Noninvasive Chromosome Screening for Evaluating the Clinical Outcomes of Patients With Recurrent Pregnancy Loss or Repeated Implantation Failure

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Abstract

Background: This retrospective cohort study determines whether noninvasive chromosome screening (NICS) for aneuploidy can improve the clinical outcomes of patients with recurrent pregnancy loss (RPL) or repeated implantation failure (RIF) in assisted reproductive technology.

Methods: A total of 273 women with a history of RPL or RIF between 2018 and 2021 were included in this study. We collected data of all oocyte retrieval cycles and single blastocyst resuscitation transfer cycles.

Results: For the RPL patients, NICS reduced the miscarriages rate per frozen embryo transfer (FET), improved the ongoing pregnancies rate and live birth rate: 17.9% vs 42.6%, adjusted OR 0.39, 95% CI 0.16–0.95; 40.7% vs 25.0%, adjusted OR 2.00, 95% CI 1.04–3.82; 38.9% vs 20.6%, adjusted OR 2.53, 95% CI 1.28–5.02, respectively. For the RIF patients, the pregnancy rates per FET in the NICS group were significantly higher than in the non-NICS group (46.9% vs. 28.7%, adjusted OR 2.82, 95% CI 1.20–6.66).

Conclusions: This study demonstrated that selection of euploid embryos through NICS can reduce the miscarriage rate of patients with RPL and improve the clinical pregnancy rate of patients with RIF. Our data suggested that NICS could be used as a diagnostic test in clinical practice.

Background

In vitro fertilization-embryo transfer (IVF-ET) is an effective method to treat infertility widely performed worldwide [1]. Nevertheless, chromosomal abnormalities often exist in early human embryos, leading to embryo implantation failure and pregnancy loss during IVF treatment [2, 3], especially in patients with a history of recurrent pregnancy loss (RPL) and repeated implantation failure (RIF) [4–6]. RPL is defined as the loss of ≥ 2 pregnancies [7], whereas RIF as the failure of ≥ 3 implantations. Trilochan et al. [8] reported that chromosome abnormalities were detected in 3,975 of 7,396 (53.7%) cases of miscarriage tissues. Besides, Kort et al. [6] analyzed 10,711 cases of blastocysts retrospectively and found that the incidence of embryo aneuploidy was significantly higher in patients with RIF than in the control group.

Currently, the most commonly used embryo selection method is morphological evaluation. However, the chromosomes of embryos cannot be identified via morphology [2, 3]. Among blastocysts with good morphology, only 42% of embryos have completely normal chromosomes. Among them, only 30% of ICM graded A Embryos, the chromosomes are normal[9]. Therefore, in some special populations (such as women with recurrent miscarriage and repeated implantation failure patients), embryos are usually evaluated through the pre-implantation genetic testing for aneuploidy (PGT-A) [10–12]. A retrospective study showed that the live birth and clinical pregnancy rate were improved through the PGT-A in women with RPL[13]. The authors included 1,389 blastocysts derived from PGT-A cycles in IVF patients with advanced maternal age, those with repeated implantation failure, those with recurrent miscarriage, and oocyte donors. Compared the control group without PGT-A, the live birth rates of these four groups are both improved[14]. However, specific equipment and extensive expertise are required for the biopsy procedure, restraining the utility of PGT-A in assisted reproduction. Moreover, the embryos are screened

for chromosomal ploidy before transferring to the uterus [15, 16], and the long-term impact of biopsy is an important concern for undetermined health risks, such as adrenal development and response to cold stress [17], epigenetic reprogramming [18], and neurological conditions [19]. Notably, implantation rates are lower with increased biopsied trophectoderm (TE) cell numbers and sizes than with appropriate cell numbers and sizes [20, 21].

Stigliani et al. first observed genomic DNA contents in embryo culture medium, [22]. Since then, multiple studies have been published using culture medium or blastocoelic fluid for analyzing chromosomal ploidy [23–30]. Xu et al. first reported a noninvasive chromosome screening (NICS) assay based on a Multiple Annealing and Looping-Based Amplification Cycle-Next Generation Sequencing (NGS) strategy using spent blastocyst culture medium, which was validated in 42 IVF and resulted in five live births among seven women [26]. Fang et al. obtained an ongoing pregnancy rate of 58% and reported 27 normal live births in a pilot clinical study using NICS [28]. Nevertheless, the clinical application of NICS has been evaluated only in small-scale trials.

In this study, we designed a retrospective cohort study including 273 patients with RPL or RIF to confirm the clinical value of NICS. To the best of our knowledge, this is the first large-scale validation study of NICS in the RPL or RIF patients.

Methods

Study Participants and Data Collection

We initially included 303 women with a history of RPL (≥ 2 events) or RIF (≥ 3 events) from July 2019 to May 2021, according to the records of the Reproductive Centre at the Second Affiliated Hospital of Wenzhou Medical University. However, ten women that abandoned embryo transfer and 20 women with chromosomal rearrangements, abnormal uterine cavity morphology, endometrial lesions, endometrial injury, intrauterine effusion, or untreated hydrosalpinx were excluded from the study. The final cohort comprised 273 women, from which we collected data of all oocyte retrieval cycles and single blastocyst resuscitation transfer cycles.

The variables analyzed in the study were: age at retrieval, the history of pregnancies, live births, and miscarriages, body mass index (BMI), hormone levels, the number of oocyte retrieval cycles, oocytes, cleavages, and D5 or D6 blastocysts, and blastocyst morphology (expansion, inner cell mass, and trophectoderm). Patients with RPL or RIF received the noninvasive chromosome screening for aneuploidy were included in the NICS group, while those underwent conventional morphology embryo transfer during the same period were included in the non-NICS group. The euploid embryos were transferred in the NICS group. The study design is illustrated in Figure 1.

Oocyte Retrieval and Embryo Culture

This study was performed at the first IVF/Intracytoplasmic Sperm Injection (ICSI) cycles after injection of 3.75 mg triptorelin for prolonged pituitary downregulation in the follicular phase of the menstrual cycle.

Ovarian stimulation with exogenous gonadotropins promoted the growth of follicles. When two or more leading follicles reached 18 mm, ovulation was induced with 10,000 IU human chorionic gonadotropin (hCG). Oocyte retrieval was performed at 35h post-hCG administration. Cumulus-enclosed oocytes were separated from the follicular fluid, placed in medium, and incubated at 37°C in a Cumulus-encl₂ atmosphere for 2h. Routine IVF or ICSI was performed based on sperm quality. The embryos were placed in droplets of G-1 PLUS medium (Vitrolife, Göteborg Sweden) in AMP-30D incubators (Bioz, Los Altos, CA, USA) in a 6.0% CO₂ and 5% O₂ balance N₂ atmosphere at 37°C.

Blastocyst Culture and Transfer

D3 embryos were placed in 30-μL droplets of G-2 PLUS medium (Vitrolife) supplemented with washed and pre-gassed mineral oil (Sage, Atlanta, GA, USA) and cultured to the blastocyst stage in AMP-30D incubators (Bioz) in a 6.0% CO₂ and 5% O₂ balance N₂ atmosphere at 37°C. At 2d of culture, the development and quality of blastocysts were evaluated according to the blastocyst scoring system, including expansion (1-6), inner cell mass (A, B, C), and trophectoderm (A, B, C). Blastocysts were categorized into good, fair, or poor quality based on the simplified SART embryo scoring system[29]. The grade is good when the ICM/TE is AA, AB or BA; the grade is fair when the ICM/TE is BB or AC; the grade is poor when the ICM/TE is CA, BC or CB. All blastocysts were previously frozen and thawed by vitrification using the Cryotop Safety kit (Kitazato, Fuji-shi, Shizuoka, Japan), according to the manufacturer's instructions. A single blastocyst was selected for transfer to each patient based on morphology (nonintervention group) or NICS results (intervention group).

Sample Collection for NICS Assay

To prevent medium cross-contamination, different Pasteur pipettes were used for each embryo. Approximately 30 μL of blastocyst medium from each embryo was transferred into RNase-DNase-free PCR tubes containing 5 μL of cell lysis buffer (Yikon Genomics, Suzhou, China). We also collected the same amount of blastocyst culture medium to be used as a negative control. All collected samples were flash-frozen in liquid nitrogen and stored at -80°C until subjected to the NICS assay.

Whole-genome amplification (WGA) and NGS Data Analysis

Whole-genome amplification was performed using culture media, followed by library preparation using ChromInst (Yikon Genomics; EK100100724 NICS Inst Library Preparation Kit). NGS was performed on an Illumina MiSeq platform that yielded approximately 2 million sequence reads from each sample. The high-quality reads were extracted and mapped to the human hg19 genome. After removing duplication reads, the read numbers were counted along the whole genome with a bin size of 1 Mb and normalized by the GC content as well as a reference dataset to represent the relative copy number. The copy number of each bin was then segmented by circular binary segmentation (CBS) algorithms to merge bins with similar trends and calculate the final copy numbers.

Assessment of Outcome Variables

Our primary outcomes included the clinical pregnancy rates, miscarriage rates, and ongoing pregnancy rates. The clinical pregnancy rate was defined as the number of cycles with gestational sacs visualized on transvaginal ultrasonography divided by the total number of transplant cycles. The miscarriage rate was calculated as the number of pregnancy failures after a gestational sac was documented by transvaginal ultrasonography divided by the total number of clinical pregnancies. The ongoing pregnancy rate was the number of cycles of any pregnancy that went beyond 12 weeks of gestation divided by the total number of transplant cycles. Ectopic pregnancy was considered a clinical pregnancy and not a miscarriage.

Statistical Analysis

Descriptive data for continuous variables are presented as means $\pm$ standard deviation, whereas categorical data as numbers and percentages. The Student's *t*-test or Mann-Whitney test were used for identifying significant differences in parametric and non-parametric data, respectively. The chi-square test or Fisher exact test were used for categorical variables in each group. Multiple logistic regression analysis was conducted to compare the outcomes of NICS and non-NICS groups after controlling the covariables at $p < 0.10$ and covariables considered clinically influential.

All analyses were carried out using the statistical software BMI SPSS, Version 25. A *P* value < 0.05 was considered to denote statistical significance.

Results

Variable Analysis

Of the 303 women initially included in the study, 10 that abandoned embryo transfer and 20 that had no transferrable embryos were excluded from the final cohort. Finally, blastocysts were transferred to 273 women during 407 transfer cycles (Fig. 1).

Of the 173 patients with a history of RPL, were enrolled in the study, of which 84 were for the NICS group and 89 were included as the non-NICS group. In total, 113 FET cycles were performed for the patients in the NICS group, and 136 high-quality blastocysts were obtained from 89 patients (Table 1). Of the 100 patients with a history of RIF, 44 were for the NICS group and 56 for the non-NICS group. In total, 64 FET cycles were performed for the patients in the NICS group, and 94 high-quality blastocysts were obtained from the non-NICS group (Table 2).

Table 1. Baseline demographics of patients with recurrent pregnancy loss (RPL) after embryo selection by non-invasive chromosome screening (NICS) versus blastocyst morphology (Non-NICS).

Variable	NICS	Non-NICS	p-value
Number of patients	84	89	
Female age (y)	32.4 ± 3.8	33.4 ± 4.7	0.109
Female body mass index (kg m ⁻²)	22.1 ± 2.8	22.4 ± 3.1	0.371
Male age (y)	34.7 ± 4.1	35.2 ± 4.7	0.381
Male body mass index (kg m ⁻²)	24.0 ± 3.0	24.5 ± 3.2	0.293
Infertility duration (y)	1.4 ± 0.7	2.1 ± 2.2	0.001
Number of prior miscarriages	2.9 ± 1.3	2.5 ± 1.1	0.023
Number of previous embryo transfers	0.63 ± 1.0	2.2 ± 1.8	< 0.001
Number of prior live births	5 (6.0%)	1 (1.1%)	0.108
Prolactin (ng m ⁻¹)	20.0 ± 44.9	21.4 ± 45.9	0.831
Testosterone	0.5 ± 0.3	0.9 ± 4.3	0.415
Progesterone	0.7 ± 0.5	0.6 ± 0.6	0.357
Follicle-stimulating hormone	6.8 ± 2.1	7.5 ± 4.2	0.158
Luteinizing hormone	4.8 ± 3.7	5.8 ± 9.6	0.349
Estradiol	62.7 ± 58.1	66.7 ± 71.88	0.673
Number of COH cycles	87	105	
Number of COH cycles per patient	1.04	1.18	0.007
Gonadotropin days ($\bar{x} \pm SD$)	10.2 ± 2.1	10.5 ± 2.9	0.416
Gonadotropin dosage (×75 IU, $\bar{x} \pm SD$)	2,065.3 ± 768.1	1,956.3 ± 856.9	0.359
Number of retrieval oocytes ($\bar{x} \pm SD$)	12.8 ± 7.0	12.8 ± 7.3	0.977
Number of cleaving zygotes ($\bar{x} \pm SD$)	8.9 ± 4.7	9.5 ± 6.0	0.441
Number of blastocysts	4.7 ± 3.3	4.4 ± 3.1	0.513
Number of FET cycles	113	136	
Embryos at d 5	80 (70.8%)	114 (83.8%)	0.014
Embryos at d 6	33 (29.2%)	22 (16.2%)	
Blastocyst quality, Good (AA/BA/AB)	36 (31.9%)	45 (33.1%)	0.837
Blastocyst quality, Fair (BB/AC)	35 (31.0%)	58 (42.6%)	0.058
Blastocyst quality, Poor (CA/BC/CB)	42 (37.2%)	33 (24.3%)	0.027

COH, controlled ovarian hyperstimulation; SD, standard deviation; FET, frozen-thawed embryo transfer.

Table 2. Baseline demographics of patients with recurrent implantation failure (RIF) after embryo selection by non-invasive chromosome screening (NICS) versus blastocyst morphology (Non-NICS).

Variable	NICS	Non-NICS	p-value
Number of patients	44	56	
Female age (y)	32.1 ± 4.7	33.3 ± 4.3	0.143
Female body mass index (kg m ⁻²)	21.0 ± 2.5	21.7 ± 2.4	0.101
Male age (y)	34.2 ± 5.2	34.9 ± 4.9	0.407
Male body mass index (kg m ⁻²)	24.2 ± 3.4	23.8 ± 3.4	0.498
Infertility duration (y)	3.5 ± 2.9	5.2 ± 3.0	0.004
Number of prior miscarriages	1.9 ± 1.2	0.8 ± 1.1	< 0.001
Number of previous embryo transfers	4.4 ± 1.3	4.2 ± 1.3	0.379
Number of prior live births	1/44 (2.3%)	0	
Prolactin (ng m ⁻¹)	26.2 ± 76.4	15.1 ± 9.9	0.231
Testosterone	0.5 ± 0.2	0.5 ± 0.3	0.973
Progesterone	0.8 ± 1.0	0.6 ± 0.3	0.287
Follicle-stimulating hormone	7.8 ± 2.4	7.5 ± 3.5	0.608
Luteinizing hormone	5.2 ± 2.2	4.5 ± 2.7	0.108
Estradiol	50.4 ± 25.8	51.2 ± 41.70	0.913
Number of COH cycles	44	70	
Number of COH cycles per patient	1	1.3	< 0.001
Gonadotropin days ($\bar{x} \pm SD$)	10.0 ± 2.4	11.0 ± 2.4	0.03
Gonadotropin dosage (×75 IU, $\bar{x} \pm SD$)	1,838.4 ± 748.3	2,192.1 ± 872.7	0.028
Number of retrieval oocytes ($\bar{x} \pm SD$)	13.9 ± 7.5	13.0 ± 7.5	0.551
Number of cleaving zygotes ($\bar{x} \pm SD$)	10.8 ± 6.1	9.7 ± 5.4	0.319
Number of blastocysts ($\bar{x} \pm SD$)	5.3 ± 2.8	4.2 ± 3.2	0.059
Number of FET cycles	64	94	
Embryos at d 5	48 (75.0%)	64 (68.1%)	0.24
Embryos at d 6	16 (25.0%)	30 (31.9%)	
Blastocyst quality, Good (AA/BA/AB)	11 (17.2%)	22 (23.4%)	0.345
Blastocyst quality, Fair (BB/AC)	31 (48.4%)	34 (36.2%)	0.124
Blastocyst quality, Poor (CA/BC/CB)	22 (34.4%)	38 (40.4%)	0.442

Data variables analyzed in this study were age at retrieval, the history of pregnancies, live births, and miscarriages, BMI, hormone levels, the number of oocyte retrieval cycles, oocytes, cleavages, and D5 or D6 blastocysts, and blastocyst morphology (expansion, inner cell mass, and trophectoderm) were comparable by Student’s t test or Mann-Whitney *U* test, each as appropriate (Tables 1 and 2).

Our analysis showed no significant differences in age, BMI and hormone levels between the NICS and non-NICS groups in RPL or RIF patients. The number of previous miscarriages in the NICS group of patients with RPL or RIF was significantly higher than the non-NICS group. Besides, infertility duration showed statistical significance ($p = 0.001$, $p = 0.004$) in the NICS group and non-NICS group no matter which feature the patients are in. The number of miscarriages and infertility duration had impact on clinical outcomes. Thus, they were included in the multiple logistic regression analysis.

Blastocyst Morphological Assessments and NICS results

The blastocyst transplantation was based on morphology and NICS results. Blastocysts were evaluated based on their development and quality using the Gardner scores system and divided into three categories: good (AA/BA/AB), fair (BB/AC), and poor (CA/BC/CB) [31]. As shown in Tables 1 and 2, no significant differences were observed between NICS group and control group of patients with RIF. Of the patients with RPL the non-NICS group was lower than NICS group (37.2% vs. 24.3%) in the poor category, however, there was no significant difference between the other two categories.

In the NICS assay, we sequenced approximately 2 million reads on each culture medium sample. The read numbers were counted along the 24 chromosomes with a bin size of 1 Mb and normalized by the mean of the corresponding bin in all samples. Examples of the results of NICS were demonstrated in Additional File 1.

Comparison of clinical outcomes between NICS and non-NICS Groups for Patients with RPL or RIF

For the RPL patients, the miscarriage rate per FET was significantly lower in the NICS group than in the non-NICS group (17.9% vs. 42.6%, adjusted OR 0.39, 95% CI 0.16–0.95), whereas the ongoing pregnancy rate (40.7% vs. 25.0%, adjusted OR 2.0, 95% CI 1.04–3.82) and live birth rate (38.9% vs. 20.6%, adjusted OR 2.53, 95% CI 1.28–5.02) were significantly higher in the NICS group compared to the non-NICS group (Fig. 2; Table 3). Nevertheless, no differences were identified in pregnancy rates per patient between the NICS and non-NICS groups (49.6% vs. 44.9%, adjusted OR 1.13, 95% CI 0.61–2.07; Table 3).

Table 3. Comparison of clinical outcomes patients with recurrent pregnancy loss (RPL).

	NICS	Non-NICS	Adjusted odds ratio (95% CI), p-value
Number of transferred cycles	113	136	
Clinical pregnancies rate (%)	56/113 (49.6%)	61/136 (44.9%)	1.13 (0.61–2.07), 0.697
Miscarriages rate (%)	10/56 (17.9%)	26/61 (42.6%)	0.39(0.16–0.95), 0.038
Ectopic pregnancies	0	1/61 (1.6%)	
Ongoing pregnancies rate (%)	46/113 (40.7%)	34/136 (25.0%)	2.0 (1.04–3.82), 0.037
Live birth rate (%)	44/113 (38.9%)	28/136 (20.6%)	2.53 (1.28–5.02), 0.008
NICS group, embryo selection by non-invasive chromosome screening; control group, blastocyst morphology.			
*Adjusted for female age, infertility duration, number of prior miscarriages, number of previous embryo transfers, number of COH cycles, embryo morphology and Day 5/6 blastocysts			

For the RIF patients, the pregnancy rates per FET were significantly higher in the NICS group than in the non-NICS group (46.9% vs. 28.7%, adjusted OR 2.82 95% CI 1.20–6.66. Fig. 3; Table 4), whereas the live birth rate and ongoing pregnancy rate per FET and per patient were higher in the NICS group than in the non-NICS group, but not significantly. Nevertheless, no differences were identified in the miscarriage rate per clinical pregnancy between the NICS and non-NICS groups (23.3% vs. 25.9%, adjusted OR 1.35, 95% CI 0.34–5.42; Table 4).

Table 4. Comparison of clinical outcomes patients with recurrent implantation failure (RIF).

	NICS	Non-NICS	Adjusted odds ratio (95% CI), p-value
Number of transferred cycles	64	94	
Clinical pregnancies rate (%)	30/64 (46.9%)	27/94 (28.7%)	2.82 (1.20–6.66), 0.018
Miscarriages rate (%)	7/30 (23.3%)	7/27 (25.9%)	1.35 (0.34–5.42), 0.671
Ectopic pregnancies	3/30(10%)	0	
Ongoing pregnancies rate (%)	20/64 (31.3%)	20/94 (21.3%)	2.19 (0.88–5.47), 0.094
Live birth rate (%)	19/64 (29.7%)	20/94 (21.3%)	1.96(0.78–4.92), 0.154
NICS group, embryo selection by non-invasive chromosome screening; control group, blastocyst morphology.			
*Adjusted for female age, infertility duration, number of prior miscarriages, number of COH cycles, gonadotropin days, gonadotropin dosage, number of blastocysts embryo morphology and Day 5/6 blastocysts			

Discussion

To our knowledge, this is the first large-scale study to confirm the effectiveness of NICS as a diagnostic test for the outcomes of IVF in women with a history of RPL or RIF. We demonstrated that the miscarriage rate was significantly lower in women with RPL ($n = 173$), whereas the pregnancy rate was significantly higher in the NICS group than in the non-NICS group of women with RIF ($n = 100$). To confirm that the improvement of clinical outcomes in the NICS group was related to the genetic testing alone irrespective of the other factors, we included the variables at $p < 0.10$ and related to the clinical outcome in the adjusted models. Our results strongly supported that the high clinical value of NICS in women with RPL prior to embryo transfer since it significantly increased the live birth rate per transfer cycle through the multiple logistic regression analysis (38.9% vs. 20.6%, adjusted OR 2.46, 95% CI 1.41–4.35). The pregnancy rates per FET in the NICS group were significantly higher than in the non-NICS control group (46.9% vs. 28.7%, adjusted OR 2.82, 95% CI 1.20–6.66) for the PIF patients. Similarly, Fang et al. obtained an ongoing pregnancy rate of 58% and reported 27 normal live births in patients with RPL or RIF after transferring 50 embryos identified as euploid by NICS [28].

In the present study, women with RPL had a history of ≥ 2 miscarriages, spontaneous abortion and/or biochemical pregnancy[30, 31]. The definition of RPL differs among organizations. For instance, the Royal College of Obstetricians and Gynecologists and the European Society of Human Reproduction and Embryology support that a miscarriage includes all pregnancy losses up to 24 weeks of gestation, whereas the American Society for Reproductive Medicine does not provide a time limit and excludes biochemical pregnancy[32]. Thus, there is no consensus on whether biochemical pregnancy should be

considered RPL. It is known that the rate of chromosome abnormality decreases with the developmental stage: 70–80% in clinical miscarriages[33], 4% in stillbirths, and 0.3% in newborn babies [34]. The selection of euploid embryos can reduce the rate of miscarriages, which are probably caused by chromosome abnormalities[35].

Women with RIF had a history of ≥ 3 failed implantations of 4–6 high-score blastomeres or > 3 high-score blastocysts. A retrospective analysis of 10,711 blastocysts showed that the incidence of blastocyst aneuploidy in RIF patients is significantly higher than that in the control group, and embryo aneuploidy is a primary reason for implantation failure [6]. A clinical study suggested that transplanting euploid embryos may help patients with RIF to achieve pregnancy[36]. A multi-center, prospective, pilot study showed that screening for embryo ploidy improved the live birth rate per embryo transfer procedure in both RPL (52.4% vs 21.6%) and RIF group (62.5% vs 31.7%)[37].

Chromosome aneuploidy is common in embryos following IVF, even in younger women, and is a major failure factor. Although morphology is clearly correlated with euploidy, it can only increase euploidy by a few percentage points at most when used to select replacement embryos. Embryos with good morphology may have chromosomal abnormalities [9]. Routine embryo screening for aneuploidy requires intensive biopsy procedures, which are costly and time-consuming. The accuracy of NICS has been verified in numerous studies, and a 58% clinical pregnancy success rate has been reported for patients with RIF and RPL [28]. Recently, the utility of niPGT-A is challenged in the article by Hanson et al. [38]. Here, we also found that NICS allowed the selection of euploid embryos, increasing the live birth rates in women with RPL and improve the clinical pregnancy rates in women with RIF based on many patients, which is meaningful to both clinicians and basic scientists on NICS assay. Our center is currently participating in a multi-unit clinical trial for women ≥ 35 years of age to further validate the clinical value of NICS since a more comprehensive application requires advanced technology such as modified whole-genome amplification and sequencing protocols as well as novel diagnostic algorithms. The validation of NICS in different populations and the accumulation of detectable sample sizes may help markedly reduce the detection cost per embryo.

There are some limitations in our study: 1) as a retrospective study, patients in the NICS groups had different clinical prognoses than those in the non-NICS groups, introducing bias in the study results, and 2) the loss and cycle cancellation caused by euploid selection after NICS was not calculated since only women with a good prognosis reach the blastocyst stage and have a euploid embryo to transfer.

Conclusions

Our large-scale retrospective study demonstrated noninvasive chromosome screening for aneuploidy improved the clinical outcomes for patients with recurrent pregnancy loss or repeated implantation failure. NICS could be used as a diagnostic test in clinical practice.

List Of Abbreviations

NICS noninvasive chromosome screening

RPL recurrent pregnancy loss

RIF repeated implantation failure

FET frozen embryo transfer

IVF-ET *in vitro* fertilization-embryo transfer

TE trophoctoderm

BMI body mass index

hCG human chorionic gonadotropin

WGA Whole-genome amplification

CBS circular binary segmentation

Declarations

Declaration of Interests

Authors declare no conflicts of interests.

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Ethics approval and consent to participate

This study was approved by the Institutional Review Board (IRB) of the Second Affiliated Hospital of Wenzhou Medical University (number: 2021-K-13-01). Informed consents were obtained in accordance with the institutional guidelines prior to embryo analysis.

Consent for publication

All authors read and approved the final manuscript.

Availability of data and materials

The original contributions presented in the study are included in the article/Supplementary Material.

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Figures

Figure 1

Inclusion and classification of patient selection in this study. NICS, non-invasive chromosome screening; RPL, recurrent pregnancy loss; RIF, repeated implantation failure; Control, blastocyst morphology (non-NICS).

Figure 2

Pregnancy outcomes of patients with recurrent pregnancy loss (RPR) after embryo selection by non-invasive chromosome screening (NICS) versus blastocyst morphology (control). MR, miscarriages rate; OPR, ongoing pregnancy rate; LBR, live birth rate. * $p < 0.05$, ** $p < 0.01$.

Figure 3

Clinical pregnancy rate of patients repeated implantation failure (RIF) after embryo selection by non-invasive chromosome screening (NICS) versus blastocyst morphology (control). * $p < 0.05$.

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