






# BMJ Open Embryo selection through non-invasive preimplantation genetic testing with cell-free DNA in spent culture media: a protocol for a multicentre, double-blind, randomised controlled trial

Jin Huang <sup>1,2,3,4</sup>, Rong Li,<sup>1,2,3,4</sup> Lin Zeng <sup>5</sup>, Liang Hu,<sup>6,7,8</sup> Juanzi Shi <sup>9</sup>, Liyi Cai,<sup>10</sup> Bing Yao,<sup>11</sup> Xiu-Xia Wang,<sup>12</sup> Yanwen Xu,<sup>13,14</sup> Yuanqing Yao,<sup>15</sup> Yan Wang,<sup>16,17</sup> Junzhao Zhao <sup>18</sup>, Yichun Guan,<sup>19</sup> Weiping Qian,<sup>20</sup> Guimin Hao,<sup>21</sup> Sijia Lu,<sup>22,23</sup> Ping Liu,<sup>1,2,3,4</sup> Jie Qiao <sup>1,2,3,4</sup>

**To cite:** Huang J, Li R, Zeng L, *et al.* Embryo selection through non-invasive preimplantation genetic testing with cell-free DNA in spent culture media: a protocol for a multicentre, double-blind, randomised controlled trial. *BMJ Open* 2022;**12**:e057254. doi:10.1136/bmjopen-2021-057254

► Prepublication history for this paper is available online. To view these files, please visit the journal online (<http://dx.doi.org/10.1136/bmjopen-2021-057254>).

JH and RL contributed equally.

Received 09 September 2021  
Accepted 23 May 2022



© Author(s) (or their employer(s)) 2022. Re-use permitted under CC BY-NC. No commercial re-use. See rights and permissions. Published by BMJ.

For numbered affiliations see end of article.

**Correspondence to**  
Dr Jie Qiao;  
Jie.qiao@263.net and  
Dr Ping Liu;  
pingliu7703@sina.com

## ABSTRACT

**Introduction** Morphological evaluation is used to select embryos for in vitro fertilisation. However, it does not fully reflect the implantation potential. Preimplantation genetic testing for aneuploidies (PGT-A) can detect embryonic aneuploidy, but biopsy procedure is invasive. Currently, a non-invasive PGT (ni-PGT) approach using spent medium is being evaluated. However, the clinical benefit of ni-PGT has not been clearly demonstrated. A multicentre randomised trial is needed to verify whether ni-PGT can be an new effective tool for evaluating embryos.

**Methods and analysis** Overall, 1148 couples aged 35–42 (women) receiving in vitro fertilization–intracytoplasmic sperm injection are planned to be enrolled. Couples will be digitally randomised to (1) ni-PGT and (2) conventional morphology groups at a 1:1 treatment ratio. The primary outcome will be the ongoing pregnancy rate related to the first transfer cycle within 6 months after oocyte retrieval.

**Ethics and dissemination** The study protocol is approved by the Ethics Committee of Peking University Third Hospital and the participating hospitals. The results will be disseminated through international conferences and scientific journals.

**Trial registration number** NCT04339166.

## INTRODUCTION

Morphological evaluation is widely used for embryo selection in in vitro fertilization–embryo transfer (IVF-ET) but is not capable of detecting chromosomal abnormalities.<sup>1–3</sup> Embryo chromosomal abnormalities occur due to errors during cell mitosis and meiosis.<sup>4 5</sup> Chromosomal abnormalities are more common in patients with an advanced maternal age. The risk of chromosome aneuploidy is approximately 20% to 31% in women between 26 and 34 years old. When the age is ≥35 years, the incidence

## STRENGTHS AND LIMITATIONS OF THIS STUDY

- ⇒ This is the first double-blinded multicentre randomised controlled trial comparing the ongoing pregnancy rate after embryo transfer selected by non-invasive preimplantation genetic testing (ni-PGT) versus conventional morphological evaluation with 1148 couples in 13 centres across China.
- ⇒ Embryo selection with ni-PGT, with a special focus on the clinical benefit of ni-PGT as a new effective indicator to evaluate embryo.
- ⇒ This study is well developed with follow-ups and procedures, and uses a dedicated electronic data capture system.
- ⇒ Trial registration and study protocol publications ensure transparency in research.
- ⇒ The cost-effectiveness analysis is not performed.

of aneuploidy in oocytes and embryos gradually increases (34% to 75%).<sup>6 7</sup> Studies have demonstrated that aneuploidies cause embryo implantation failure or embryonic development arrest;<sup>8</sup> therefore, the probability of successful natural and IVF pregnancy is significantly reduced in older women with increased miscarriage rates. To improve IVF outcomes, embryos were evaluated before implantation through preimplantation genetic testing for aneuploidy (PGT-A) under specific indications, such as elderly women or patients with recurrent miscarriage or implantation failure. Before transferring into the uterus, embryos were biopsied and tested for chromosomal ploidy. Clinical studies have shown that the clinical outcomes of IVF were indeed improved by PGT-A.<sup>9 10</sup> Munné *et al*<sup>11</sup> conducted a multinational multicentre clinical trial, compared the PGT-A with non-PGT-A. The results showed

the ongoing pregnancy rate of PGT-A group aged 35–40 years was statistically increased, but there was no such trend for people <35 years old.<sup>11</sup> Chang *et al*<sup>12</sup> compared 5471 PGT-A cycles with 97069 non-PGT-A cycles, the people age  $\geq 35$  in the PGT-A group significantly reduced the rate of miscarriage. The problem, however, is that such a biopsy procedure is invasive and conveys unknown health risks in the long-term development of embryos.<sup>13–16</sup> Therefore, a less invasive method that evaluates the ploidy status of embryos is highly preferred in the field of IVF.

The cell-free DNA (cfDNA) in spent culture media (SCM) was first demonstrated in 2013.<sup>17</sup> Then, several studies evaluated the non-invasive SCM-based PGT-A approaches. A non-invasive chromosome screening (NICS) technology, named ni-PGT, was first reported in 2016. It is also the first time that NICS was used in balanced translocation patients and five live births were obtained from seven couples.<sup>18</sup> PGT-A by trophoctoderm biopsy or whole embryo and SCM showed a consistency rate between 78.2% and 100%.<sup>18–23</sup> However, other studies reported a consistency rate between 32.2% and 56.3%.<sup>24 25</sup>

Non-invasive PGT was preliminarily applied by several studies.<sup>18 21 26</sup> The cfDNA-based ni-PGT showed a potential ability to ameliorate the ongoing pregnancy rate and lower miscarriage. However, the scale of these

clinical trial was small, larger trials will further support ni-PGT as a satisfactory tool evaluating potential embryo implantation.

## METHODS AND ANALYSIS

### Study design

This is a multicentre, randomised, controlled trial evaluating the ongoing pregnancy rate of first embryo transfer in 1148 aged couples undergoing intracytoplasmic sperm injection (ICSI). Participants will be enrolled at 13 hospitals in China. This clinical trial was approved by the Ethics Committee of Peking University Third Hospital Medical Science Research Ethics Committee and the participating hospitals. The informed consent will be collected before any procedure from the enrolled couples. **Table 1** shows the schedule of enrolment, interventions and assessments during the study period. **Figure 1** is the flowchart of this randomised controlled trial.

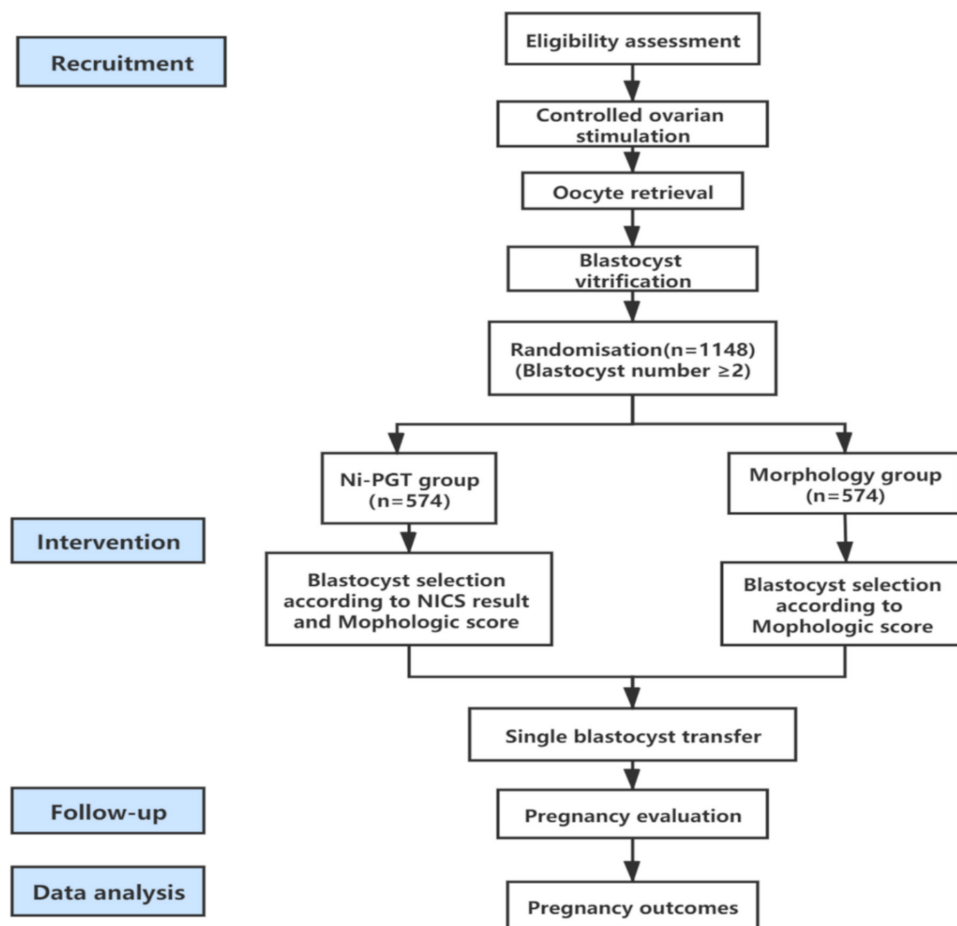
### Study setting

This study will be conducted in 13 Chinese hospitals: Peking University Third Hospital; Affiliated Jinling Hospital, Medicine School of Nanjing University; Reproductive Medical Centre of Hebei Maternity Hospital; Northwest women's and Children's Hospital;

**Table 1** Enrolment, interventions and assessments

Content	Study period								
	Enrolment	Pre-allocation		Allocation	Post-allocation			Close-out	
	Screening and baseline assessment	Oocyte retrieval	Embryo culture	Blastocysts vitrification and randomisation	Embryo transfer	Evaluation of pregnancy	Follow-up of pregnancy		
Time point	T0	T1	T2	T3	T4	T5	T6	T7	
	–3 month	–1 month	–1 weeks	0 day		2–6 months	3–7 months	6–10 months	12 months
Enrolment									
Eligibility	x	x	x						
Informed consent	x	x							
Allocation				x					
Interventions									
NICS+morphology				x					
Morphology				x					
Assessments									
Baseline data	x	x							
Laboratory tests	x	x			x	x	x	x	
Fertilisation			x						
Embryo quality			x		x				
Pregnancy tests						x			
Pregnancy outcomes							x	x	
Fetus information							x	x	
Neonate information							x	x	
Safety assessment	x	x			x	x	x	x	

NICS, non-invasive chromosome screening.



**Figure 1** Flowchart of this randomised controlled trial. NICS, non-invasive chromosome screening; ni-PGT, non-invasive preimplantation genetic testing.

Chinese PLA General Hospital; Shengjing Hospital of China Medical University; First Affiliated Hospital; Sun Yat-sen University; Reproductive and Genetic Hospital of Citic-Xiangya; Peking University Shenzhen Hospital; The Second Hospital of Hebei Medical University; West China Second Hospital of Sichuan University; The Second Affiliated Hospital of Wenzhou Medical University and The Third Affiliated Hospital of Zhengzhou University. Yikon Genomics and Xukang Medical Science & Technology (Suzhou) will provide ni-PGT testing using NICS. The progression of the trial will be observed by an independent data and safety monitoring board (DSMB).

### Eligibility criteria

Women who come to reproductive medical centres of the involved hospitals will be screened to determine eligibility for our trial.

### Inclusion criteria

1. Infertile couples receiving ICSI procedures for assisted reproduction.
2. Women age between 35 and 42.
3. Women with body mass index from 18 to 30 kg/m<sup>2</sup>.
4. Women receiving controlled ovarian hyperstimulation treatment, including ultra-long proto-

col, long-protocol, short protocol treatment with gonadotrophin-releasing hormone agonist (GnRH-a) protocol and GnRH antagonist (GnRH-ant) protocol; and the number of oocytes was  $\geq 6$ .

5. Culture embryos to blastocyst stage and all the blastocysts will be individually cryopreserved.
6. Single thawed blastocyst will be transferred for the first time.
7. Only patients whose blastocysts are  $\geq 2$  and whose blastocyst morphology is greater than 4BC/4 CB will be included.<sup>27</sup>
8. Written informed consent.

### Exclusion criteria

1. Contraindications for IVF or ICSI, such as liver diseases, kidney diseases, type I or type II diabetes, heart diseases, anaemia, uncontrolled hypertension, history of cerebrovascular events, history of deep venous thrombosis and/or pulmonary embolism, history of cervical, endometrial or breast cancer and undiagnosed vaginal bleeding.
2. Preimplantation genetic testing cycles, including PGT-A, PGT for monogenic disorders (PGT-M) or PGT for structural chromosome defect (PGT-SR).

3. Pathologies potentially affecting pregnancy outcomes, including genital malformations, hydrosalpinx, intrauterine infections, myoma of uterus greater than 4 cm, benign tumour of pelvic or abdominal cavity greater than 4 cm, endometrial thickness less than 8 mm, pituitary tumours, and malignant tumours.
4. Untreated hyperprolactinaemia, thyroid disease and adrenal disease.
5. Women with untreated endometrial polyps before the thawing transfer cycle.

### Determination of dropout

All patients who signed the informed consent form will have the right to withdraw their consent and quit the trial at any stage. Drop-outs are only considered after the randomisation. Those who withdraw their consent before the randomisation, have the stimulation cancelled, have poor fertilisation, have suboptimal embryo development or whose embryos do not reach the 4BC/4CB stage are not drop-outs.

Reasons for dropout:

Researchers decide to drop out:

1. Serious adverse events (SAEs) occur, and the patients are not suitable to continue the study according to the researchers' judgement.
2. Before the blastocyst transfer, the patients have other diseases or special physiological changes and are not suitable to continue.
3. Patients who do not have the first blastocyst transfer within 6 months after oocyte retrieval.
4. Patients who do not complete the first transfer in the preferred order due to thawing failure.
5. In case of emergencies during the study, the blindness of the patients are broken.

Patients withdraw:

1. For whatever reason, the patients refuse to continue the trial process or withdraw the consent.
2. Although the patients do not withdraw the consent, but they no longer receive visits and follow-up.

### Recruitment

Outpatient infertile couples will be screened by dedicated researchers. The trial details will be explained by a research member. Couples will have time to consider participation to the research project. The couples with plans to attend will sign the consent form on or before the day of oocyte retrieval. Ineligible patients will continue the conventional clinical practices.

### Randomisation

Allocation and randomisation will be performed when the couples have  $\geq 2$  blastocysts. They involve an online software producing a randomisation list that allocates participants at a 1:1 ratio to the ni-PGT or morphology group, with a block size of four. The procedures will be executed by staff who are not involved in the study treatment.

### Blinding

Participants and clinicians/nurses involved in experimental procedures, as well as investigators and data analysts will be blinded to participant allocation. Embryologists ranking the thawing blastocysts will know the allocation. In this study, Yikon Genomics and Xukang Medical Science & Technology will also be blinded.

### Interventions

#### Controlled ovarian hyperstimulation and oocyte retrieval

The controlled ovarian hyperstimulation will be performed through standard routine procedures according to each centre. The selection of either the GnRH-a or GnRH-ant protocol will be performed by medical personnel involved in the study.

The oocyte retrieval is arranged for 36 hours ( $\pm 2$ ) after human chorionic gonadotrophin (hCG) injection. The oocyte retrieval will be executed using a 17–18 G oocyte aspiration needle under transvaginal ultrasound guidance. Immediately after oocyte retrieval, the retrieved cumulus oocyte complexes will be cultured in a 37°C with 5% or 6% CO<sub>2</sub> incubator. Considering the existing processes of the multiple centres, the culture medium is from different manufacturers (Vitrolife, Cook, SAGE and Quinns).

### ICSI

The ICSI will be performed as previously described.<sup>28</sup> In short, the integrity and maturity of denuded oocytes will be examined. An enzymatic removal will be performed during oocyte preparation. Only metaphase-II oocytes will be injected.

### Assessment of fertilisation

The assessment of fertilisation will be performed approximately 16–18 hours after fertilisation. The zygotes will be cultured until day 3, while the embryo quality will be evaluated after fertilisation, approximately at 67–69 hours. The number and size of blastomeres and the anucleate fragmentation will be scored.

### Removal of cumulus cells and sample collection

Before ICSI, the oocytes will be denuded. On day 3, if cumulus cells remain stick to the oocyte, the residue will be removed with an appropriate stripper gently before blastocyst culture. Blastocyst culture is done with sequential media in three-gas system in all centres. On day 4, the embryos will be individually transferred into a new blastocyst culture dish. These operations can effectively remove exogenous cfDNA contamination, and also ensures the accuracy of ni-PGT results.<sup>29</sup> The volume of each droplet will be around 25  $\mu$ l. When embryos develop to the blastocyst freezing standard, the blastocyst will be vitrified, and about 20  $\mu$ l culture medium from each corresponding blastocyst will be collected through an RNase/DNase-free PCR tube. All embryos were morphologically evaluated before freezing.<sup>27</sup>

## Groups

The intervention is different embryo selection methods, the ni-PGT group selects the embryo by NICS grade, and the morphological group selects the embryo with highest morphological score.

### Ni-PGT group

The spent culture media will be collected and tested using NICS. Blastocysts will be classified into three grades from A to C according to the NICS results, with euploid probabilities of  $\geq 0.94$ ,  $0.7-0.94$  and  $\leq 0.7$  for A, B and C, respectively. In the pilot study for this trial,<sup>30</sup> the embryos with no result for amplification failure or non-informative results were included in grade B. The outcomes of transferring B-grade embryos were worse than A-grade embryos and better than C-grade embryos. Moreover, no or non-informative results were neither euploidy nor aneuploidy, so these embryos were not suitable to be graded as A or C. Therefore, embryos with no result for amplification failure or non-informative results are graded B. A single blastocyst will be thawed and transferred in the preference order of A>B>C. The grading system had an area under the curve value of 0.92 and a negative predictive value of 0.93.<sup>30</sup> If the patients have only one blastocyst, the ni-PGT result will not be able to provide a reference for embryo selection. So randomisation and allocation will be performed when couples have  $\geq 2$  blastocysts. In blastocyst with the same grade, blastocysts with a higher morphology grade will be preferentially transferred.

### Morphological group

Only the first thawed blastocyst of the morphology group will be determined through the Gardner grading system. If a patient has multiple embryos with the highest rating, embryologists selected and thawed the best embryo between the highest rating according to the centre standards. Embryo morphology rank is 5AA>5AB>5BA>4AA>4AB>4BA>6AA>6AB>6BA>5BB>4BB>6BB>5AC>5BC>4AC>4BC>6AC>6BC>5CA>5CB>4CA>4CB>6CA>6CB, which is the consensus of the 13 centres participating in this trial.

The spent culture media from the morphological group blastocysts will also be collected and tested using NICS. The second and later thawed blastocysts will be ranked according to the 'NICS result grade combined with morphological standard'.

### Blastocyst preparation

As previously described, the expanded blastocysts will be vitrified and warmed.<sup>31</sup> If the blastocyst needs to shrink before freezing, it is transferred in another droplet instead of the culture dish. The vitrification and warming procedure will be performed according to the standard of each centre.

### Blastocyst transfer and luteal support

In this study, we focused on the first blastocyst thawing cycle, whether the ni-PGT group or the morphological group. The endometrium preparation, blastocyst transfer

and luteal support methods will be performed according to the standard of each centre. Endometrial preparation includes natural cycle and artificial cycle. Embryo transfer was performed during the window of implantation. The details are implemented in accordance with the standards of each centre.

### Follow-up

Measurements of blood and urinary hCG will be performed 12 days after blastocyst transfer. Participants will be diagnosed with a clinical pregnancy according to the ultrasonography evaluation 28 days after transfer. An ongoing pregnancy is defined as a fetal heartbeat occurring 12 weeks after the embryo transfer. After delivery, the following information will be collected within 2 weeks: pregnancy complications, gestational age, delivery, newborn sex and weight and birth complications.

### Outcome measures

Our primary outcome will be the ongoing pregnancy rate of the first transfer cycle within 6 months after oocyte retrieval. The time frame will be 12 weeks after the transfer of the first embryo. The ongoing pregnancy rate is defined as the number of women with a clinical pregnancy/ number of women randomised to each group.

The secondary outcomes of our trial include the clinical pregnancy, miscarriage and live birth rates. However, only the culture medium will be tested, and there will be no additional treatment for patients and embryos. The embryos will only be ranked according to the study criterion, and no embryos will be discarded. As a result, there will be few AEs.

### Data management

The data will be collected at the baseline and during follow-up from medical records. To guarantee authenticity of the study results, all researchers and clinicians involved in the study will master all details of the research. All participant-identifiable data will be stored in dedicated files and only delegated members will have access to them.

### Safety reporting

AEs: any adverse experience to a patient during the trial. Adverse experience can be symptoms (eg, nausea, pain), signs (eg, tachycardia, hepatomegaly) or abnormal test results (eg, laboratory tests, ECG). SAEs: any adverse events occurring during the trial that meets one or more of the following criteria: resulting in death; immediate life-threatening; requiring hospitalisation or prolongation of current hospitalisation time; resulting in permanent or apparent disability/insufficiency, or severely impairing daily life; resulting in congenital malformations or birth defects.

All AEs and SAEs will be reported to the DSMB and accredited by Medical Education Technology Committee, according to the study protocol requirements.

## Statistical analysis

### Sample size calculation

We hypothesised that ni-PGT results will increase the ongoing pregnancy rate, decreasing the miscarriage rate. According to the average ongoing pregnancy rate in the 13 participating centres, in the 35–42 age group, the ongoing pregnancy rate of embryos selected according to ‘morphology’ was approximately 38.8%, and it is expected that there will be a 10% difference in the ongoing pregnancy rate between groups. Accordingly, we used a two-sided test with a 5% alpha error and 90% statistical power, at least 516 subjects will be included in each group, with a total of 1032 subjects (ni-PGT group: morphology group=1:1). Considering fully completed the clinical trial with a relatively generous estimation method, we calculated the sample size according to the 10% dropout rate, each group included 574 participants (1148 participants in total).

### Statistical analysis

The results will be analysed according to the intention-to-treat (ITT) principle. As sensitivity analysis, the per-protocol (PP) method will also be implemented. The ITT populations refer to all randomised patients who experience at least one intervention and have post-intervention evaluation data. The PP populations refer to those who are randomised and meet at least the following criteria: (1) Meet the inclusion criteria and follow the protocol; (2) Complete all planned visits; (3) No drugs or treatments that may affect the evaluation of efficacy were used during the trial. Those cases when the embryo does not survive the thawing for the first transfer within 6 months after oocyte retrieval, were included as the ITT population but not PP population.

The missing data will be considered as randomly missing. The last-observation-carried-forward method will impute missing data. A sensitivity analysis will test best-case and worst-case hypotheses of distribution of the missing values. All secondary outcomes are considered exploratory.

The statistical analysis will be performed by using SPSS, V.25.0 (SPSS, Chicago, Illinois, USA). The significance is defined as  $p < 0.05$ , two-sided.

### Trial status

In each study centre, the recruitment started in April 2020. The last recruitment is estimated in December 2022.

### Patient and public sector involvement

Neither patients nor the public sector was involved in the research project. Patients will not participate in interpretation of the results or writing the final manuscript. As study interventions are routine procedures, the burden of the interventions will be assessed by enrolled couples.

### Ethics and dissemination

The study protocol was approved by the Ethics Committee of Peking University Third Hospital and the participating

hospitals. The informed consent will be collected before any study procedure from the enrolled couples. The Research Electronic Data Capture will store the collected data. The results will be presented at international conferences and published in peer-reviewed journals.

### Author affiliations

- <sup>1</sup>Centre for Reproductive Medicine, Department of Obstetrics and Gynecology, Peking University Third Hospital, Beijing, China
- <sup>2</sup>National Clinical Research Center for Obstetrics and Gynecology, Peking University Third Hospital, Beijing, China
- <sup>3</sup>Key Laboratory of Assisted Reproduction (Peking University), Ministry of Education, Beijing, China
- <sup>4</sup>Beijing Key Laboratory of Reproductive Endocrinology and Assisted Reproductive Technology, Beijing, China
- <sup>5</sup>Research Centre of Clinical Epidemiology, Peking University Third Hospital, Beijing, China
- <sup>6</sup>Institute of Reproductive and Stem Cell Engineering, NHC Key Laboratory of Human Stem Cell and Reproductive Engineering, School of Basic Medical Science, Central South University, Changsha, China
- <sup>7</sup>Clinical Research Centre for Reproduction and Genetics in Hunan Province, Reproductive and Genetic Hospital of CITIC-XIANGYA, Changsha, China
- <sup>8</sup>Hunan International Scientific and Technological Cooperation base of Development and Carcinogenesis, Changsha, China
- <sup>9</sup>Northwest Women's and Children's Hospital, Xi'an, China
- <sup>10</sup>Reproductive Medical Center of Hebei Maternity Hospital, Shijiazhuang, China
- <sup>11</sup>Affiliated Jinling Hospital, Nanjing, China
- <sup>12</sup>Shengjing Hospital of China Medical University, Shenyang, China
- <sup>13</sup>Centre for Reproductive Medicine, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou, Guangdong, China
- <sup>14</sup>Guangdong Provincial Key Laboratory of Reproductive Medicine, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou, Guangdong, China
- <sup>15</sup>Department of Obstetrics and Gynecology, Chinese People's Liberation Army General Hospital, Beijing, China
- <sup>16</sup>Department of Obstetrics and Gynecology, Sichuan University West China Second University Hospital, Chengdu, Sichuan, China
- <sup>17</sup>Key Laboratory of Birth Defects and Related Diseases of Women and Children, Sichuan University, Chengdu, Sichuan, China
- <sup>18</sup>Department of Obstetrics and Gynecology, Wenzhou Medical College Second Affiliated Hospital, Wenzhou, China
- <sup>19</sup>Reproductive Medicine Center, The Third Affiliated Hospital of Zhengzhou University, Zhengzhou, China
- <sup>20</sup>The Center of Reproductive Medicine, Peking University Shenzhen Hospital, Shenzhen, Guangdong, China
- <sup>21</sup>Second Hospital of Hebei Medical University, Shijiazhuang, Hebei, China
- <sup>22</sup>Xukang Medical Technology (Suzhou) Co., Ltd, Suzhou, China
- <sup>23</sup>Yikon Genomics Company, Ltd, Suzhou, China

**Correction notice** The article has been corrected since it was published online. The co-author Rong Li's name was published incorrectly as Li Rong which has been amended now.

**Contributors** JQ, PL and RL conceived the study idea. JQ, PL, RL, JH and SL participated in the design of the study, recruitment of participants and drafting of the manuscript. JQ, PL, RL and JH participated in the recruitment of participants and the assessment of clinical outcomes. JQ, PL, RL, JH, LH, JS, LC, BY, X-XW, YX, YW, JZ, YG, YY, WQ and GH supervised patient diagnosis and recruitment in each study centre. JH and LZ formed the data management team responsible for collecting and analysing all data. The manuscript was drafted by JH. All authors participated in reviewing, curating and approving the final manuscript.

**Funding** This study was supported by the National Key Research and Development Program of China (2018YFC1003100) and the National Science Foundation of China (82071721). The study funders had no role in the study design, implementation, analysis, manuscript, preparation or decision to submit this article for publication.

**Competing interests** None declared.

**Patient and public involvement** Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

**Patient consent for publication** Not applicable.

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

**Open access** This is an open access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited, appropriate credit is given, any changes made indicated, and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>.

#### ORCID iDs

Jin Huang <http://orcid.org/0000-0003-1879-4219>

Lin Zeng <http://orcid.org/0000-0001-8707-5854>

Juanzi Shi <http://orcid.org/0000-0002-9040-7829>

Junzhao Zhao <http://orcid.org/0000-0002-2385-205X>

Jie Qiao <http://orcid.org/0000-0003-2126-1376>

#### REFERENCES

- Alfarawati S, Fragouli E, Colls P, *et al*. The relationship between blastocyst morphology, chromosomal abnormality, and embryo gender. *Fertil Steril* 2011;95:520–4.
- Fragouli E, Alfarawati S, Spath K, *et al*. Morphological and cytogenetic assessment of cleavage and blastocyst stage embryos. *Mol Hum Reprod* 2014;20:117–26.
- Capalbo A, Rienzi L, Cimadomo D, *et al*. Correlation between standard blastocyst morphology, euploidy and implantation: an observational study in two centers involving 956 screened blastocysts. *Hum Reprod* 2014;29:1173–81.
- McCoy RC, Demko ZP, Ryan A, *et al*. Evidence of selection against complex Mitotic-Origin aneuploidy during preimplantation development. *PLoS Genet* 2015;11:e1005601.
- Webster A, Schuh M. Mechanisms of aneuploidy in human eggs. *Trends Cell Biol* 2017;27:55–68.
- Yan J, Wu K, Tang R, *et al*. Effect of maternal age on the outcomes of in vitro fertilization and embryo transfer (IVF-ET). *Sci China Life Sci* 2012;55:694–8.
- Franasiak JM, Forman EJ, Hong KH, *et al*. The nature of aneuploidy with increasing age of the female partner: a review of 15,169 consecutive trophoctoderm biopsies evaluated with comprehensive chromosomal screening. *Fertil Steril* 2014;101:656–63.
- Scott RT, Ferry K, Su J, *et al*. Comprehensive chromosome screening is highly predictive of the reproductive potential of human embryos: a prospective, blinded, nonselection study. *Fertil Steril* 2012;97:870–5.
- Chen M, Wei S, Hu J, *et al*. Can comprehensive chromosome screening technology improve IVF/ICSI outcomes? A meta-analysis. *PLoS One* 2015;10:e0140779.
- Rubio C, Bellver J, Rodrigo L, *et al*. In vitro fertilization with preimplantation genetic diagnosis for aneuploidies in advanced maternal age: a randomized, controlled study. *Fertil Steril* 2017;107:1122–9.
- Munné S, Kaplan B, Frattarelli JL, *et al*. Preimplantation genetic testing for aneuploidy versus morphology as selection criteria for single frozen-thawed embryo transfer in good-prognosis patients: a multicenter randomized clinical trial. *Fertil Steril* 2019;112:1071–9.
- Chang J, Boulet SL, Jeng G, *et al*. Outcomes of in vitro fertilization with preimplantation genetic diagnosis: an analysis of the United States assisted reproductive technology surveillance data, 2011–2012. *Fertil Steril* 2016;105:394–400.
- Zhang S, Luo K, Cheng D, *et al*. Number of biopsied trophoctoderm cells is likely to affect the implantation potential of blastocysts with poor trophoctoderm quality. *Fertil Steril* 2016;105:1222–7.
- Guzman L, Nuñez D, López R, *et al*. The number of biopsied trophoctoderm cells may affect pregnancy outcomes. *J Assist Reprod Genet* 2019;36:145–51.
- Middelburg KJ, Heineman MJ, Haadsma ML, *et al*. Neurological condition of infants born after in vitro fertilization with preimplantation genetic screening. *Pediatr Res* 2010;67:430–4.
- Middelburg KJ, van der Heide M, Houtzager B, *et al*. Mental, psychomotor, neurologic, and behavioral outcomes of 2-year-old children born after preimplantation genetic screening: follow-up of a randomized controlled trial. *Fertil Steril* 2011;96:165–9.
- Stigliani S, Anserini P, Venturini PL, *et al*. Mitochondrial DNA content in embryo culture medium is significantly associated with human embryo fragmentation. *Hum Reprod* 2013;28:2652–60.
- Xu J, Fang R, Chen L, *et al*. Noninvasive chromosome screening of human embryos by genome sequencing of embryo culture medium for in vitro fertilization. *Proc Natl Acad Sci U S A* 2016;113:11907–12.
- Kuznyetsov V, Madjunkova S, Antes R, *et al*. Evaluation of a novel non-invasive preimplantation genetic screening approach. *PLoS One* 2018;13:e0197262.
- Rubio C, Navarro-Sánchez L, García-Pascual CM, *et al*. Multicenter prospective study of concordance between embryonic cell-free DNA and trophoctoderm biopsies from 1301 human blastocysts. *Am J Obstet Gynecol* 2020;223:751 e1–51.
- Rubio C, Rienzi L, Navarro-Sánchez L, *et al*. Embryonic cell-free DNA versus trophoctoderm biopsy for aneuploidy testing: concordance rate and clinical implications. *Fertil Steril* 2019;112:510–9.
- Huang L, Bogale B, Tang Y, *et al*. Noninvasive preimplantation genetic testing for aneuploidy in spent medium may be more reliable than trophoctoderm biopsy. *Proc Natl Acad Sci U S A* 2019;116:14105–12.
- Jiao J, Shi B, Sagnelli M, *et al*. Minimally invasive preimplantation genetic testing using blastocyst culture medium. *Hum Reprod* 2019;34:1369–79.
- Yin B, Zhang H, Xie J. Validation of preimplantation genetic tests for aneuploidy (PGT-A) with DNA from spent culture media (SCM): concordance assessment and implication. *Reproductive biology and endocrinology : RB&E* 2021;19:41.
- JR H, Arrach N, Rhodes-Long K. Pushing the limits of detection: investigation of cell-free DNA for aneuploidy screening in embryos. *Fertil Steril* 2018;110:467–75.
- Fang R, Yang W, Zhao X, *et al*. Chromosome screening using culture medium of embryos fertilised in vitro: a pilot clinical study. *J Transl Med* 2019;17:73.
- Gardner DK, Lane M, Stevens J, *et al*. Blastocyst score affects implantation and pregnancy outcome: towards a single blastocyst transfer. *Fertil Steril* 2000;73:1155–8.
- Oehninger S, Veeck L, Lanzendorf S, *et al*. Intracytoplasmic sperm injection: achievement of high pregnancy rates in couples with severe male factor infertility is dependent primarily upon female and not male factors. *Fertil Steril* 1995;64:977–81.
- Huang J, Yao Y, Jia J. Chromosome screening of human preimplantation embryos by using spent culture medium: sample collection and chromosomal ploidy analysis. *Journal of visualized experiments. J Vis Exp* 2021:175.
- Chen L, Li W, Liu Y, *et al*. Non-Invasive embryo selection strategy for clinical IVF to avoid wastage of potentially competent embryos. *Reprod Biomed Online* 2022;45:26–34.
- Chen Y, Zheng X, Yan J, *et al*. Neonatal outcomes after the transfer of vitrified blastocysts: closed versus open vitrification system. *Reprod Biol Endocrinol* 2013;11:107.