa approximately 39–42h after hCG injection. Gametes are then co-incubated overnight at 37°C under 5% O₂ and 6% CO₂ in the conventional incubators. Assessment of fertilization are carried out about 16–18h (day 1) after fertilization. Then zygotes are left in conventional incubators for a further 48 hours or are transferred to the preequilibrated Embryoslide for culture within the Embryoscope according to the randomization results. For couple who take ICSI, the oocytes will be denuded by hyaluronidase before micromanipulation. Only the mature, metaphase-II (MII) oocytes with an extruded first polar body are microinjected. The procedure of ICSI has been previously described (17). After injection, oocytes are transferred to the preequilibrated Embryoslide for culture within the Embryoscope or standard culture dishes in conventional incubators according to the randomization results. Assessments of fertilization and embryo quality after ICSI for the conventional incubators group are identical with IVF. The images taken by the TLS are reviewed at 16–18 h post injection for fertilization assessment. The cleavage embryo quality for both groups will be observed at 48 (day 2) or 72 (day 3) hours after oocyte retrieval. The embryos are scored according to the quality, numbers, size of the blastomeres and the amount of anucleate fragmentation.

For participants receive fresh embryo transfer, embryo transfer will be performed on Day 3 after oocyte retrieval under ultrasound guidance. Surplus embryos will be frozen according to the routines at the study centre. Luteal support is administered in the form of vaginal progesterone (Crinone) 90 mg/d until the confirmation of biochemical pregnancy, and will be maintained to 10 weeks of gestation. The progesterone will be used until the menses when the biochemical pregnancy is not observed. Oral progesterone (20mg b.i.d.; Duphaston) will be offered for women who appear vaginal bleeding in case.

For participants undergo frozen thawed embryo transfer, patients with irregular menses will receive oral E2 valerate (Progynova) 4–6mg/d on 2–3 days of subsequent artificial menstrual cycle (by the use of oral contraceptives (OC) or progestins) within 6 months after oocyte aspiration. Oral progesterone will be added if the endometrial thickness is ≥ 8 mm. Patients with regular menses will have ovulation monitoring by transvaginal ultrasound from day 12 of menstrual cycle. Oral progesterone will be added on the day of ovulation. Frozen-thawed embryos will be transferred on Day 3 after progesterone initiation. The transfer procedure will be the same as that used for the fresh embryo transfer. Oral medications will

be continued at an unchanged dose until the confirmation of biochemical pregnancy, and will be maintained to 10 weeks of gestation, and it will be used until the menses when the biochemical pregnancy is not observed.

Follow-up

Urine and blood hCG will be measured 14 days after embryo transfer, and positive results indicate biochemical pregnancy. If the gestational sac is observed with ultrasonography on 7 weeks after transfer, clinical pregnancy will be confirmed. Ongoing pregnancy is defined by the presence of a gestational sac with fetal heartbeat after 12 weeks of gestation.

For women who are confirmed as ongoing pregnancy, they will be required to notify researchers of the time of delivery. In 2 weeks after delivery, the information of pregnancy (pregnancy complications, and fetus information), delivery information (gestational age, delivery mode, placenta abnormality and/or delivery complications), infant information (such as sex, birth weight, birth defect) will be collected by completing forms.

Outcome measures

The primary outcome will be cumulative live birth of the trial IVF/ICSI cycle within 12 months of randomization. Live birth will be defined as a delivery of one or more living infants (≥ 22 week's gestation or birth weight more than 500g).

For the effectiveness of the treatment, we will record these secondary outcomes in terms of effectiveness:

- Fertilisation: defined as number of zygotes with 2PN (per woman randomised and per oocyte retrieved).
- Available embryo: defined as number of embryos ≥ 4 cells and $\leq 30\%$ fragmentation on day 3 observation.
- Good quality embryo: defined as number of embryos with ≥ 6 cells and $\leq 30\%$ fragmentation developed from 2PN embryos on day 3 observation.
- Biochemical pregnancy: defined as blood hCG ≥ 10 U/L at 14 days after embryo transfer.
- Implantation: defined as the number of gestational sacs observed per embryo transferred.
- Clinical pregnancy: defined as one or more observed gestational sac or definitive clinical signs of pregnancy under ultrasonography at 7 weeks after embryo transfer (including clinically documented ectopic pregnancy).
- Multiple pregnancy: defined as a pregnancy with two or more gestational sacs or positive heart beats at 7 weeks of gestation.
- Ongoing pregnancy: defined as the presence of a gestational sac and fetal heartbeat after 12 weeks of gestation.

For the safety of the treatment, we will record the following treatment complications as secondary outcomes:

- Ovarian hyperstimulation syndrome (OHSS): defined as exaggerated systemic response to ovarian stimulation characterized by a wide spectrum of clinical and laboratory manifestations. It is classified as mild, moderate, or severe according to the degree of abdominal distention, ovarian enlargement, and respiratory, hemodynamic, and metabolic complications.
- Miscarriage: defined as the spontaneous loss of an intra-uterine pregnancy prior to 22 completed weeks of gestational age.
- Ectopic pregnancy: defined as the implantation takes place outside the uterine cavity, confirmed by sonography or laparoscopy.

We will also collect the following obstetric and perinatal complications:

- Gestational diabetes mellitus (GDM)
- Hypertensive disorders of pregnancy (comprising pregnancy induced hypertension (PIH); pre-eclampsia (PET) and eclampsia)
- Antepartum haemorrhage, including placenta previa, placenta accreta and unexplained
- Preterm birth: defined as birth of a fetus delivered after 22 and before 37 completed weeks of gestational age in participants confirmed ongoing pregnancy.
- Birth weight, including low birth weight (defined as weight < 2500 gm at birth), very low birth weight (defined as < 1500 gm at birth), high birth weight (defined as >4000 gm at birth) and very high birth weight (defined as >4500 gm at birth)
- Large for gestational age (defined as birth weight >90th centile for gestation, based on standardized ethnicity based charts) and small for gestational age (defined as less than 10th centile for gestational age at delivery based on standardized ethnicity based charts)
- Congenital anomaly (any congenital anomaly will be included)
- Perinatal mortality: defined as fetal or neonatal death occurring during late pregnancy (at 22 completed weeks of gestational age and later), during childbirth, or up to seven completed days after birth.

Data management and monitoring

The data collected for the trial will be a mixture of routinely clinical data (such as demographic data, fertility history, ART records), which are verifiable from the medical record and questionnaire data. All researchers and physicians are required to receive training classes and pass the test. Each participant will be assigned an appropriate code number that is consistent with the allocated intervention, which will appear on all report forms to maintain confidentiality.

All data are collected at baseline and follow-up through a standard clinical electronic data collection system (EDC). Initially, all of the researchers and physicians will be required to keep accurate and verifiable source notes in the medical record relevant to each participant's eligible criteria of this trial. After recruitment of eligible participants, trained assessors will take charge of the data input: they can log on to a secure data portal with the individual ID, and upload the data from medical record to eCRF with the personal trail ID of each participant. When the trial is close-out, all participant-identifiable data, such as consent forms, screening and identification logs will be stored in the investigator site files, accessible only to delegated members of the study team.

Sample size calculation

According to the literature (18) and the data of our centre, live birth rates among women with DOR in the control arm were around 25.0%. Based on other studies within fertility care as well as the discussion by gynaecologist and epidemiologists, we assume that the minimal clinically important difference to make time-lapse system preferable over conventional incubator would be 10.0%. To demonstrate this difference with two-sided test, 5.0% alpha-error, 80% statistical power, and taking consideration of dropout as 10%, the lowest numbers of participants we need to enrol for the study are 730. The ratio between test and control group will be 1:1.

Statistical analysis

Baseline characteristics will be described by descriptive analysis, and the balance between the two arms will be assessed. For continuous variables, the normality test will be estimated using frequency histograms and the Shapiro test initially. If the parameters are normally distributed then they will be presented as mean with standard deviation (SD). If the parameters are non-normally distributed, their medians and inter-quantile ranges (IRQs) will be reported. For categorical variables, we will present the proportions of the two arms. In addition, we will also report the numbers of recruitment, participants lost to follow-up, protocols violation, and other relevant descriptive data.

Data analysis of this trial will follow the intention-to-treat principle, which includes all randomized women in the primary comparison between the two arms. Per-protocol analysis may be conducted as a secondary analysis. The primary outcome, cumulative live birth rate, will be compared between the two arms using Pearson's chi-square test or Fisher's exact test for the purpose of unadjusted analysis. We will also compute unadjusted risk ratio (RR) and its 95% confidence interval (95% CI). In the event of prominent imbalance of potential confounders between the two arms, we will perform multivariable Poisson Regression or Log-Binomial model to compute adjusted RR and its 95% CI. Secondary outcomes will be compared between the two arms using the similar approach described for the primary outcome.

For missing values regarding baseline characteristics, we will first perform analysis by excluding missing values, we will then perform multiple imputation to impute missing values and conduct subsequent analysis to estimate the robustness of the findings. For loss to follow-up and protocol violation, we will attempt sensitive analyses to explore the effect of these factors on the trial findings.

Primary and secondary outcomes will be compared between the two arms within several clinically important subgroups including different ages (<35y vs. ≥35y), embryo transfer (fresh vs. frozen) and COH protocols in which the effects on outcomes might be modified. Due to the concern over multiplicity of sub-group analysis, we will place limited importance on subgroup findings.

All tests will be two-tailed, and differences with p value <0.05 will be considered statistically significant.

Safety

All observed or volunteered adverse events, regardless of treatment group or suspected causal relationship to intervention, will be recorded and reported to an independent Data and Safety Monitoring Board (DSMB).

The investigator will inform subjects and the reviewing accredited medical research ethics committee if anything occurs, on the basis of which it appears that the disadvantages of participation may be significantly greater than was foreseen in the research proposal. The study will be suspended pending further review by the accredited medical research ethics committee, unless suspension would jeopardize the subjects' health. The investigator will take care that all subjects are kept informed.

Interim analysis

The DSMB will perform an interim analysis within 3 months after the first 365 randomised participants have completed embryo transfer. They will do so using the endpoint ongoing pregnancy, as data on live birth will not be available. The interim analysis will be conducted using a two-sided significant test with the Haybittle–Petospending function and a Type I error rate of 5% with stopping criteria of $P < 0.001$ ($Z_{\alpha} = 3.29$). The study could be stopped prematurely based on the advice of the DSMB.

Discussion

Patients with DOR usually exhibit poor response to controlled ovarian stimulation, and are classified as a population of poor prognosis in ART cycles and categorized into group 3 and group 4 according to the POSEIDON criteria (15). To our best knowledge, only one small RCT study included poor-prognosis patients (16 in the TLS arm and 15 in the convention incubator arm) (13). The data showed that no differences in day 3 embryo scores, implantation and clinical pregnancy rates were observed between the two groups. However, this study did not provide the detail information about these participants. To date, no reported RCT studies have been conducted in DOR patients. Therefore, a large RCT study in DOR patients is warranted to investigate if the TLS could improve embryo quality and selection as well as clinical outcomes in this population.

Strengths of this trial include its randomised, controlled design and relatively large sample size, which should minimise bias and increase validity and reliability of data. The results of this study will provide evidence for the efficacy and safety of time-lapse system compared with conventional incubators in DOR patients undergoing IVF/ICSI.

Abbreviations

ART: Assisted reproductive technology; COH: controlled ovarian hyperstimulation; CRF: case report form; DOR: diminished ovarian reserve; DSMB: Data Safety and Monitoring Board; E2: estradiol; EDC: electronic data collection; FET: Frozen embryo transfer; FSH: follicle stimulating hormone; GDM: gestational diabetes mellitus; GnRH: gonadotrophin-releasing hormone; IVF: In vitro fertilization; ICSI: intracytoplasmic sperm injection; ITT: Intent-to-treat; LH: luteinizing hormone; OHSS: ovarian hyperstimulation syndrome; OC: oral contraceptives; P: progesterone; PCOS: polycystic ovarian syndrome; PPOS: progestin-primed ovarian stimulation; PIH: pregnancy induced hypertension; RCT: Randomized controlled trial; SD: standard deviation; TLS: time-lapse imaging system.

Declarations

Ethics approval and consent to participate

This trial was approved by the institutional ethical committee of Shanghai First Maternity and Infant Hospital on 8 December 2019 (Reference No.: KS1958). All participants in the trial will provide written informed consent. The study was registered on Chinese Clinical Trial Registry (ChiCTR1900027746) and will be conducted according to the principles outlined in the Declaration of Helsinki and its amendments, in accordance with the Medical Research Involving Human Subjects Act, and using Good Clinical Practice.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and analysed during the current study are available from the corresponding author on reasonable request. The principle investigator will publish the results of the study as soon as appropriate.

Competing interests

The authors have no conflicts of interest to declare.

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Authors' contributions

Study concept and design: MC, W-TL and B.W.M. Acquisition of data: MC, YW, XH, CS, ZM, AA, LH, CT, KL, YF, ZC, PK, YG and W-QL. Analysis and interpretation of data: MC, YW, XH, W-TL and B.W.M. Drafting of the manuscript: MC, W-TL and B.W.M. Critical revision of the manuscript for important intellectual content: YW, XH, CS, ZM, AA, LH, CT, KL, YF, ZC, PK, YG, W-QL and XT. Statistical analysis: MC, YW, XH, W-TL and B.W.M. Study supervision: B.W.M and XT.

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Not applicable.

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Table

Please see the supplementary files section to access the table.

Figures

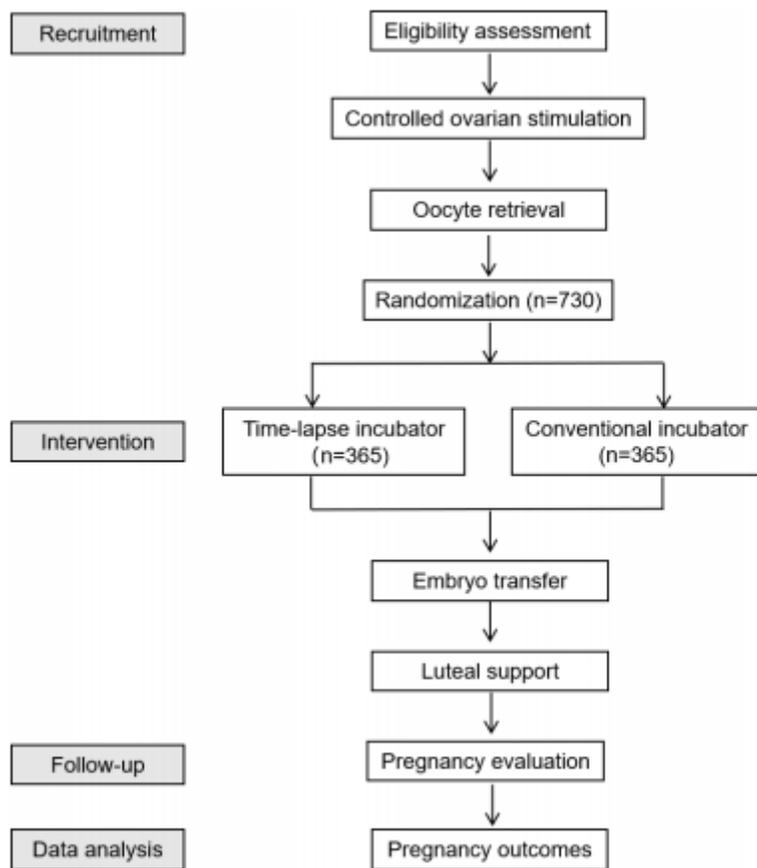


Figure 1

Flowchart followed the checklist of Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) showing patient enrolment, allocation, treatment and follow-up of participants.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Table1Scheduleofenrolment.pdf](#)