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Final title: PGT for aneuploidy does not enhance live birth in young patients (≤ 35 years): a randomized controlled trial of single blastocyst frozen embryo transfers (ClinicalTrials.gov ID: NCT03095053)

Short title: PGT-A versus morphological score blastocyst selection

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Registered Title: Conventional blastocyst morphology versus comprehensive chromosome screening selection: a randomized control superiority trial

Short title: CCS versus blastocyst morphology selection

Secondary identifiers: Optimizing implantation in FET

Sponsor: Antalya IVF

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Changes to original registration:

Primary outcome measure: the primary outcome measure was changed from ongoing pregnancy (i.e., clinical pregnancy at >14 weeks of gestation) to the preferred outcome of live birth (LB, a delivery at >20 weeks, with a live infant).

The AFC cut-off was changed to ≥5 (changed from ≥10) to be in line with the definition of decreased ovarian reserve and increase the eligible population.

Trial registration: ClinicalTrials.gov ID: NCT03095053

Protocol submission: 23 March 2017

Protocol revised submission: 28 March 2017

Randomized Controlled Trial registered and posted: 29 March 2017

Data collection start: 21 February 2018

Data collection was commenced at >20 weeks from the date of last patient randomized had undergone FET

Data analysis start: 25 April 2018

Description

Brief description

Introduction

Embryo aneuploidy is likely the leading cause of implantation failure in IVF cycles. Since the inception of IVF, non-invasive morphology based scoring has been the most widely used embryo selection method, resulting in relatively low embryo implantation rates. Our understanding of the optimal conditions required for *in vitro* embryo culture in IVF has advanced significantly over the past two decades. The implementation of improved *in vitro* embryo culture technologies (i.e., culture media and incubators) has resulted in an increase in the number of good quality embryos and consequently in increased numbers of blastocysts. While blastocyst transfers have seemingly improved the reproductive outcomes of IVF, they still remain suboptimal. The main objective of this randomized controlled trial (RCT) will be to investigate whether preimplantation genetic testing (i.e., PGT with comprehensive chromosome screening (CCS)) for aneuploidy is a superior embryo selection method, with the live birth outcomes of euploid blastocyst frozen embryo transfers (FET) compared with the LB outcomes of unknown-ploidy blastocyst FET, with blastocysts selected on (standard) morphological score.

Methods

This RCT will be conducted at a single private IVF centre performing routine segmented-IVF, with intracytoplasmic sperm injection (ICSI), blastocyst freeze-all, and artificial frozen embryo transfer (art-FET). Normo-ovulatory infertile patients, with maternal age ≤35 years and at least two blastocysts with a morphology score of 2BB cryopreserved, will be randomized by computer-generated randomized allocation to either the PGT or morphology arm of the trial. All transfers will be single embryo transfers (SET), with only the first FET cycles following freeze-all to be analyzed.

Consent and Ethics

Akdeniz University Medical Faculty Clinical Research Ethics Committee has approved the trial (reference number: 2015/399), with anonymized results to be released in ClinicalTrials.gov. All patients will provide informed consent, which included an agreement for the use of anonymised data for research and SET.

Full description

Introduction

Embryo aneuploidy is likely the leading cause of implantation failure in IVF cycles. Since the inception of IVF, non-invasive morphology based scoring has been the most widely used embryo selection method, resulting in relatively low embryo implantation rates. Embryo morphology assessment methods have significant limitations (i.e., the assessments are subjective and the method uses fixed time-point assessments to define dynamic embryo development) and shortcomings (i.e., exposes embryos to sub-optimal conditions during assessment). Notwithstanding the limitations and shortcomings of this method its use worldwide has continued, because it is a relatively simple and non-invasive method and its scores have been shown to be (moderately) positively correlated to embryo euploidy, ongoing pregnancy, and live birth (Van Royen *et al.,* 1999, Ahlstrom *et al.,* 2011, Forman *et al.,* 2013, Capalbo *et al.,* 2014, Oron *et al.,* 2014, Rhenman *et al.,* 2015).

However multiple gestations still represents one of the most significant complications in IVF, which are mainly the result of multiple-embryo embryo transfers, with multiple-embryo transfers used to overcome the relatively low embryo implantation rates in IVF. Our understanding of the optimal conditions required for *in vitro* embryo culture in IVF has advanced significantly in the past two decades. The implementation of improved *in vitro* embryo culture technologies (i.e., culture media and incubators) has resulted in an increase in the number of good quality embryos and consequently in the numbers of blastocysts. While blastocyst transfers have seemingly improved the reproductive outcomes in IVF, the use of SET and PGT technologies have revealed embryo implantation still to be sub-optimal (Schoolcraft *et al*., 2013). New CCS platforms are a major breakthrough in PGT, allowing 24-chromosome screening to be performed with high degree of accuracy from single cells (Harper and Harton, 2010). The evidence that morphology scores were only moderately associated with euploidy and that the transfer of PGT predicted euploid embryos resulted in higher implantation rates (Dahdouh *et al.,* 2015), has seen the continued use of morphology based scoring methods increasingly being challenged.

In addition to all the other advances in IVF, significant improvements have also been made in cryopreservation technologies. These improvements have resulted in significant improvements in frozen-thawed embryo survival (i.e. minimizing of risks), post-thaw developmental competence (Cobo *et a*l., 2012; Balaban *et a*l., 2008), and in the reproductive outcomes of FET (Evans *et al*., 2014; Ozgur *et al*., 2015). The benefits of FET include; the transfer of embryos to a more physiologic endometrium (i.e., early luteal phase), the ability to time transfers more accurately, and

the ability to use patient specific endometrial preparations (Casper and Yanushpolsky, 2016, Franasiak *et al.,* 2016; Groenewoud *et al.,* 2013, Yarali *et al.,* 2016).

Moreover, the hypotheses of PGT require to be confirmed in further robust RCT before its implementation in routine IVF.

Objectives

The primary objective of this RCT will be to investigate whether PGT for aneuploidy as a blastocyst selection method is superior to standard morphology scoring blastocyst selection, comparing the reproductive outcomes in FET cycles. The decision to transfer all blastocysts in FET will eliminate any potential impact of ovarian stimulation confounding on endometrial receptivity and to use freeze-all cycles will allow the use of the primary blastocysts of blastocyst cohorts. The primary outcome measure of this trial will be LB, with a LB defined as a pregnancy cycle delivering at >20 weeks of gestation.

Secondary objectives

The secondary objective of the RCT will be to investigate whether euploid blastocyst transfer results in reduced miscarriage, with a miscarriage defined as a clinical pregnancy lost at <20 weeks of gestation.

Keywords: blastocyst; comprehensive chromosome screening; euploidy; frozen embryo transfer

Funding: This RCT will not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors, and therefore will be wholly funded by centre running the trial.

Declaration of Interests: The authors declare no conflicts of interest.

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Materials and methods

Study design

The RCT will be performed as a single private IVF center trial. The patient population of the trial will include young patients undergoing autologous blastocyst freeze-all IVF cycles from March 2017. In this trial, a patient will undergo a blastocyst-freeze-all cycle after ICSI and only the reproductive outcomes of the first FET following the freeze-all will be analyzed. Currently at the trial centre more than 95% of cycles are extended culture cycles and more than 80% of these cycles result in freeze-all cycles, with a live birth rate after the first FET of >45% per oocyte retrieval.

Setting

Antalya IVF is located in Antalya, Turkey.

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Study population/Participants and recruitment

The trial participants will consist of infertile patient-couples attending Antalya IVF for autologous segmented-IVF treatment. Patients will be informed of the trial at their clinical consultation before commencing treatment, at which the trial procedures and the likely reproductive outcomes will be discussed. All patients who consent to participation and who satisfy the inclusion criteria will undergo the same standard IVF procedures; ovarian stimulation (OS), oocyte retrieval, ICSI, extended embryo culture, blastocyst vitrification, and artificial FET.

Eligibility criteria

Patient-couples eligible for inclusion in the trial must satisfy the following criteria; female age of ≤35 years, female body mass index (BMI) of ≥18 or ≤35 kg/m2, antral follicle count (AFC) of ≥5 (changed from ≥10), normo-ovulatory, intend to use autologous oocytes, and have ≥2 blastocysts with a morphological score of 2BB on day 5 of embryo development.

Exclusion criteria

Patient couples will be excluded from the trial for the following reasons, patients with drug contraindications, patients with pathophysiology unrelated to reproduction, patients with intrauterine pathophysiologies, patients with no blastocysts, patients with <2 blastocysts with a morphological score of 2BB.

Randomisation

Patient couples who satisfy the inclusion criteria and who completed an informed consent will randomized by computer generated number in a 1:1 ratio on day 5 of in vitro embryo culture to the two arms, with the FET drug prescription indicating allocation to the ***the PGT group***, in which all blastocysts will be assessed according to standard morphological parameters, with the best scoring blastocyst to undergo biopsy and PGT for aneuploidy or ***the morphology group***, in which all blastocysts will be assessed according to standard morphological parameters, with the best scoring blastocyst selected for transfer (figure 1). Patient couples in the PGT group will be subgrouped

according to whether the blastocyst biopsied is predicted to be euploid (**the euploid subgroup**) or aneuploid (**the aneuploid subgroup**). In the aneuploid group the next best morphology scoring blastocyst will be transferred.

Interventions

Controlled ovarian stimulation, oocyte pickup, and embryo culture

All patients will have an ultrasound examination on cycle day 2 or 3 to assess the state of the patients ovaries and uterus, and perform an antral follicle count (follicles >2≤10 mm), before commencing flexible start gonadotropin-releasing (GnRH) antagonist (0.25mg, Cetrotide, Merck Serono, Istanbul, Turkey) co-treatment OS protocols. Follicular development will be stimulated using a combination of rFSH (150-375 IU, Gonal-F, Merck Serono, Istanbul, Turkey) and hMG (75-150 IU, Menopur, Ferring Pharmaceuticals, Mumbai, India). The gonadotropin doses will be based on maternal age, BMI, AFC, and previous OS outcome. Final oocyte maturation will be triggered when three or more follicles reach ≥17 mm, with GnRHa (0.2 mg, Gonapeptyl®, Ferring Pharmaceuticals, India).

Oocyte retrievals will be performed 36 hours after triggering using transvaginal ultrasound (TVS) guided follicle aspiration (follicular aspiration needle, 461230LF, Rheinbach, Germany) procedures. Oocyte collection and manipulation will be performed using Cook Medical media (Sydney IVF, Brisbane, Australia) and embryo culture will be performed using SAGE 1-Step™ medium (67010010A, SAGE, Origio, Malov, Denmark), with no change of media during culture. Incubation conditions were set at 6% CO_2, 5% O_2 and 37.0ºC (G185 Long Term Flat Bed Incubators, K-Systems, Kivex Biotec ltd, Birkerod, Denmark). All oocyte inseminations will be performed using ICSI, with daily embryo development assessments, thereafter.

Blastocyst assessment

Blastocysts will be graded according to the 3-part grading system (Gardner *et al.,* 2000), with a blastocyst grade including an assessment of blastocyst expansion (grade 1 to 6), inner cell mass morphology (ICM, A to C, according to the number and degree of compaction of the cells), and trophectoderm morphology (TE, A to C, according to the number, size and contiguous arrangement of the trophectoderm cells). Blastocysts with a grade of ≥2BB were regarded as usable.

Gardner DK, Lane M, Stevens J, Schlenker T, Schoolcraft WB. Blastocyst score affects implantation and pregnancy outcome: towards a single blastocyst transfer. Fertil Steril 2000; 73: 1155-1158.

Biopsy

Blastocysts with morphological grades of ≥2BB will be biopsied on day 5 of in vitro embryo culture. Biopsies will be performed using using a Hamilton Thorne Zilos laser (Hamilton Thorne, MA, USA), with 3-10 trophectoderm cells removed from the blastocysts.

Comprehensive chromosome screening

All trophectoderm biopsies will be processed for analysis by Next-Generation Sequencing (NGS, illumina, California, USA).

Blastocyst cryopreservation

Vitrification and warming of blastocysts will be performed using ultra-rapid technologies (Cryotop, Kitazato BioPharma Co. Ltd, Fuji-city, Japan), according to the manufacturer's guidelines. Blastocysts were warmed and transferred on the same day; after warming the blastocysts were placed in incubation for approximately 2 hours and then morphologically reassessed.

Cycle programming and endometrial preparation

All patients will undergo a standard endometrial preparation protocol, using GnRHa to programme the start of the endometrial preparation (Ozgur *et al.,* 2016).

Ozgur K, Berkkanoglu M, Bulut H, Humaidan P, Coetzee K. Agonist depot versus OCP programming of frozen embryo transfer: a retrospective analysis of freeze-all cycles. J Assist Reprod Genet 2016; 33: 207-214.

Frozen embryo transfer art-FET

All transfers will be SET and performed in art-FET. Embryo transfers will be performed with glass syringes (50µL, Hamilton, Giarmata, Romania) attached to embryo replacement catheters (Wallace, Smiths Medical, Kent, UK), with trans-abdominal ultrasound guidance. All blastocyst FET will be scheduled to be performed on the 6th day of progesterone administration (90 mg, twice-a-day, Crinone® 8%, Merck Serono, Turkey) to time the day of transfer, with progesterone administration commencing on day 15 of endometrial preparation.

Outcome measures

Patient variables recorded will be maternal age, infertility duration and etiology, BMI, AFC, and endometrial thickness. The treatment cycle variables recorded were oocyte number, mature oocyte number, blastocyst number (i.e., the number usable blastocysts, with grade ≥2BB), oocyte maturity, oocyte fertilization, blastocyst rate (i.e., the ratio of the number of blastocysts to the number of 2PN zygotes). The primary outcome measure recorded will be LB (changed from ongoing pregnancy),

with clinical pregnancy and miscarriage as secondary outcomes. A LB cycle will be defined as a pregnancy cycle that delivered at >20 weeks of gestation, resulting in a live infant. A clinical pregnancy will be defined as a cycle with a fetal sac observed on ultrasound after 5 weeks of gestation. A biochemical pregnancy will be defined as a cycle with an arbitrary serum βhCG concentration of >29 IU/L. Pregnancy tests will be performed 9 days after the FET.

Statistics

Statistical Package for Social Science 11.5 (SPSS version 11.5) was used for the statistical analysis of characteristics and outcome measures (i.e., p values and Risk Ratios (RR)). Continuous data were analyzed either with the student's t-test or Mann-Whitney-U test, depending on outcome of normality testing (Shapiro-Wilk). Categorical data were analyzed using either the chi-square test or Fisher's Exact-test (i.e., for low sample numbers). A $p < 0.05$ indicated outcomes of significant difference.

Sample size calculation

At the trial centre the overall ongoing pregnancy rate was 66% for the transfer CCS euploid blastocysts, while the overall ongoing pregnancy rate was 52% for blastocysts selected for transfer by morphology only. The sample sizes required to have 80% chance of detecting a difference of 20% at a significance level of 0.05 was calculated to be 74.

Ethics

Akdeniz University Medical Faculty Clinical Research Ethics Committee has approved the trial (reference number: 2015/399), with anonymized results to be released in ClinicalTrials.gov. All patient will be provided with trial information, with all patients wishing to participate in the trial providing informed consent, which included an agreement for the use of anonymised data for research, before commencing treatment.

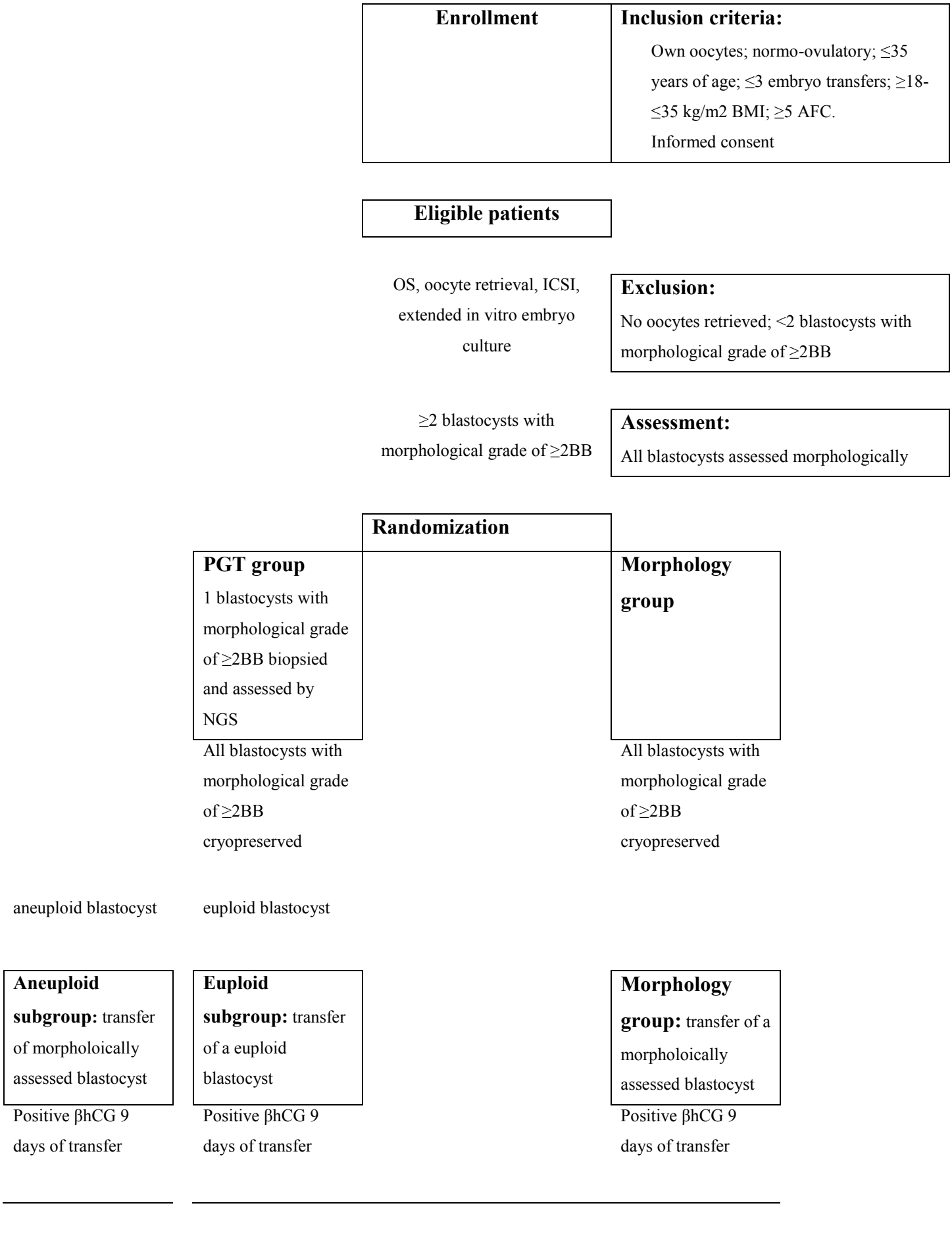
All treatment procedures and medical devices used in treatments have been approved by the Turkish Health Ministry and the management and quality control of the procedures performed accredited by TÜV Rheinland Cert GmbH (Assisted Reproductive Technologies; ISO 9001:2008; Cert No. 01 100 062424).

Data

All data pertaining to the trial and required for anlysis will be collected by the principal investigator from the IVF database of Antalya IVF at >20 weeks after the last patient randomized had

undergone FET. A LB cycle will defined as a pregnancy cycle delivered at >20 weeks of gestation. A clinical pregnancy will defined as a pregnancy cycle with a fetal sac observed on ultrasound at >5 weeks of gestation. A miscarriage will be defined as a clinical pregnancy lost at <20 weeks of gestation.

Figure 1: Study design



Fetal sac on ultrasound >5 weeks	**Fetal sac on ultrasound >5 weeks**	**Secondary outcome measure**	**Fetal sac on ultrasound >5 weeks**
Live birth: Delivery at >20 weeks	**Live birth:** Delivery at >20 weeks	**Primary outcome measure**	**Live birth:** Delivery at >20 weeks