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A method for determining potential parental contamination: linkage disequilibrium-based log-likelihood ratio analysis for IVF-PGT

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Abstract

Background At present, embryologists are attempting to use conventional in vitro fertilization (cIVF) as an alternative to intracytoplasmic sperm injection (ICSI) for preimplantation genetic testing (PGT). However, the potential parental contamination origin of sperm cells and cumulus cells is considered the main limiting factor in the inability of cIVF embryos to undergo PGT.

Methods In this study, we established an IVF-PGTA assay for parental contamination tests with a contamination prediction model based on allele frequencies and linkage disequilibrium (LD) to compute the log-likelihood ratio (LLR) under competing ploidy hypotheses, and then verified its sensitivity and accuracy. Finally, comparisons of the effectiveness of SNP-based analysis and LLR-based IVF-PGTA among 40 cIVF embryos was performed, based on both statistical analysis of the parental contamination rate and chromosomal ploidy concordance rate between TE biopsy and ICM isolations.

Results With IVF-PGTA assay, biopsies with 10% maternal contamination could be detected accurately, and contamination caused by sperm cells could be eliminated completely. Utilizing LLR-based or single Nucleotide Polymorphism (SNP) -based analyses, our comprehensive examination of 40 clinically discarded fresh cIVF embryos revealed an absence of paternal contamination. Strikingly, the LLR-based analysis uniquely revealed a mere instance of 24% maternal contamination within the trophectoderm cell (TE) biopsy of 5* embryo. Furthermore, it was solely through this analysis that embryo (9-F) was identified as a triploid of paternal origin.

Conclusions In this study, we developed a new bioinformatics analysis method for identifying parental contamination during IVF-PGT, especially for couples with nonmale factor infertility.

Keywords Conventional in vitro fertilization (cIVF), Potential parental contamination, IVF-PGTA assay, Linkage disequilibrium (LD), Log-likelihood ratio (LLR) analysis

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Introduction

In preimplantation genetic testing (PGT), intracytoplasmic sperm injection (ICSI) is widely used in clinically assisted reproductive technology (ART) and conventional in vitro fertilization (cIVF) insemination. The intention is to ensure single sperm insemination, which can avoid paternal contamination owing to attachment of the sperm to the zona pellucida during cIVF insemination [4, 6, 9, 15, 17, 19, 22, 32, 36]. In addition to efficacy, safety concerns have been raised by practitioners. According to many studies, ICSI can lead to an increase in birth defects, genetic abnormalities, and developmental delay in ART [1, 5, 10, 16, 20, 27, 30]. Compared to cIVF insemination, ICSI is also considered to be a cost- and resource-dependent technique [7, 21, 33]. To date, several retrospective studies and a single-centre prospective study have demonstrated that compared to ICSI, cIVF may be more appropriate for PGT for couples with nonmale factor infertility [3, 12, 13, 28, 31, 34].

It has been reported that the tightly packed DNA of sperm cannot be amplified with appropriate single-cell whole-genome amplification (WGA) methods, such as PicoPLEX and multiple annealing and loop-based amplification cycle (MALBAC) approaches [12, 14, 14, 25, 26] detected maternal contamination (MC) in cryopreserved cumulus cells. However, the real effect of cumulus cells on PGT in clinical practice requires further investigation. Moreover, single nucleotide polymorphism (SNP) genotyping information or quantitative fluorescent PCR (QF-PCR) of short tandem repeat (STR) markers has been used to track parental genetic information in the genetic materials of offspring [12, 14, 25, 26]. However, the high DNA demand or nonspecificity of WGA products preclude their widespread use in clinical practice. Recently, a new method termed the quantitative parental contamination test (qPCT) has been reported for parental contamination tests in cIVF embryos. However, the embryonic karyotype and contamination were analysed according to sequencing data from preimplantation genetic screening (PGS) and informative SNPs from the Infinium Asian Screening Array (ASA) bead chip, respectively [14]. Based on the MALBAC amplification product, the quality of the informative SNP loci obtained by the bead chip was poor, which may have resulted in inaccurate detection of the contamination ratio. Therefore, a simple and effective detection method is needed for IVF-PGT.

Daniel Ariad et al. [2] described a statistical method, termed linkage disequilibrium (LD)-informed PGT-A, that can distinguish different forms of aneuploidies due to meiosis or mitosis errors, and this method retained high accuracy at a coverage of 0.05× [2]. In this study, we established a prediction model for parental contamination by algorithm optimization, termed IVF-PGTA. Herein, the genome-wide log-likelihood ratio (LLR) was computed based on the leveraged allele frequencies and linkage disequilibrium in a population reference panel including 504 references. The LLR-based contamination analysis maintained high accuracy at a coverage of only 0.03× and was not limited by the amplification approaches.

In this study, we developed an IVF-PGTA assay for simultaneous chromosomal status and contamination analysis according to low-coverage next-generation sequencing (NGS) data. Moreover, 50 clinically fresh cIVF embryos were used to further verify the sensitivity and accuracy of this method.

Materials and methods

Whole-genome amplification of sperm cells and cumulus cells

A freshly ejaculated semen sample was obtained from a normal adult male who provided informed consent. After liquefaction, the semen sample was processed according to the semen preparation requirements for ICSI insemination, and a single spermatozoon with an apparently normal morphology was selected for tubing (Fig. 2A). Samples of 3 ~ 23 sperm cells were prepared and amplified with PicoPLEX and multiple displacement amplification (MDA) WGA protocols according to the corresponding manufacturers' instructions. The amplification products of all the samples were quantified with a Qubit™ dsDNA HS Assay Kit (Thermo Fisher Scientific, 2471823) according to the manufacturer's instructions. The cryopreservation of 1 ~ 10 cryopreserved (Fig. 2E) or cocultured cumulus cells (Supplementary Fig. 2) for single-cell amplification was performed with the same procedure used for the sperm cells. Because of amplification bias and nonspecific amplification [39], qPCR was used to further verify the quality of the MDA amplification products using 6 pairs of specific primers targeting different genes distributed on different chromosomes.

Establishment of the artificial parental contamination model

Next, 3 ~ 23 sperm cells and 1 ~ 10 cryopreserved or cocultured cumulus cells were added to the corresponding biopsied TE cells of the cIVF blastocysts. Several TE cell lines were also prepared as negative controls. To eliminate the impacts of sperm cells or cumulus cells, the zona pellucida was stripped completely before biopsy. One embryo was subjected to TE biopsy up to three times. All the artificially contaminated samples were further subjected to an IVF-PGTA assay. Both chromosomal ploidy and parental contamination were analysed with a regulated PGT-A analysis algorithm (algorithm-PGT-A) and LLR-based analysis, respectively.

Sample collection

Biologically low-potential blastocysts from conventional fresh IVF cycles, such as blastocysts with an unfulfilled inner cell mass (ICM), a small size (< 160 μm), an incomplete trophoblast layer, a few trophectoderm cells, or low-grade (BC or CB) blastocysts that were discarded voluntarily by patients on Day 7 after fertilization, were selected for this study. Blastocysts were washed at least three times before biopsy in 10-μl drops of G-PGD (Vitrolife, SE). When a clear view of the ICM was observed, the blastocyst was fixed, held, and positioned using a holding pipette. Then, the zona pellucida was perforated to induce blastocyst collapse via three to five of 2.5-ms laser pulses (Saturn Active Laser System, Research Instrument Ltd., UK). Five to 10 TE cells were obtained in the biopsy pipet, followed by three laser pulses to disrupt the intercellular junctions. Herein, the mechanical 'flicking' method was used to cut the TE cells inside both the biopsy pipette and the holding pipette. Biopsied TE cells were washed and further checked for the presence of additional sperm or cumulus cells under a high-power microscope and then placed in a 0.2-ml PCR tube containing less than 2.5 μl 1PBS. The corresponding ICM fraction was isolated using the same method. The tubes were then clearly marked, and all samples were stored at − 80 °C for further processing.

IVF-PGTA and LLR-based analysis

The artificial contamination specimens, TE biopsies, and their corresponding ICM samples were amplified with PicoPLEX. After whole-genome sequencing, libraries were prepared for sequencing. Here, parental genomic DNA (gDNA) was extracted from their peripheral blood and was used directly for library preparation. All libraries were subsequently sequenced using an MGI-200 sequencer (MGI Tech Co., Ltd., Shenzhen, China) with a single-end read length of 100 base pairs. More than 10 million raw reads of each sample were generated, and the quality score of 30 (Q30) of the output sequencing data was required to be above 80%. To determine the blastocyst ploidy status, blastocysts were defined as euploid, mosaicism, or aneuploid with algorithm-PGT-A. Embryos with 30% > the extent of mosaicism < 70% were categorized under the term "mosaicism". The embryo was diagnosed as "euploid" when the extent of mosaicism was < 30%, and the embryo was diagnosed as "aneuploid", when the extent of mosaicism was > 70%.

The LLR-based contamination prediction model was used for potential parental contamination tests. Briefly, the Z score, which represents the difference in the LLR between a sample and a normal diploid (2PN), was used to quantify the anomaly. Specifically, the Z score represents the standard deviation, and the larger the absolute value above and below the mean, the more significant is the difference. For example, a Z score of 3 indicated 3 standard deviations above the mean. A Z score of -3 indicated 3 standard deviations below the mean.

PGT-Plus assay and SNP-based analysis

The PGT-Plus assay was performed as described previously [38]. In brief, all embryonic biopsy samples were amplified with PicoPLEX. The amplified products and their corresponding parental genomic DNA (gDNA) were digested with two restriction enzymes, to generate 2- kb ~ 5 kb endonuclease sites per 1 Mb region genome wide. After library preparation, all libraries were sequenced using an MGI-200 sequencer (MGI Tech Co., Ltd., Shenzhen, China) with a paired-end read length of 100 base pairs. Approximately 80 million raw reads were generated for each sample, and the quality score was 30 (Q30) > 80%. The informative SNPs were then identified according to the output sequencing data.

Interpretation of the SNP data has been reported previously [14, 23, 24, 38]. Briefly, interpretation of the SNP data was based on the B allele frequency (BAF). The theoretical BAFs for genotypes AA, AB, and BB were approximately 0, 0.5, and 1, respectively. For a SNP with maternal AA and paternal BB, the genotype of the embryo was expected to be heterozygous AB, and the corresponding B allele frequency was 0.5. When the BAF of the genotypes was approximately 0.33, the pattern of heterozygous SNPs was AAB, suggesting that maternal contamination might exist. When the BAF of the genotypes was approximately 0.66, the pattern of heterozygous SNPs was ABB, suggesting that paternal contamination might be responsible for this result.

Statistical analysis

The experiments were repeated at least three times. GraphPad Prism 8.3.0 software was used to analyse all the data for each experiment. Multiple t tests for each row were used to compare differences between two groups. A p value < 0.05 was considered to indicate statistical significance.

Results

Design of this study

First, 28 clinical blastocyst TE biopsy samples were used to develop a prediction model for contamination with the IVF-PGTA. Then, 10 discarded fresh cIVF embryos were used to verify the sensitivity and accuracy of the contamination prediction model. Finally, comparisons of the effectiveness of SNP-based analysis and LLR-based IVF-PGTA among 40 cIVF embryos were performed. Both statistical analysis of the parental contamination rate

and chromosomal ploidy concordance rate between TE biopsy and ICM isolations were conducted (Fig. 1).

Amplification of sperm and cumulus cell DNA with two different WGA protocols

It has been reported that up to 60 sperm DNA fragments failed to amplify using PicoPLEX [12], and the rate of per-base per-cycle error was lower than that for MALBAC [11]. Herein, sperm cells with a normal morphology, cryopreserved cells, or cocultured cumulus cells were subjected to PicoPLEX-based and multiple displacement amplification-based (MDA) WGA protocols (Fig. 2). As a result, none of the samples were successfully amplified with PicoPLEX, resulting in significantly greater DNA amplification yields with MDA (Fig. 2A-D, Supplementary Table 1). Copy number variant (CNV) analysis further indicated that up to 25 sperm cells failed to amplify (Fig. 2H, Supplementary Table 1). Due to amplification bias and nonspecific amplification [39], qPCR was used to verify the quality of the MDA amplification products. The amplification curve revealed that fewer than 10 sperm cells were unsuccessfully amplified with MDA (Supplementary Fig. 1A and B, Supplementary Table 3).

Generally, cumulus cells are considered the origin of maternal contamination. Herein, single cumulus cells were isolated before fertilization (Fig. 2E, Supplementary Fig. 2A-F), followed by cryopreservation or coculture with blastocysts until TE biopsy. Unlike sperm cells, all

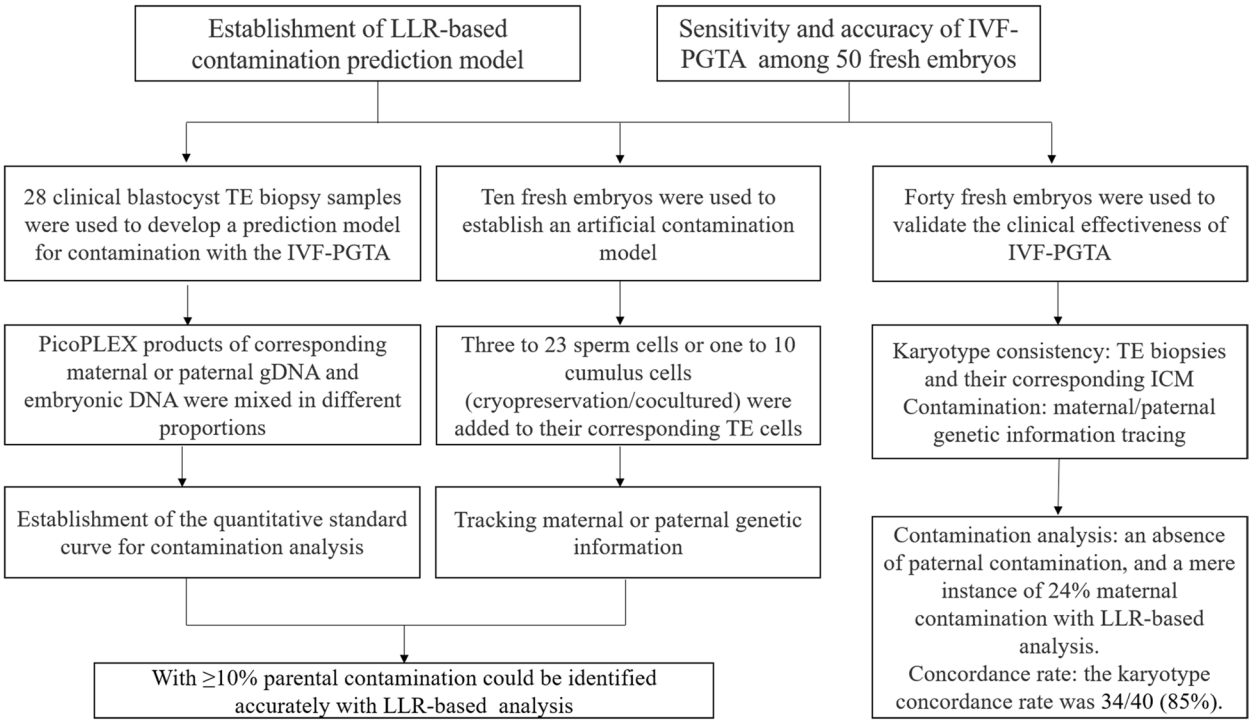

Fig. 1 Flow diagram for establishment of the IVF-PGTA method. Abbreviations: cIVF, conventional in vitro fertilization; TE, trophectoderm; ICM, inner cell mass; gDNA: genomic DNA

(See figure on next page.)

Fig. 2 Validation of the effects of parental cells subjected to different single-cell WGA approaches. **A** We selected 1, 5, 10, 15, 20, and 25 individual mature sperm cells for tubing (black arrowheads); the WGA products of different numbers of sperm cells obtained with PicoPLEX (**B**) and with tenfold dilutions of MDA (**C**) were analysed by gel electrophoresis. PBS served as the negative control (NC); 5–10 biopsied TEs were used as the positive control; M: 15 kb DNA ladder. **D** Statistical analysis of the amplification yield of sperm cells with PicoPLEX and MDA. **E** We isolated 1, 2, and 3 single cumulus cells from the tubing (black arrowheads). **F** WGA products of 1, 2, and 3 cumulus cells with PicoPLEX and MDA were analysed by gel electrophoresis. The cycle values of all samples are shown in Supplementary Table 4. **G** Statistical analysis of the amplification yield of cumulus cells with PicoPLEX and MDA. CNV analysis of 25 sperm cells (**H**), one cryopreserved cumulus cell (**I**), and 10 cocultured cumulus cells (**J**). The scale bars represent 75 μm. M: 15 kb DNA marker. The above data are representative of three independent experiments, and the error bars indicate the means ± SEMs

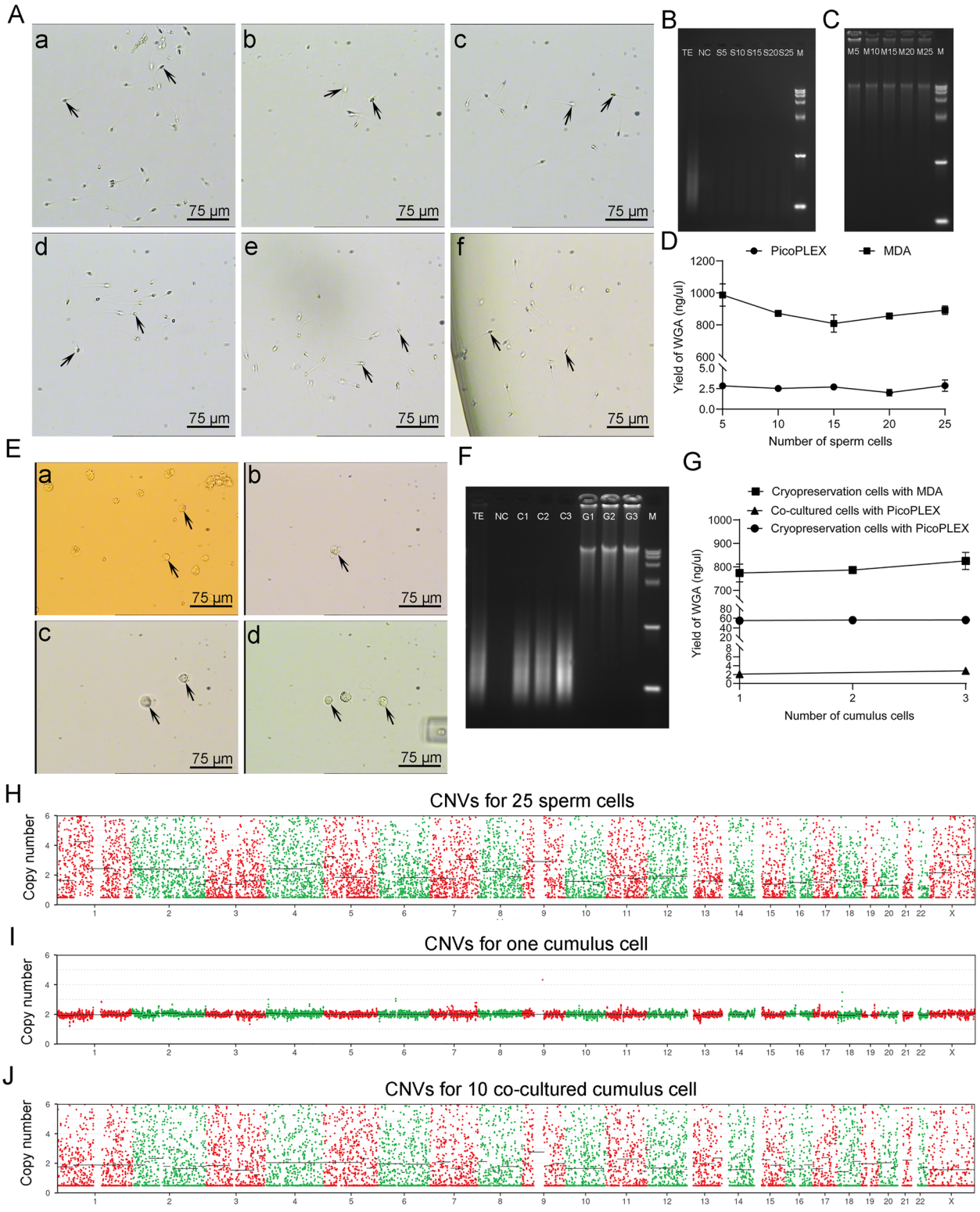

Fig. 2 (See legend on previous page.)

cryopreserved cumulus cells were successfully amplified with MDA and PicoPLEX (Fig. 2F, G). The DNA amplification yield with PicoPLEX was significantly lower than that with MDA (Fig. 2G, Supplementary Table 1). Surprisingly, 10 cocultured cumulus cells failed to amplify with PicoPLEX (Supplementary Fig. 2G, Supplementary Table 2). CNV analysis further indicated that one cryopreserved cumulus cell sample was amplified successfully, but 10 cocultured cells failed to expand (Fig. 2I, J). These results indicated that the paternal contamination origin of sperm cells could be effectively eliminated with PicoPLEX. To some extent, maternal contamination caused by several cocultured cumulus cells was likely to have little effect on the results of PGT.

LLR-based contamination prediction model for parental contamination detection

Twenty-eight TE biopsy PicoPLEX products from 10 families, defined as aneuploidies or aneuploidy mosaicism without parental contamination, were collected (Supplementary Table 4). PicoPLEX products of corresponding maternal or paternal gDNA and embryonic DNA were mixed in different proportions. All the artificially contaminated samples were processed according to the PGT-A procedure shown in Fig. 3A. Both chromosomal ploidy and contamination tests were performed. The results showed that with LLR-based analysis, the artificial parental/embryonic proportions were accurately detected. A qualitative standard curve was produced, and the correlation coefficient reached more than 0.99 (95% CI: 99.46%-99.98%) (Fig. 3B). To track the origin of the contamination, we further optimized the algorithm for LLR analysis. Raw sequencing data of maternal and paternal origin were mixed with those from embryos, and the log-likelihood ratio was calculated. LLRs greater than zero were selected, and the ratio of the medians of the two groups was obtained. Herein, the R value represented the origin of contamination. A value of $R > 0.8$ suggested the presence of paternal contamination (Fig. 3C, E), whereas a value of $R > 1.4$ indicated the presence of

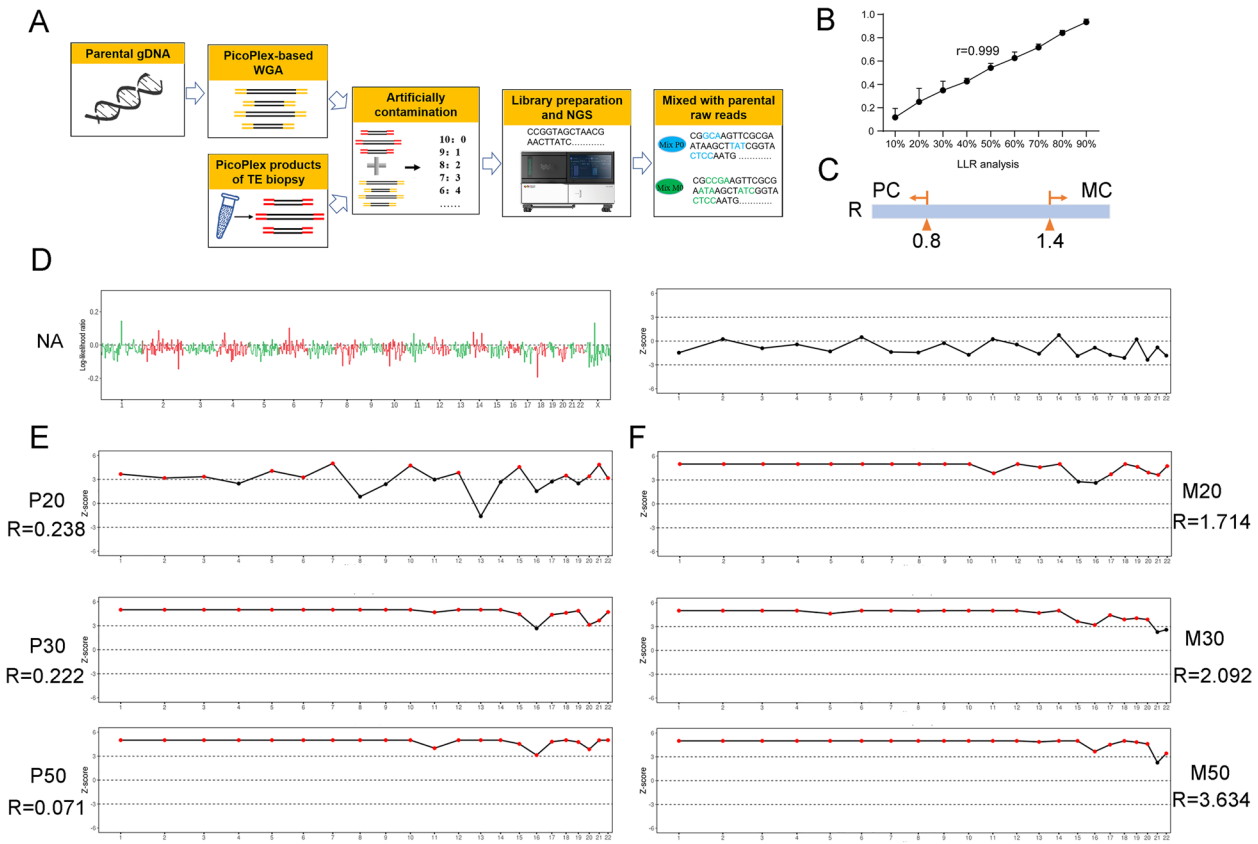

Fig. 3 Establishment of the prediction model for contamination of IVF-PGTA. **A** Workflow of artificially mixing embryo/maternal or embryo/paternal DNA in different proportions for contamination analysis. **B** Schematic of the contamination origin based on maternal or paternal analysis. MC represents the LLR value for maternal bias, and PC represents the LLR value for paternal bias. **C** Establishment of the quantitative standard curve for contamination analysis. **D**-**F** LLR analysis for interpretation of parental contamination; the Z score was used to quantify the anomaly. NA: 5–10 biopsied trophectoderm cells without parental contamination; P20/M20, P30/M30, P50/M50: paternal or maternal gDNA/embryo DNA = 2:8, 3:7, 5:5, respectively. The above data are representative of three independent experiments, and the error bars indicate the means ± SEMs

maternal contamination (Fig. 3C, F). A sample of biopsied TE cells without contamination was used as a negative control (Fig. 3D). Moreover, we also assessed the performance of the LLR-based predictive model for contamination in a set of artificially contaminated parental specimens. The initial findings suggested that the IVF-PGTA assay exhibited promising performance [sensitivity: 100%, specificity: 97.2%, positive predictive value (PPV): 98%, negative predictive value (NPV): 100%, false-positive rate (FPR): 2.86%, false-negative rate (FNR): 0%] (Supplementary Tables 5 and 6).

Sensitivity and accuracy of IVF-PGTA in clinical practice

Fifty discarded fresh cIVF embryos at different stages (stage IV-VI) [18] were collected for this study (Fig. 4A, a-c). TE biopsy was performed on blastocysts at different

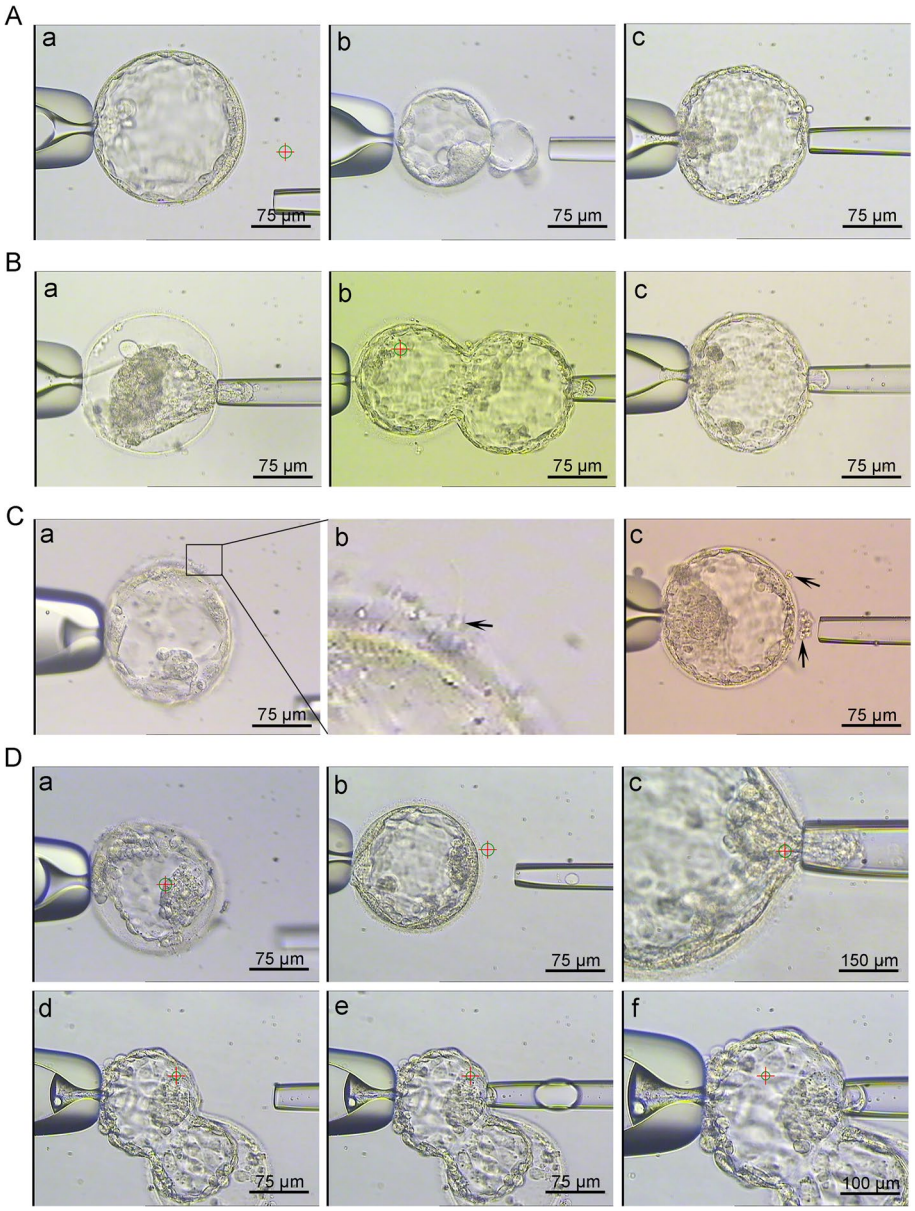

Fig. 4 TE and ICM biopsy procedure for typical discarded cIVF embryos. **A** Types of clinically typical discarded cIVF embryos. a-c: IV-, V-, and VI-stage cIVF embryos, respectively; (**B**) TE biopsy of discarded cIVF embryos at different stages. a-c: IV-, V-, and VI-stage cIVF embryos, respectively; (**C**) TE biopsy of discarded cIVF embryo-attached sperm (a, b, black arrowhead) or cumulus cells (c, black arrowheads), where b shows a magnified view of a. **D** The ICM biopsy procedure for discarded cIVF embryos, a-c: IV-stage cIVF embryo, d-f: V-stage cIVF embryo. Scale bars represent 150 μm, 100 μm, 75 μm or 50 μm

stages as a routine laboratory procedure (Fig. 4B, a-c). For blastocyst-attached sperm (Fig. 4C, a-b, black arrowhead) or cumulus cells (Fig. 4C, c, black arrowhead), visibly contaminated cells could be easily eluded under a high-power microscope. Moreover, the corresponding ICM fractions were isolated as positive controls (Fig. 4D).

Ten fresh embryos were used to establish an artificial contamination model. During conventional IVF insemination, redundant sperm cells stuck to the zona pellucida, their heads were usually left, and the cumulus cells were also incompletely ablated. To eliminate the effects of parental cells, the zona pellucida was peeled off completely before biopsy (Fig. 5A). Then, five to 23 sperm cells or one to 10 cumulus cells (cryopreservation/cocultured) were added to their corresponding TE cells. Both artificial contamination samples and parental gDNA were subjected to IVF-PGTA (Fig. 3A). Paternal contamination caused by sperm cells was not detected in any of the samples, and chromosomal ploidy was not affected (Fig. 5B, E and Supplementary Fig. 3A).

Unlike spermatozoa, biopsies with 10% maternal contamination caused by one cryopreserved cumulus cell could be accurately detected, and the more cumulus cells there were, the greater maternal contamination was present (Fig. 5C, F and Supplementary Fig. 3B). However, contamination caused by up to 10 cocultured cumulus cells could not be detected (Fig. 5D and Supplementary Fig. 3C). These results indicated that the IVF-PGTA assay showed high sensitivity and accuracy for detecting contamination. In clinical practice, with IVF-PGTA, the paternal contamination origin of sperm cells can be effectively eliminated, and the chromosomal status may not be strongly affected by several cumulus cells.

Comparison of the clinical effectiveness of both the LLR-based IVF-PGTA assay and SNP-based analysis in cIVF embryos

Forty fresh cIVF embryos were used to further validate the clinical effectiveness of IVF-PGTA (Supplementary Table 7). All embryonic samples were subjected to both chromosomal status determination and contamination tests with both the IVF-PGTA assay and SNP-based analysis (Fig. 6A). As expected, no paternal contamination was detected with LLR analysis (Supplementary Table 7). Notably, the embryo (9-F) was defined as aneuploid with paternal contamination by SNP analysis, but a triploid (3PN) result of paternal origin was further revealed by LLR analysis (Fig. 6C and Supplementary Table 7). The LLR-based analysis revealed a 24% maternal contamination in the TE biopsy of 5 embryo, a finding that was not replicated by the SNP analysis, which indicated no presence of contamination (Fig. 6B and Supplementary Table 7). These results indicate that LLR analysis might be more sensitive than SNP analysis for contamination, and LLR could initially distinguish triploid from parental contamination according to the distribution trends of the genome-wide Z scores.

In addition, the chromosomal ploidy concordance rate between biopsied TE and the corresponding ICM was 85% (34/40), which was comparable to that reported for ICSI [8, 29, 35]. Among 40 cIVF embryos, three (36-F1/2, FQ1-1/2, and Z2-1/2) could not be diagnosed because of poor quality sequencing data or poor tissue relatedness (Supplementary Table 7, Supplementary Fig. 5A-C). The chromosomal status of TEs from two embryos (PT2 and X3) was consistent with that of their corresponding ICMs, although the mosaicism rate was slightly different (Supplementary Table 7 and Supplementary Fig. 4A, 4B). The chromosomal ploidy of the biopsied TEs of three embryos (2N1, 2N2, and M1) was not consistent with that of the ICM, which is the gold standard (Supplementary Table 7 and Supplementary Fig. 4C-F). Moreover, the tissue relatedness between the TE and corresponding ICM from the embryo (Z2-1) was diagnosed as first degree (parent–offspring or full sib), potentially due to exogenous pollution of the biopsied TE cells (Supplementary Table 7, Supplementary Fig. 6H).

Discussion

This is the first study to develop an IVF-PGTA assay for both chromosomal ploidy and contamination tests according to the NGS data obtained for samples with only 0.03 × coverage, and the LLR-based contamination prediction model did not require parental genetic information for contamination detection. In clinical practice, LLR-based analysis has shown high accuracy and effectiveness for contamination tests. Biopsies with 10% contamination can be detected, although the detection efficiency may not reach 100%. Moreover, compared with SNP-based analysis, LLR-based analysis can be used to distinguish triploids from parental contamination. However, the clinical feasibility of IVF-PGTA was further validated.

Potential parental contamination in cIVF embryos is considered a main limiting factor for PGT. Consistent with previous studies [12, 14, 25, 26], we also demonstrated that sperm DNA was successfully amplified with MDA but not with PicoPLEX. In contrast to a previous study [14], we demonstrated that cocultured cumulus cells failed to amplify with PicoPLEX, and the amplification product yields were comparable to those of the negative control. Moreover, the results of artificial contamination samples showed that contamination caused by cryopreserved cumulus cells could be detected, but no contamination was detected in samples with cocultured

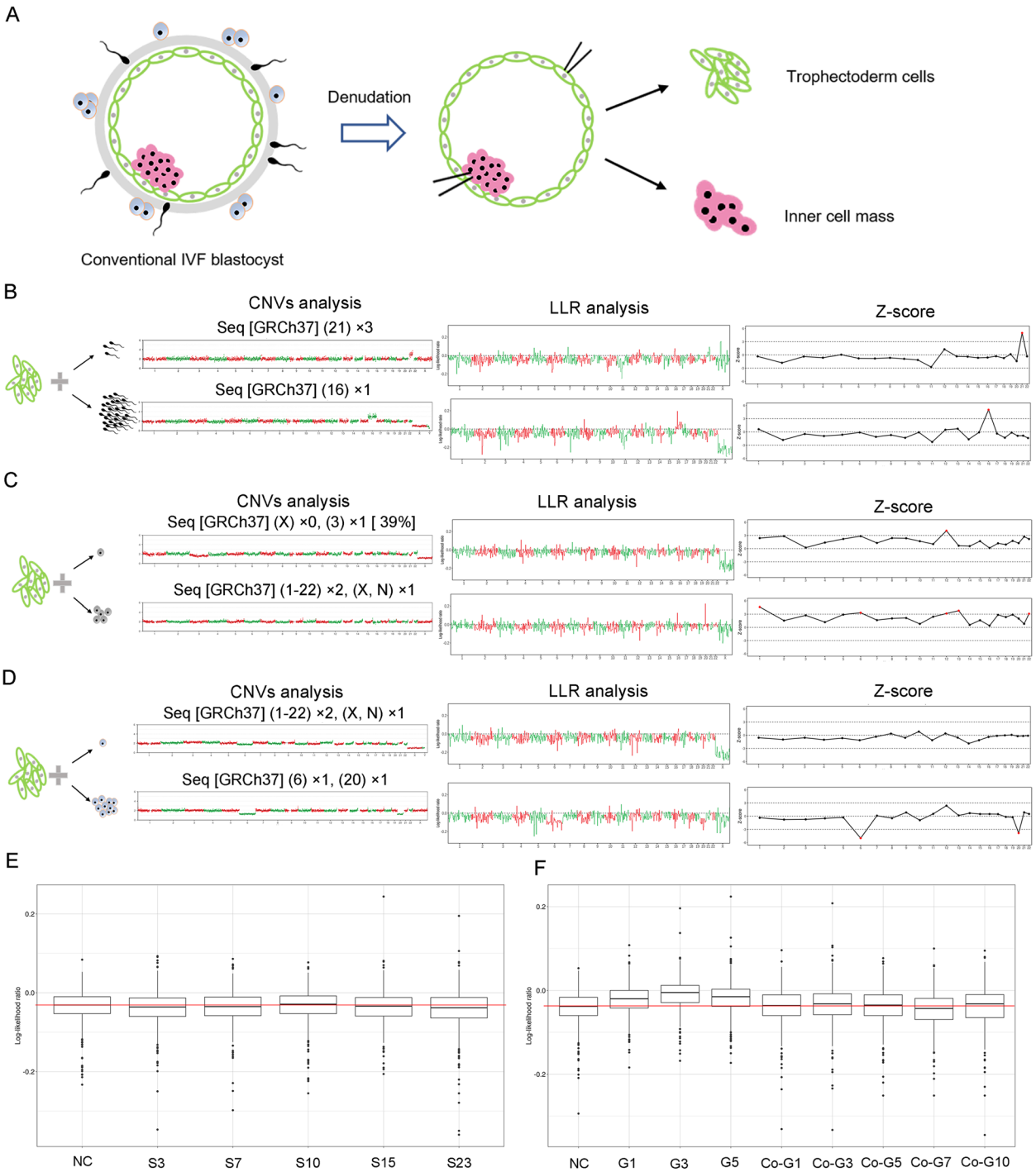

Fig. 5 Validation of the effectiveness of IVF-PGTA using the artificial parental contamination mode. **A** Both TE and corresponding ICM fractions were isolated from denuded cIVF blastocysts; (**B-D**) 3 ~ 23 frozen sperm cells, 1 ~ 5 cryopreserved cumulus cells, and 1 ~ 10 cocultured cumulus cells were artificially added to several biopsied TE cells; LLR analysis to determine the origin of paternal contamination from 3 ~ 23 frozen sperm cells (**E**) and the origin of maternal contamination from 1 ~ 5 cryopreserved or 1 ~ 10 cocultured cumulus cells (**F**), where NC represents TE biopsy without parental cells

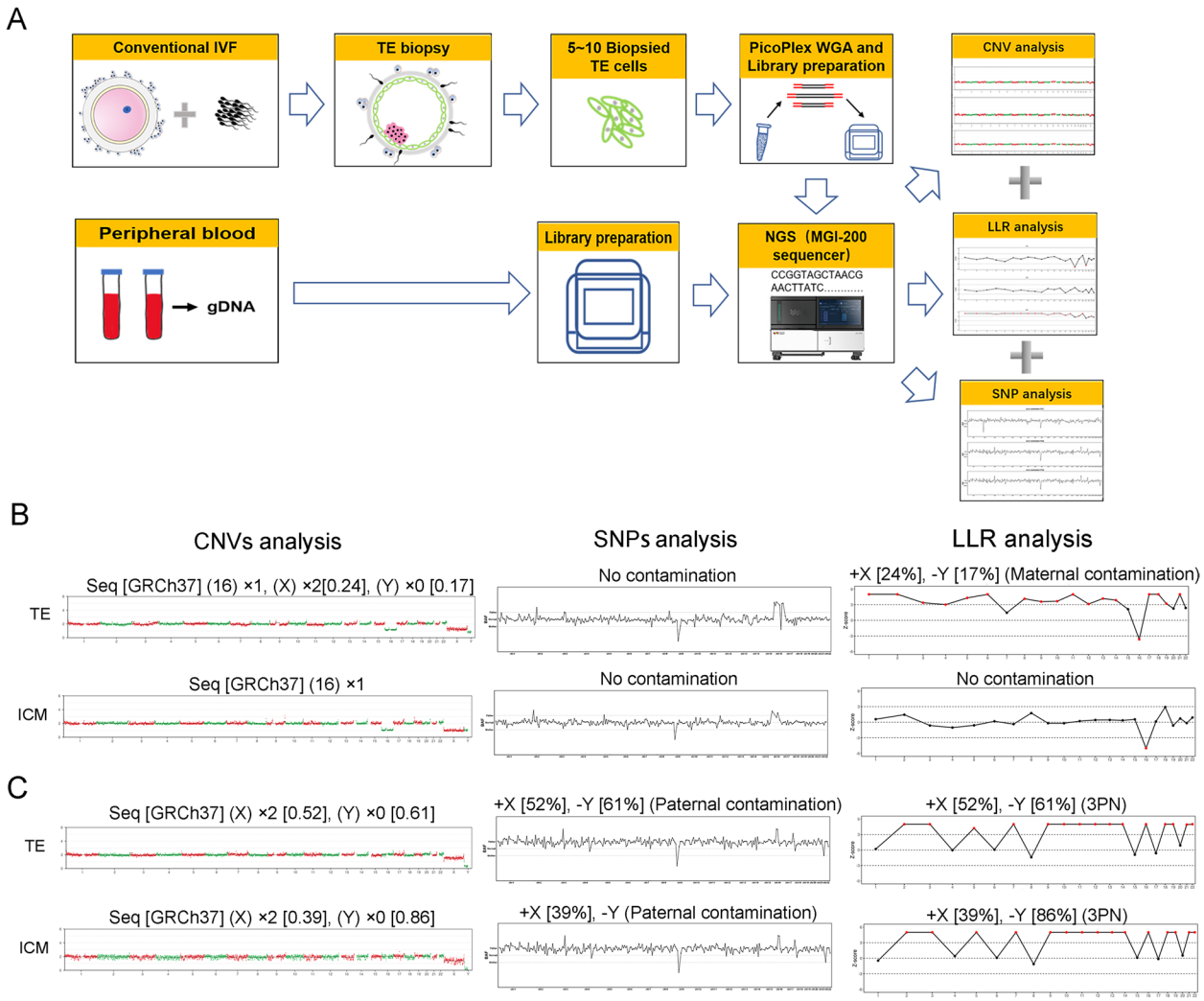

Fig. 6 The clinical feasibility of IVF-PGTA (**A**) Workflow for clinical embryos for both PGT-A and contamination tests. **B** Biopsied-TE with 24% maternal contamination confirmed by LLR analysis. **C** The results obtained using 3PN for the embryo (9-F) paternal origin

cumulus cells, which might be related to cell apoptosis during coculturing.

SNP-based analysis has been used to track parental genetic information in corresponding offspring materials [14, 23, 24]. However, due to sufficient DNA requirements or the poor quality of informative SNPs, this analysis requires further optimization. Recently, the LLR-based LD-PGTA method was used to trace the origin of aneuploidy [2, 37]. Herein, we optimized an algorithm to develop an LLR-based IVF-PGTA method. Compared to SNP analysis, the major advantages of LLR analysis are that (*i*) it is based on whole-genome sequencing data at a coverage of just 0.03×; (*ii*) contamination analysis can be performed without parental genomic DNA (gDNA) from the embryos; and (*iii*) it retains high accuracy down to lower coverage and is not restricted by WGA approaches. Moreover, data from 50 clinical cIVF embryos showed that LLR analysis may be more effective for detecting contamination than SNP analysis, and even TE biopsies with 10% contamination could be identified accurately.

Among 80 embryonic specimens, only one embryo with 24% maternal contamination was detected with IVF-PGTA. Although most cumulus cells are usually removed before TE biopsy, the remaining cumulus cells are likely to undergo apoptosis during culture. The maternal contamination origin of several cumulus cells (less than 10) did not appear to have a large impact on the PGT results. Nevertheless, a randomized controlled multicentre clinical trial is needed to further validate the feasibility of IVF-PGT.

In conclusion, we developed a novel method for parental contamination testing for IVF-PGT, termed IVF-PGTA. Compared to SNP-based analysis, optimized LLR analysis showed greater sensitivity and accuracy for

detecting contamination. In clinical practice, the IVF-PGTA is an alternative to the ICSI-PGT and provides a simple and effective treatment for patients, especially for couples without male factor infertility.

Abbreviations

cIVF	Conventional in-vitro fertilization
ICSI	Intracytoplasmic sperm injection
PGT	Preimplantation genetic testing
SNP	Single nucleotide polymorphism
LD	Linkage disequilibrium
LLR	Log-likelihood ratio
TE	Trophectoderm cells
ICM	Inner cells mass
ART	Assisted reproductive technology
WGA	Whole-genome amplification
MALBAC	Multiple annealing and loop-based amplification cycle
MDA	Multiple displacement amplification
MC	Maternal contamination
QF-PCR	Quantitative fluorescent PCR
STR	Short tandem repeat
qPCT	Quantitative parental contamination test
PGS	Preimplantation genetic screening
ASA	Asian Screening Array
NGS	Next generation sequencing

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12958-024-01300-z.

Supplementary Material 1.
Supplementary Material 2.
Supplementary Material 3.
Supplementary Material 4.
Supplementary Material 5.
Supplementary Material 6.
Supplementary Material 7.
Supplementary Material 8.
Supplementary Material 9.
Supplementary Material 10.
Supplementary Material 11.
Supplementary Material 12.
Supplementary Material 13.

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Authors' contributions
B. L., C. M., L.B., F. D: design and investigation the study. B. L., C. M: project administration. L.B., Z.W., A. Z., Y. Z., L. Z.: data collection and methodology. L.B., F. D., L. K., J. Z., N. L., L. Q., T. S: data analysis and interpretation. L.B., F. D: writing-original draft. L.B., F. D., Z.W., A. Z., Y. Z., L. K., L. Z., J. Z., N. L., L. Q., T. S., B. L., C. M: writing-review and editing.

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Data availability
Data is available upon reasonable request to the corresponding author.

Declarations

Ethics approval and consent to participate
Approval for this study was obtained from the Medical Ethics Committee of the First Affiliated Hospital of Soochow University (Approval No. Y2022389). All discarded fresh conventional IVF embryos were collected from the Reproductive Medical Center of the First Affiliated Hospital of Soochow University. All patients provided informed consent before participating in the study.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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