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Less-invasive chromosome screening of embryos and embryo assessment by genetic studies of DNA in embryo culture medium

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Abstract

Purpose To perform a preliminary exploration of a new embryo rank in clinical practice by combining the embryo chromosome copy number and mitochondrial copy number analysis of DNA extracted from embryo culture medium and blastocoel fluid.

Method Eighty-three ICSI embryos from day 2 or day 3 were cultured to day 5 or day 6. Thirty-two blastocysts of 3 cc or above were obtained. Culture medium and blastocoel fluid were collected at 24 h before blastocyst formation. The genomic DNA and mitochondrial DNA (mtDNA) from the culture medium combined with blastocoel fluid and the whole blastocyst were amplified and sequenced by MALBAC-NGS. We compared the chromosomal information generated by the new protocol from the culture medium and the information employed by the whole embryo method. A multivariable linear regression was performed to study the impact of the blastocyst morphological score, chromosomal abnormality, embryo mtDNA copy number, and female age on the culture medium mtDNA copy number.

Results (1) The DNA from 31 blastocysts was successfully amplified, and the successful amplification rate was 96.9% (31/32). The success rate of the amplification of genomic DNA extracted from the culture medium was 87.5% (28/32). (2) There were 18 blastocysts in which the less invasive method and the whole embryo method revealed the same results. The consistency rate was 66.7% (18/27). (3) The culture medium mitochondrial DNA copy number (MCN) had a significantly positive correlation with the blastocyst mitochondrial DNA copy number ($P = 0.001$), female age ($P = 0.012$), and blastocyst score ($P = 0.014$), but there was no obvious correlation with blastocyst chromosome ($P = 0.138$).

Conclusions The preliminary exploration result of the less invasive approach for having an embryo rank was not satisfying, which still awaits further long-term evaluation.

Keywords Medium · Genomic DNA · Mitochondrial DNA · MALBAC · NGS · Preimplantation genetic testing · In vitro fertilization and embryo transfer · Less-invasive

Introduction

In IVF/ICSI (in vitro fertilization/intracytoplasmic sperm injection), how to select an embryo that is most likely to result in a healthy baby remains an important challenge. The morphological score is a commonly utilized embryo assessment; however, approximately 44.4% of the morphologically high-quality day-3 embryos were still chromosomally abnormal. A morphologically high-quality embryo was not equal to chromosome normal or high implantation potential. Then, preimplantation genetic testing (PGT) emerged, which could help select an embryo with a normal chromosome to improve the pregnancy rate and reduce the abortion rate of ART [1–5]. However, PGT requires cell biopsy, and the polar body, cleavage cell, and blastocyst trophectoderm are all invasive to the developing embryo and might induce long-term harm to the offspring [6]. Recent animal studies have found that biopsy may cause offspring epigenetic changes [7, 8] and nervous system or ovarian dysfunction [9, 10]. Biopsy is prohibited by law in some countries, such as Germany, Italy, and Switzerland [11], which has also prevented PGT from widespread application. Therefore, a less invasive technique to assess the genetic and chromosomal defects of human embryo preimplantation is urgently needed and desirable. The DNA extracted from the blastocoel fluid was first detected by Palini

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et al. in 2013 [12]. Studies have shown that embryos may release low-level genomic DNA (gDNA) and high-level mitochondrial DNA (mtDNA) into the culture medium and in blastoceles [13]. Stigliani S et al. found that the success rates for the detection of mitochondrial DNA and genomic DNA extracted from the culture medium were 98.8% and 63.0%, respectively [14]. Embryo culture medium-based less invasive preimplantation genetic screening recently obtained a satisfying result [15]. Although the correlation between mitochondrial DNA and embryo quality, embryo implantation potential, embryo chromosome abnormality, and embryo development potential, collectively defined in our study as "embryo-associated structures," has not reached an agreement until now, we hope that embryo culture medium mitochondrial DNA could be used as a reference index to predict the development potential of the embryo. In our study, we utilized culture medium combined with blastocoel fluid (ECB) to assess chromosomal abnormality and mitochondrial DNA copy number (MCN) and evaluate whether this strategy could be used as a less invasive method for embryo selection, not only with normal chromosomes but also with high development potential.

Materials and methods

A total of 83 ICSI embryos from day 2 or day 3 were cultured, and 32 blastocysts were obtained (Fig. 1). The embryos were donated by couples who already had a healthy baby via ART at the Reproductive Medical Center, Department of Gynecology and Obstetrics, General Hospital of Tianjin Medical University. Ethical approval was obtained from the Scientific and Ethical Committee of Tianjin Medical University for the experimental protocol (IRB2017-155-01). A detailed patient consent form was signed for each embryo sample used in this study.

Embryo culture Each embryo was naked again post-thaw and sequentially cultured to day 5 or day 6 until blastocyst formation separately. The culture media (Vitrolife, Sweden) were replaced every 24 h.

All blastocysts were evaluated using a scoring system based on the recommendation by Gardner and Schoolcraft in 1999 [16]. Only blastocysts scoring 3BB or higher by day 5 or day 6 were considered as high-quality embryos [17]. A specific numerical score was given in our center. The blastocyst scores were as follows: The blastocysts of grade 1 and 2 were given 10 and 11 points respectively, grade 3 or above were given as different degrees multiplied by 4. The grades of the inner cell mass and trophectoderm were as follows: grades A, B, and C were given 4, 3, and 2 points, respectively, and the sum was the score of the blastocyst. For example, a 3BB blastocyst was given a mark of 18 (3 multiplied by 4 + 3 + 3).

Sample collection Embryo culture medium and blastocoel fluid were collected. An infrared laser (Saturn Active Laser System, UK, RI) was used to create a small breech in the zona pellucida (ZP) to release the blastocoel fluid into the culture medium. The location of the breach was far from the inner cell mass. The whole blastocyst was placed in RNase–DNase-free PCR tubes and culture media with the released blastocoel fluid (20–30 μl) were also transferred to RNase–DNase-free PCR tubes.

Negative control (NC): The culture medium, which was identically processed but did not contain embryos, was used as a negative control (NC1), and unused culture medium was also used as a negative control (NC2).

Whole genome amplification and NGS

The whole genome amplification (WGA) was performed on the media and whole embryos and the culture medium,

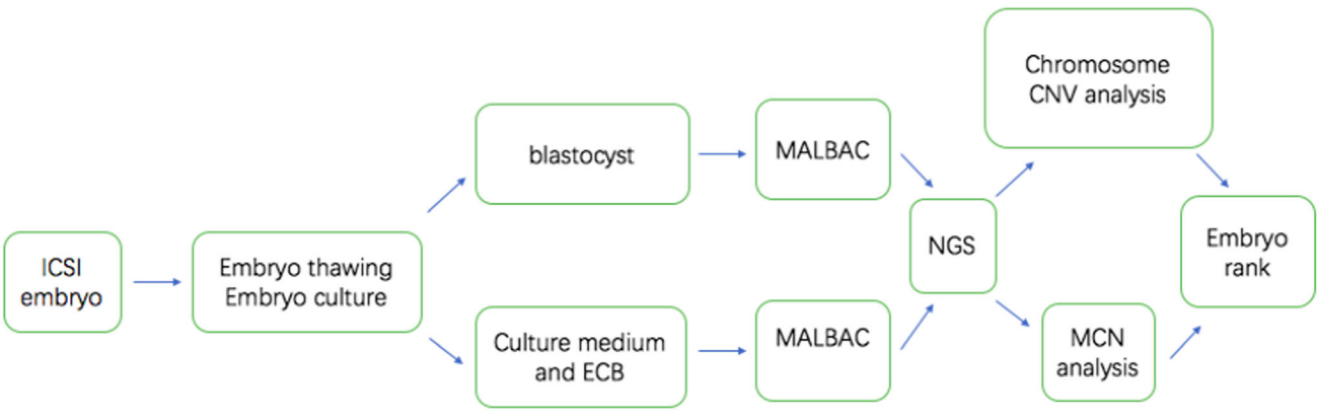

Fig. 1 Experimental operation flow chart: ICSI D2 or D3 embryos, embryo thawing and embryo culture, whole blastocyst, and culture medium collection at 24 h before blastocyst formation, DNA amplification through MALBAC-WGA, and sequencing the amplification products by Illumina Hiseq 2500 platform. The embryo chromosome copy number variation and mitochondrial DNA copy number variation were analyzed, combining both values to obtain an embryo rank

followed by library preparation by chrominst (Yikon Genomics, EK100100724 NICSInst™ Library Preparation Kit). The amplification products were purified and quantified with a Qubit® dsDNA HS Assay Kit (cat. no. Q32851; Invitrogen) and then sequenced with an Illumina HiSeq 2500 platform, which yielded approximately 2 million sequencing reads on each sample. Single end reads 55 (SE55), and the sequencing depth is approximately 0.02~0.03×. The chromosomal CNV analysis was performed as previously described (Zong et al., 2012) [18]. The read numbers were counted along the whole genome with a bin size of 1 Mb. A copy number gain from 2 to 3 copies results in a 50% increase in read counts, while a copy number loss from 2 copies to 1 copy results in a 50% decrease in read counts.

Mitochondrial data analysis

The mitochondrial content was quantified in the form of copy number/nuclear genome. Sequencing reads mapped to the mitochondrial genome were counted and normalized by the read count mapped to the autosomal chromosomes, and mitochondrial copy numbers per nuclear genome (MCN) were calculated and displayed according to the following formula:

MCN = (mitochondrion mapped reads/mitochondrion mappable region)/(autosomal mapped reads/2 × autosomal mappable region)

Result analysis

The chromosomal information generated by the new protocol from the culture medium and the information employed for the whole embryo method were compared, and we used the information employed for the whole embryo as the gold standard. However, opinions on how to define the concordance vary [19]; here, we utilized the following criteria. Whole chromosomal CNV concordance was defined in embryos with normal chromosomes. In embryos with abnormal chromosomes, the whole blastocyst and spent media shared the same chromosomal abnormality.

A multivariable linear regression was performed to study the impact of blastocyst morphological score, chromosomal abnormality, embryo mtDNA copy number, and female age on the culture medium mtDNA copy number. The statistical analysis was performed using SPSS 19.0.

Results

A total of 32 blastocysts were obtained. The age of the female donors was between 26 and 40 years old (29.7 years old average). Detailed information about the embryo morphological score, blastocyst morphological grade, DNA amplification, MCN of blastocysts, and culture media are listed in Table 1.

Chromosome copy number variation results The success rate of amplification of blastocysts was 96.9% (31/32). Blastocyst number 47 failed to amplify; however, the culture medium was amplified successfully. Four embryo DNA samples from culture media failed to amplify. The success rate of DNA amplification from culture media was 87.5% (28/32) (Table 1).

Twenty-seven paired samples (DNA extracted from the culture medium and the whole embryos) were successfully sequenced. There were 18 blastocysts in which the less invasive method (less invasive chromosome screening (NICS) and the whole embryo demonstrated the same results. Using the chromosome status of the whole embryos as the standard, the consistency rate was 66.7% (18/27). There were 9 blastocysts in which the NICS differed from the whole embryo, among which 4 blastocysts showed chromosomal normal and NICS abnormal, 2 samples with gender inconsistency, 2 blastocysts revealing abnormal chromosomal and normal NICS, and 1 sample with inconsistent abnormal chromosomes (Table 2).

MCN results (1) A total of 26 pairs of samples (the mitochondrial DNA extracted from the blastocyst and culture medium) were detected successfully, with a successful detection rate of 100% (26/26). The blastocyst mitochondrial DNA copy number was 86~2495, and the culture medium was 20~809.

(2) Through multiple linear regression analysis, we found that the culture medium MCN had a significantly positive correlation with the blastocyst mitochondrial DNA copy number ($P = 0.001$), female age ($P = 0.012$), and blastocyst scores ($P = 0.014$) but had no obvious correlation with the blastocyst chromosome ($P = 0.138$) (Table 3).

Discussion

Possibility of using DNA extracted from culture medium for embryo genetic analysis

Wu H et al. found that gDNA in culture medium may differ in different embryonic development phases [20]. The successful rates of detection of gDNA among day 4, day 5, and day 6 embryos were 19.7%, 90.2%, and 88.5%, respectively. Gianaroli L et al. found that the rate of successful detection of gDNA based on blastocoel fluid was 76.5%, with a 98.1% consistency rate with embryonic DNA [21]. The gDNA of culture medium and blastocoel fluid is released by apoptotic cells during the embryo self-repair process. However, whether the released free DNA in embryo culture medium was consistent with the embryo DNA varies, as some studies have proposed high concordance, while others have suggested moderate or lower concordance [22–24]. Therefore, this discrepancy has become the focus of interest and concern.

Table 1 Sample data and mitochondrial DNA copy number results

ID	Female age	D2/3	Blastocyst	Blastocyst grade	Blastocyst score	Amplify	Blastocyst MCN	Culture medium MCN
2	34	4C1I	3CC	Poor	16	No	Undetected	Undetected
7	34	5C1II	3CC	Poor	16	Yes	Undetected	Undetected
12	29	11C1I	3CB	Poor	17	No	Undetected	Undetected
17	29	5C1I	3CC	Poor	16	No	Undetected	Undetected
22	26	8C1II	3CC	Poor	16	Yes	2042	40
27	26	8C1I	3CC	Poor	16	Yes	1696	43
32	32	5C1II	4CC	Poor	20	Yes	369	44
37	29	6C1I	3CC	Poor	16	Yes	1017	72
42	30	5C1II	3CC	Poor	16	No	Undetected	Undetected
47	29	12C1I	4CC	Poor	20	Yes	Undetected	Undetected
52	29	12C1I	5CC	Poor	24	Yes	2253	809
57	29	6C1I	3CC	Poor	16	Yes	2246	435
62	32	8C1I	3 BC	Poor	17	Yes	86	32
67	32	8C1II	3CC	Poor	16	Yes	1454	41
72	29	4C1I	4CC	Poor	20	Yes	1850	160
77	28	6C1I	3CC	Poor	16	Yes	2017	42
82	28	5C1III	4CC	Poor	20	Yes	1967	46
87	28	4C1I	3CC	Poor	16	Yes	2495	38
91	26	11C1I	4BA	Good	23	Yes	1438	92
94	29	8C1I	3 BC	Poor	17	Yes	1566	75
96	29	7C1I	3CC	Poor	16	Yes	1671	106
98	29	8C1I	4CC	Poor	20	Yes	1045	189
102	29	6C1I	4CC	Poor	20	Yes	1544	74
105	29	8C1I	4CC	Poor	20	Yes	1633	295
106	29	8C1I	4CC	Poor	20	Yes	1633	295
108	40	8C1I	3CC	Poor	16	Yes	2111	426
110	30	12C1I	5CB	Poor	25	Yes	1121	72
113	30	6C1I	3CC	Poor	16	Yes	1216	22
115	29	11C1I	3CC	Poor	16	Yes	816	118
118	29	8C1I	4CC	Poor	20	Yes	957	32
120	29	8C1I	4CC	Poor	20	Yes	705	20
122	29	8C1I	3CC	Poor	16	Yes	782	37

DNA in the culture medium may not represent the whole embryo, as has been shown in 9 cases of this study. Our research found that there were 18 blastocysts in which the NICS and the whole embryo revealed the same results. Using the chromosome status of the whole embryos as the standard, the consistency rate was 66.7% (18/27). This rate is lower than that reported by Xu et al. in 2016 (85.71% (36/42) [23], which can be explained by a number of possibilities. First, we have different opinions on the definition of the concordance. In embryos with abnormal chromosomes, they thought that aneuploidy–aneuploidy in both samples shows aneuploidy concordance, and we proposed that the whole blastocyst and spent media share the same chromosomal abnormality, representing true consistency. The consistency rate of Xu et al. in 2016 was 71.4% (30/42) based on our standards of the concordance. Then, the embryos we utilized were donated for scientific research. As shown in Table 1, only one of the 32 blastocysts was defined as a high-quality blastocyst, and the rest were all poor-quality blastocysts. Poor-quality embryos easily release more abnormal cell components or apoptotic cells into the blastocoel fluid and culture medium, which may contribute to the lower consistency rate. Maternal contamination, mosaicism, self-repair mechanism during embryo development, and other factors may also explain these results.

Maternal contamination is a common problem among discordant results. The majority of false-negative results have been attributed to maternal contamination. In our study, 2 paired samples (blastocysts chromosomal abnormal and

Table 2 27 samples NICS and whole blastocyst sequencing results

16 normal embryos (NICS and blastocyst consistent)			2 abnormal embryos (NICS and blastocyst consistent)		
ID	Blastocyst	NICS	ID	Blastocyst	NICS
32	46,XY	46,XY	96	45,XY, − 16 (× 1)	45,XY, − 16 (× 1)
37	46,XY	46,XY	108	44,XX, − 20 (× 1), − 21 (× 1)	44,XX, − 20 (× 1), − 21 (× 1)
52	46,XX	46,XX	9 embryos (NICS and blastocyst inconsistent)		
57	46,XY	46,XY	4 false-positive embryos (NICS abnormal, blastocyst normal)		
62	46,XX	46,XX	7	46,XY	47,XY, + 17 (*3)
72	46,XX	46,XX	22	46,XX	45,XX, − 7 (× 1)
77	46,XX	46,XX	87	46,XX	45,XX, − 15 (× 1)
91	46,XX	46,XX	113	46,XY	44,XY, − 4(× 1), − 10 (× 1)
94	46,XX	46,XX	2 false-negative embryos (NICS normal, blastocyst abnormal)		
102	46,XX	46,XX	82	45,X, − X (× 1)	46,XX
105	46,XY	46,XY	110	45,XX, − 7 (× 1)	46,XX
106	46,XX	46,XX	2 gender inconsistent		
115	46,XX	46,XX	67	46,XX	46,XY
118	46,XY	46,XY	98	46,XX	46,XY
120	46,XX	46,XX	1 abnormal embryo inconsistent		
122	46,XY	46,XY	27	45,XY, − 5 (× 1)	45,X, − Y (× 0)

NICS normal) were analyzed (Fig. 2): the 2 NICS were 46,XX, which may be caused by maternal contamination. Prevention is very critical. In our study, we carefully removed and washed the cumulus-corona colliculus complex before ICSI and again after embryo thawing. Washing and replacing the culture medium every 24 h may also decrease the likelihood of maternal contamination. Clinically validating less-invasive or noninvasive PGT requires the development of strategies to parse maternal and embryonic DNA to report accurate aneuploidy calls. Vera-Rodriguez et al. discovered that samples of spent media with percentages of embryonic DNA ranging from 0 to 100% by SNP analysis, which suggested that the embryonic genome, may not be uniformly represented in the spent culture media of all embryos [25]. Recent advances in prenatal sampling have shown that DNA fragments from the fetus differ in size and contain different preferred ends than maternal fragments [26]; thus, future studies are needed to determine whether the difference could be used to discriminate between embryonic and maternal DNA in spent culture medium.

Mosaicism has drawn significant attention in IVF in recent years. Many studies have reported varied mosaicism rates [27–29]. The mosaicism rates of blastocysts compared with cleavage-stage embryos (74% versus 62%) might explain why normal cells tend to survive and abnormal cells tend to be eliminated [30]. The false-positive results of NICS might also be a reflection of mosaicism, which was reported in human embryos [31, 32]. Vera-Rodriguez et al. discovered a majority of samples with imperfect or partial concordance between NICS and blastocyst biopsy originating from mosaic blastocysts. However, FISH analysis of whole blastocysts showed concordance with the blastocyst biopsy results in most cases, suggesting a potential preferential release of certain types of aneuploidy cells [25]. Clinically, if the patient did not have a completely normal embryo preimplantation after PGT, then whether mosaicism embryos can be implanted is

Table 3 Culture medium MCN and influencing factors

	Non-normalized coefficient		Normalized coefficient		
	B	Standard error	A trial version	*t*	Sig.
(Constant)	− 1378.121	404.335		− 3.408	.003
Blastocyst chromosome normal or not	− 112.977	73.261	− .251	− 1.542	.138
Female age	30.456	11.030	.447	2.761	.012
Blastocyst score	27.284	10.149	.418	2.688	.014
Blastocyst MCN	.177	.048	.596	3.724	.001

a. Dependent variable: The culture medium, MCN

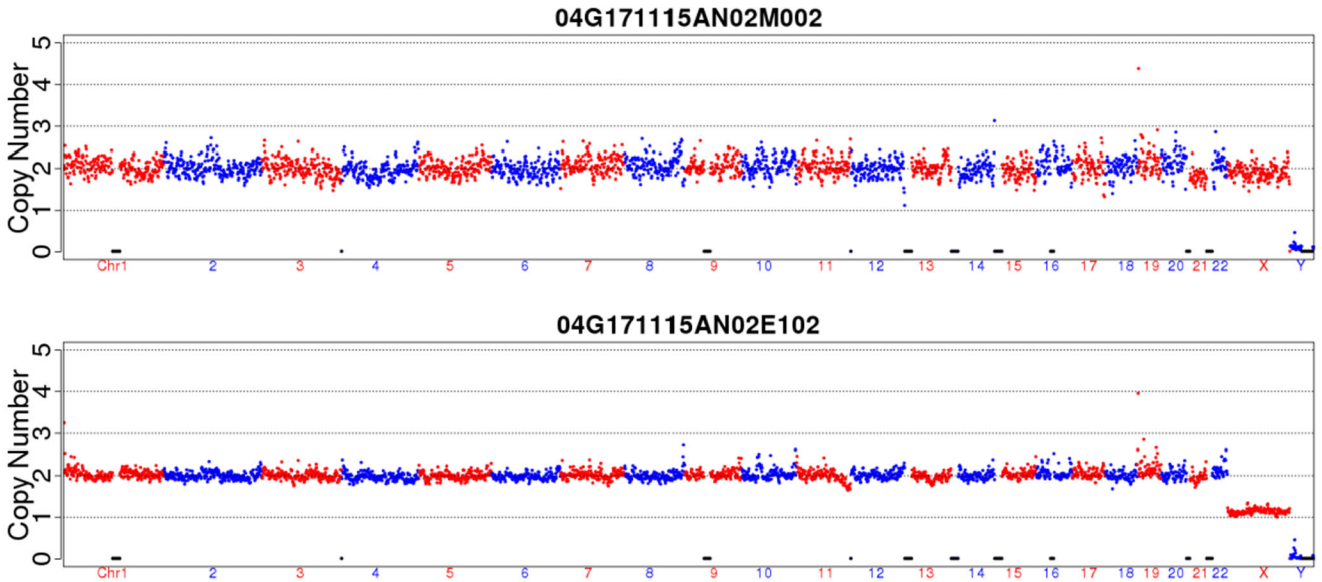

Fig. 2 Sample 82, chromosomal copy number analysis by MALBAC – NGS, blastocyst chromosomal was abnormal (45,X, −X(× 1)) and NICS was normal (46,XX) (false-negative)

controversial. Some studies have been reported; Fragouli et al. in 2017 [33] found significant differences in the miscarriage rate and ongoing pregnancy rate between euploid and mosaicism. Greco et al. [34] performed mosaicism to implant the uterus in 18 patients, and 6 patients obtained clinical pregnancy and delivered a healthy baby, which was certified to have a normal chromosome. Therefore, if the patient strongly requires the embryo to implant with the absence of euploid, then mosaicism would be a choice based on detailed explanation and genetic consultation.

The false-positive result of NICS was also considered to be caused by the self-repair mechanism during embryo development. In our study, 4 paired samples had normal blastocyst chromosomal and abnormal NICS (Fig. 3). Taylor T H et al. [35] suggested that abnormal cells will be released into blastocoel liquid or culture medium during embryo development, which leads to false-positive results. To decrease the impact of apoptotic cells, we replaced the embryo culture medium every 24 h. One study collected from an embryo cultured in monophasic media grown in the same media droplet from day 1 until day 5/6 (blastocyst) may lead to a higher quantity of DNA released into the media, but it also results in increased DNA degradation over the 5–6 days of culturing [36]. Valeriy Kuznyetsov et al. found that 24–48 h of culture gives a good enough yield to achieve consistent DNA amplification while minimizing cell-free DNA degradation [37].

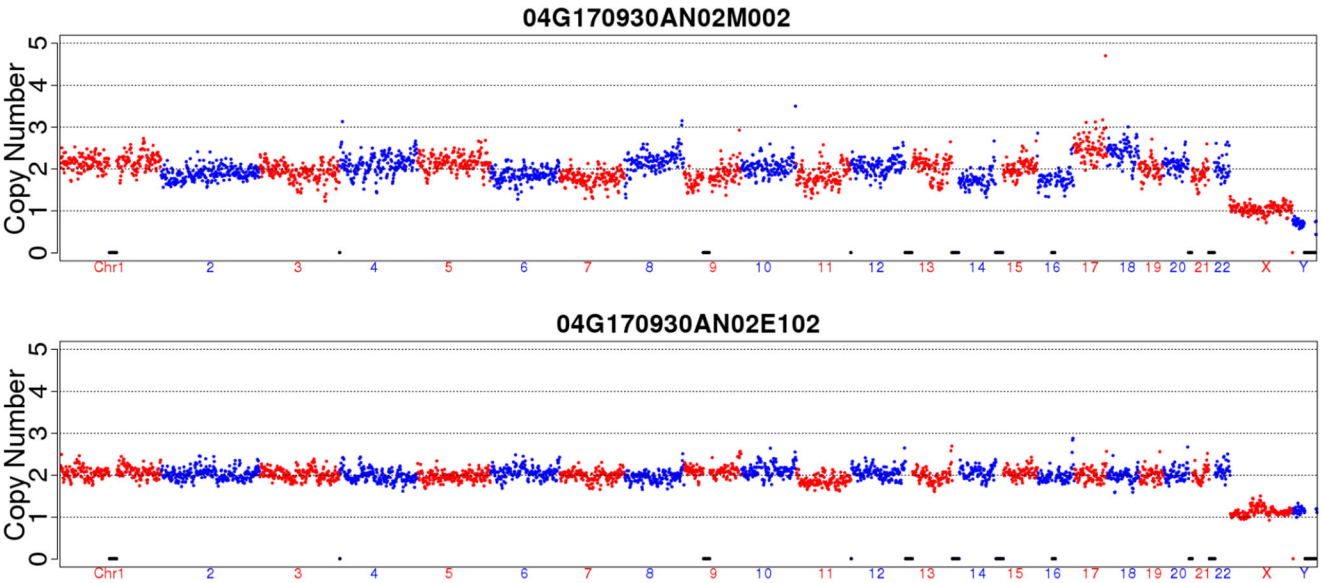

Fig. 3 Sample 7, chromosomal copy number analysis by MALBAC – NGS, blastocyst

Human serum albumin in the embryo culture medium contains human DNA. The content of human DNA in different embryo culture medium batches was slightly different, and a high human DNA content may affect the sequencing result. Our research found that 2 samples had gender inconsistency (Fig. 4), the blastocyst chromosome was 46,XX, and the NICS was 46,XY, which may be due to this reason or the influence of mosaicism. Decreasing the volume of embryo culture medium or using serum free medium may be helpful for this issue.

We also found that the successful rate of amplification of embryo DNA from culture medium was 87.5% (28/32), which is lower than that of blastocyst 96.9% (31/32). In our investigation, the initial 4 embryo culture media that were collected with no laser drilling, among which 3 samples' amplification failed, suggesting that the lower amplification rate may be because the blastocoel liquid did not release into the culture medium, which could not satisfy the sufficient amount of DNA for subsequent detection. Subsequently, we collected samples after systemically laser drilling without causing any other harm to the embryo. The blastocoel fluid DNA can be released into the embryo culture medium, which significantly increases the concentration of embryo DNA. The rate of successful amplification reached 96.4% (27/28). Valeriy Kuznyetsov et al. also reported that extracting cell-free embryonic DNA through combining embryo culture medium and blastocoel fluid, a 100% successful amplification of DNA from these NICS samples can be achieved [37]. We proposed that combining both together as one sample would increase the amount of cell-free DNA obtained to satisfy subsequent detection. Indeed, because of the laser drilling utilized in our study, we thought that the protocol was called less invasive and may be more appropriate than noninvasive. However, in the clinic, before blastocyst vitrification, we release the blastocoel fluid through laser drilling so that to decrease the formation of ice crystals, which may affect the survival of the blastocyst; Mukaida et al. also reported that the artificial shrinkage of blastocoels by microneedle or a laser pulse before vitrification improves the survival rate and clinical outcome of the embryo [38].

Currently, as a less invasive technology, the chromosome consistency rate between the culture medium and the whole blastocyst is not very satisfying. More studies involving the optimization of the sample collection procedure are needed to improve the accuracy of NICS.

chromosomal was normal (46,XY) and NICS was abnormal (47,XY, + 17(*3)) (false-positive)

Mitochondrial DNA and embryo quality

In IVF-ET, how to select an embryo that is most likely to result in a healthy baby remains an important challenge. Morphologic evaluation remains the primary method for embryo selection. However, a high-quality embryo does not equal an embryo with a normal chromosome or high implantation potential. Mitochondria play an important role in maintaining cell metabolism and function and are indispensable for embryo development, a process requiring a large energy supply [39, 40]. Regarding the correlation between embryo mitochondria and female age, embryo quality, embryo implantation potential, or other embryo-associated structures, previous studies have studied; however, there is still no consensus. Moreover, most of the studies focus on embryo mitochondrial DNA rather than embryo culture medium mitochondrial DNA, which requires embryo biopsy and is invasive. In addition, most of the previous studies utilized PCR or array-CGH

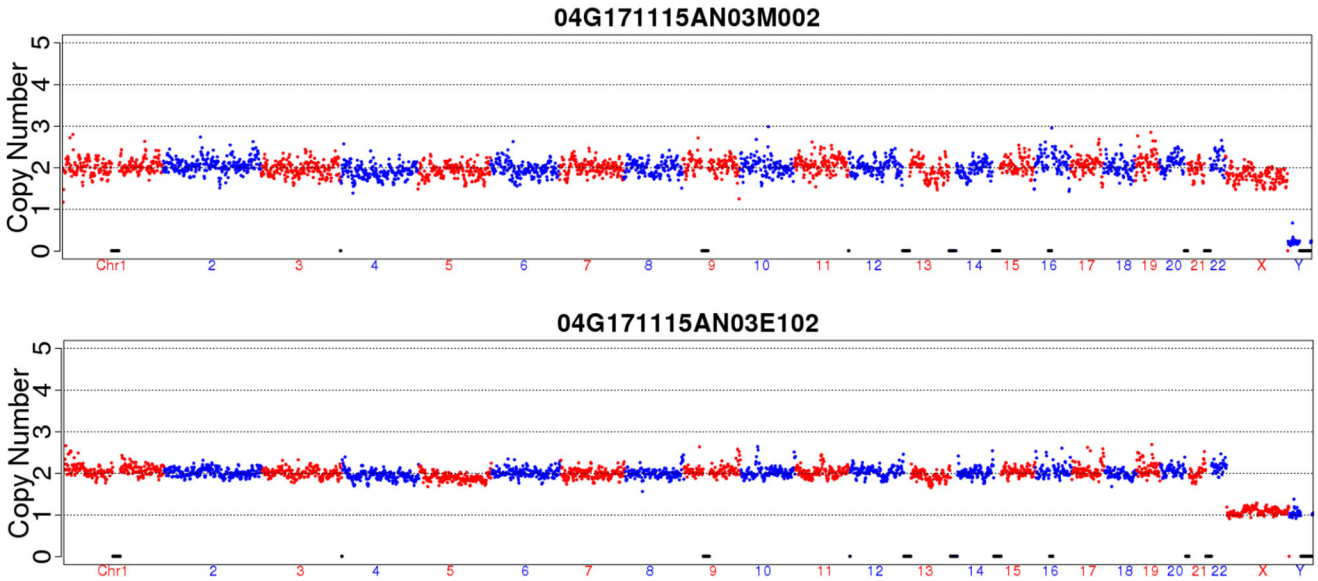

Fig. 4 Sample 67, chromosomal copy number analysis by MALBAC – NGS, blastocyst chromosomal was 46,XX, and the NICS was 46,XY

as a research approach, and MALBAC-NGS is rarely used. Our study extracted mitochondrial DNA by combining the embryo culture medium and blastocoel fluid less invasively and then through MALBAC-NGS to study the correlation between the embryo culture medium MCN and female age, morphological grade, embryonic growing potential, development potential, and embryo chromosome to select an optimal embryo in clinical practice.

Our research showed that the mitochondrial DNA of all 26 blastocysts extracted from the culture medium was 100% successfully detected. Embryo culture medium MCN has a significantly positive correlation with blastocyst mitochondrial DNA copy number ($P = 0.001$), female age ($P = 0.012$), and blastocyst scores ($P = 0.014$) but has no obvious correlation with blastocyst chromosome ($P = 0.138$). Wei Shang et al. [41] found that oocyte MCN increased as female age increased, and embryo MCN had no obvious correlation with embryo morphological score. They thought it may be a metabolic stress response mechanism, as female age increases, cells are gradually aged and need more ATP to meet their needs. As a compensatory mechanism, more mitochondria are produced to meet the needs of the body. Other studies [14, 20] have reported that embryo culture medium MCN increases as the embryo fragment increases, which indicates that embryo culture medium MCN is negatively correlated with the embryo morphology score. The relationship between the embryo morphological score and the embryo culture medium MCN has not reached agreement. This discrepancy may be explained by a number of possibilities. First, although the blastocyst morphological score has a uniform standard, it is still evaluated by the embryologist, which is subjective, and in our study, we used the scoring system as described before, which is different from that of others, which may have shown some differences. Then, some studies use embryos, and some use embryo culture medium. The collection time of the culture medium is also different and lacks a uniform standard. The collection time also needs to be further certified by more studies and uniform standards. Some studies [41] have also stated that embryo MCN has no significant correlation with embryo chromosome. Additionally, cell MCN at the cleavage stage was significantly lower than that at the blastula stage by real-time PCR [42], which suggested that cell MCN was positively correlated with embryo development potential.

Previous studies on mitochondrial DNA, whether on material (day 3 embryo, day 5 embryo, embryo culture medium) or detection method (PCR, array-CGH, MALBAC- NGS), all lack a uniform standard. In our research, there was no relevant research on embryo implantation potential because of the preliminary trials phase. More research and uniform standards are still needed to perfect it in the future.

In our study, the consistency rate of whole blastocyst and NCIS was moderately efficient, and the embryo culture medium MCN showed a significantly positive correlation with blastocyst scores. Although the preliminary results of the examination of less-invasive approach for having an embryo rank were not satisfying, the results also give us a new idea to select an optimal embryo in the future.

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Authors' roles X.B. designed and coordinated the study. H.X., C.Y., L.F., and X.S. collected the clinical cases. J.Z. and H.X. performed and analyzed the data. J.Z. wrote the draft of the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Ethical approval was obtained from the Scientific and Ethical Committee of Tianjin Medical University for the experimental protocol (IRB2017-155-01). A detailed patient consent form was signed for each embryo sample used in this study.

Conflict of interest The authors declare that they have no conflict of interest.

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