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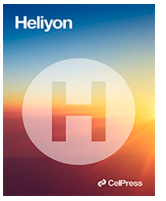

Research article

Performance of preimplantation genetic testing for aneuploidy for patients with unexplained recurrent pregnancy loss and repeated implantation failure

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ABSTRACT

Objective: The primary objective was to investigate whether the utilization of next-generation sequencing (NGS) for preimplantation genetic testing for aneuploidy (PGT-A) could enhance the reproductive outcomes in patients with unexplained recurrent pregnancy loss (uRPL) or unexplained repeated implantation failure (uRIF) undergoing intracytoplasmic sperm injection (ICSI) cycles.

Materials and methods: We studied the reproductive outcomes of uRPL or uRIF sufferers in Chengdu women and children's central hospital from July 2020 to Jan 2024 retrospectively. These patients were categorized into two groups based on whether they underwent PGT-A or not. As the patients in the PGT-A group all had ICSI and frozen-thawed embryo transfer (FET), only patients who underwent ICSI and FET were included in the non-PGT-A group for comparison. Demographic characteristics and reproductive outcomes were compared in uRPL or uRIF sufferers.

Results: For uRPL group, a significant increased ongoing pregnancy rate (63.6 % *vs* 26.1 %, $p = 0.002$) and reduced pregnancy loss rate (18.4 % *vs* 73.3 %, $p < 0.001$) were found in the PGT-A group in comparison with those in the non-PGT-A group. For uRIF group, no significant difference was noted in the HCG-positive rate, ongoing pregnancy rate, or pregnancy loss rate between the two groups. It is noteworthy that the maternal age in the PGT-A group was significantly higher than that in the non-PGT-A group ($p = 0.048$).

Conclusions: NGS-based PGT-A effectively optimized the reproductive outcomes in uRPL sufferers. Although its benefits in uRIF appeared to be limited, there is a potential advantage for those with advanced maternal age. Considering the small sample size, further randomized controlled trials are warranted to validate these findings.

1. Introduction

In the realm of assisted reproductive technology (ART), recurrent pregnancy loss (RPL) and recurrent implantation failure (RIF) are commonly observed in patients who received in vitro fertilization (IVF) treatment [1]. RPL, defined as the occurrence of two or more

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pregnancy losses before 20–24 gestational weeks [2], affecting up to 1–2% of all couples [3]. Meanwhile, RIF, characterized by three or more unsuccessful embryo transfers with good-quality embryos [4], affecting approximately 10 % of couples seeking IVF treatment [5]. RPL and RIF both brought great emotional and psychological burden for the suffered couples [6], which linked to increased risk of infertility and pregnancy loss [7]. Several factors, including maternal age, chromosomal aberrations, infections, endocrine disorders, uterine abnormalities, and autoimmune diseases, have been implicated in the pathogenesis of RPL and RIF. However, some cases remained unexplained, termed unexplained RPL (uRPL) or unexplained RIF (uRIF), representing a significant challenge in assisted reproductive technology (ART) [8,9].

Embryo aneuploidy is a primary contributor to ART failures [10]. It was reported that abnormal karyotypes are detected in at least 55 % of conceptions from RPL sufferers [11], while embryo aneuploidy accounts for 30–50 % of RIF cases [12]. Consequently, euploid embryo transfer has been proposed as a potential strategy to optimize reproductive outcomes in patients with uRPL or uRIF. Fortunately, euploid embryo could be selected by preimplantation genetic testing for aneuploidy (PGT-A). Currently, next-generation sequencing (NGS)-based PGT-A is the latest and widely utilized technique globally [13]. In previous technique, NGS-based PGT-A was reported to dramatically improve the IVF reproductive outcomes [14].

However, the impact of NGS-based PGT-A for patients with uRPL or uRIF remains controversial, necessitating further exploration. In this study, we made a comparative analysis of the reproductive outcomes of uRPL or uRIF sufferers in intracytoplasmic sperm injection (ICSI) cycles between those underwent NGS-based PGT-A and those who did not. The purpose of the study was to furnish some evidence-based insights into the applicability of NGS-based PGT-A.

2. Materials and methods

2.1. Patient selection

A retrospective analysis was conducted between July 2020 and Jan 2024 by using the data of couples with uRPL or uRIF in Chengdu's women and children's central hospital. These couples were divided into PGT-A group and non-PGT-A group. All patients in the PGT-A group had undergone ICSI and EET in frozen-thawed embryo transfer (FET) cycles. To avoid any bias, the control group consisted of similar patients who did not have PGT-A. In our study, uRPL sufferers referred to those who had $\geq$2 pregnancy losses or miscarriages aged from 20 to 44 years old. And uRIF sufferers were those who had failed embryo transfers with good-quality for more than three times aged from 20 to 44 years old. Patients who had PGT for chromosomal structural rearrangement or monogenic inherited disease, infections, endocrine disorders, uterine abnormalities, or autoimmune diseases were excluded. All pregnant patients were followed up to 12 gestational weeks to ascertain the occurrence or absence of pregnancy loss. The study was approved by the Ethics Committee of Chengdu Women and Children's Central Hospital (20240119).

2.2. Blastocyst culture and biopsy

MII oocytes were fertilized through ICSI, and zygotes with two pronuclei were cultured

In separate micro-drops individually. All biopsy procedures were conducted on blastocyst (day 5 or 6) on a heated stage of a Nikon Diaphot 300 inverted microscope. After opening the zona pellucida by a laser system (MTG company, Germany), 5–10 trophectoderm cells were retrieved and stored at low temperatures (−20 °C) in RNAsee DNAse-free PCR tubes before analysed by NGS technology (Next Seq550, Illumina, USA). After biopsy, blastocysts were cryopreserved through vitrification.

Patients who possessed at least one euploid embryo eligible for transfer were included in the PGT-A group. In FET cycles, a single euploid blastocyst was transferred in the PGT-A group, while in the non-PGT-A group, one or two cleaved embryos or blastocysts were transferred. Serum human chorionic gonadotropin (HCG) levels were assessed on day 14 after embryo transfer, and an ultrasound scan was conducted at 6–7 gestational weeks. Clinical pregnancy was confirmed by the observation of an intrauterine gestational sac on the ultrasound scan. Ongoing pregnancy was defined as a viable intrauterine pregnancy persisting for 12 weeks following embryo transfer. Pregnancy loss was defined as any loss that occurred before 12 gestational weeks. The ongoing pregnancy rate was the primary outcome, while HCG-positive rate, clinical pregnancy rate and pregnancy loss rate were served as the secondary outcomes.

2.3. Statistics

The data were presented in the format of mean $\pm$ standard deviations for parametric continuous variables and median (range) for nonparametric continuous variables. For categorical variables, the data were expressed as n (%). The Student's T test was used for comparing parametric continuous data, while the Mann-Whitney *U* test for nonparametric continuous data. Categorical variables were analysed using the Chi-square test or Fisher's exact test. All statistical analyses were conducted using SPSS 22.0 software, and a p-value of less than 0.05 was considered significant.

3. Results

There were 72 uRPL sufferers (PGT *vs* no-PGT = 49 *vs* 23) and 25 uRIF sufferers (PGT *vs* no-PGT = 10 *vs* 15) included in our study. For the RPL group, there were no significant differences in various maternal factors such as age, body mass index (BMI), gestational and previous pregnancy loss times, smoking status, AMH, FSH, as well as paternal factors like age, total sperm count, sperm motility, morphology, and DNA fragment index between those who underwent PGT-A and those who didn't. Additionally, the following

variables in terms of controlled ovarian hyperstimulation (COH) protocol, gonadotropin dosage, number of retrieved oocytes, MII oocytes and 2 PN embryos, endometrial preparation, endometrial thickness and endometrial type at transfer were also comparable between the two groups. However, the PGT-A group had more women with educational level > senior high school (85.7 % *vs* 47.8 %, $p = 0.002$), and shorter infertility duration (1(1–1.25) *vs* 3.33 (1.96–4.69), $p < 0.001$)). Notably, all patients in the PGT-A group underwent a single euploid blastocyst transfer, whereas in the non-PGT-A group, 8 patients received a single blastocyst, and the remaining patients had one or two (n = 1/14) cleaved embryos transferred. As for the reproductive outcomes, there was no significant difference in terms of HCG-positive rate and clinical pregnancy rate. However, a remarkable enhancement in the ongoing pregnancy rate (65.3 % *vs* 26.1 %, $p = 0.002$) and lower pregnancy loss rate (17.9 % *vs* 52.9 %, $p < 0.001$) were observed in the PGT-A group in comparison with the non-PGT-A group (Table 1).

For the RIF group, the PGT-A group exhibited a somewhat older maternal age in comparison to the non-PGT-A group (40 (34.5–41) *vs* 34 (32–37), $p = 0.048$)). With regards to other baseline characteristics, there were no notable differences between the PGT-A and non-PGT-A groups. In the PGT-A group, all patients underwent a single euploid blastocyst transfer. In contrast, 8 patients received a single cleaved embryo transfer, while 7 patients had two cleaved embryos transferred in the non-PGT-A group. For the reproductive outcomes, there were no significant differences observed in the HCG-positive rate, clinical pregnancy rate, ongoing pregnancy rate, or

Table 1
Baseline demographics and reproductive outcomes of PGT-A group and control group in RPL sufferers.

	RPL PGT(n = 49)	RPL NO PGT(n = 23)	*p*
Maternal Age (years)	34.73 ± 4.23	35.96 ± 4.27	0.262
Maternal Education level			0.002
<Senior high school	7 (14.3 %)	12 (52.2 %)	
≥Senior high school	42 (85.7 %)	11 (47.8 %)	
Maternal BMI (kg/m2)	22.89 ± 4.15	23.68 ± 3.83	0.44
Infertility type			
Primary			
Secondary	49(100 %)	23(100 %)	
Infertility years	1(1–2.25)	3.33 (1.96–4.69)	<0.001
Gestational times	3(2–4)	3(2–4)	0.58
Previous pregnancy loss times	3(2–3)	3(2–3.25)	0.885
Smoke	4(8.2 %)	0	0.159
AMH (ng/mL)	2.19(1.33–3.67)	2.68(0.67–3.38)	0.62
FSH (IU/L)	7.33(6.47–9.18)	6.59(5.23–9.07)	0.197
Paternal Age (years)	36.10 ± 5.22	38.35 ± 6.54	0.121
Paternal Education level			0.578
≤Senior high school	8(16.33 %)	5(21.74 %)	
>Senior high school	41(83.67 %)	18(78.26 %)	
Total sperm count	212.74(131.29–358.47)	174.5(76.35–263)	0.104
Prog motility (%)	44.05 ± 17.68	36.86 ± 13.58	0.09
Normal Morphology (%)	5(4.28–7)	3.7(2.74–6.1)	0.758
DFI	13.15(7.95–21.86)	17.82(5.5–32.11)	0.439
COH Protocol			0.277
Long GnRH agonist	10(20.41 %)	8(34.78 %)	
Antagonist	6(12.25 %)	4(17.39 %)	
PPOS	33(67.35 %)	11(47.83 %)	
Gonadotropin dosage	2887.5(2118.75–3300	2700(2250–3000)	0.671
Number of retrieved oocytes	14.02 ± 6.79	13.26 ± 8.61	0.991
Number of MII oocytes	11.44 ± 6	11.09 ± 7.28	0.842
Number of 2 PN embryos	9.58 ± 4.78	7.61 ± 4.85	0.109
Endometrial preparation (%)			0.939
NC (%)	4(8.16 %)	2(8.7 %)	
HRT (%)	45(91.84 %)	21(91.3 %)	
Endometrial thickness at transfer(mm)	9(8–10)	8(7–9)	0.057
Endometrial type at transfer			0.197
A-B	25(51 %)	5(21.74 %)	
B	24(49 %)	18(78.26 %)	
Embryo of development days (%)			<0.001
D3	0	15(65.22 %)	
D5	30(61.22 %)	6(26.87 %)	
D6	19(38.78 %)	2(8.7 %)	
Number of embryo transfer			<0.001
1	49(100 %)	9(39.1 %)	
2	0	14(60.9 %)	
HCG-positive rate (%)	39/49 (79.6 %)	17/23 (74 %)	0.589
Clinical pregnancy rate	38/49 (77.5 %)	15/23 (65.2 %)	0.268
Ongoing pregnancy rate (%)	32/49 (65.3 %)	6/23 (26.1 %)	0.002
Pregnancy loss rate (%)	7/39 (17.9 %)	9/17 (52.9 %)	<0.001

Abbreviations: HCG, human chorionic gonadotropin; BMI, body mass index; DFI, DNA fragment index; COH, controlled ovarian hyperstimulation; PPOS, progestin primed ovarian stimulation; NC, natural cycle; HRT, hormone replacement cycle.

pregnancy loss rate between the two groups (Table 2).

4. Discussion

Our study demonstrated that NGS-based PGT-A was beneficial for uRPL sufferers, evidenced by its ability to lower the pregnancy loss rate and increase the ongoing pregnancy rate. This finding aligns with previous research, which has established the positive impact of PGT-A on individuals experiencing RPL [15–20]. While the HCG-positive and clinical pregnancy rates were similar between the PGT-A and non-PGT-A groups, the notable increase in the ongoing pregnancy rate was primarily attributed to PGT-A's potential to reduce the pregnancy loss rate in uRPL sufferers. It is noteworthy that despite the use of PGT-A, the pregnancy loss rate was still 17.9 %, suggesting that factors other than euploid embryo transfer alone contributed significantly to pregnancy loss. In terms of baseline characteristics, the PGT-A group exhibited a higher level of maternal education and a shorter duration of infertility. This could be attributed to the fact that women with higher educational level tend to seek medical assistance sooner, which may lead them to opt for PGT-A testing.

For uRIF sufferers, our study did not find significant differences in reproductive outcomes between the PGT-A group and the control

Table 2
Baseline demographics and reproductive outcomes of PGT-A group and control group in RIF sufferers.

	RIF PGT(n = 10)	RIF NO PGT(n = 15)	*p*
Maternal Age (years)	40(34.5–41)	34(32–37)	0.048
Maternal Education level			1
<Senior high school	2(20 %)	3(20 %)	
≥Senior high school	8(80 %)	12(80 %)	
Maternal BMI (kg/m2)	22.1(18.78–25.88)	22.5(21.2–27.6)	0.292
Type of infertility			0.405
Primary	3(30 %)	7 (46.7 %)	
Secondary	7(70 %)	8(53.3 %)	
Years of infertility	4.84 ± 3.67	6.83 ± 3.82	0.208
Gestational times	1(0–2.5)	1(0–1)	0.892
Previous pregnancy loss times	0(0–1)	0(0–0)	0.807
Smokers (%)	2	0	0.071
AMH (ng/mL)	1.8(1.46–6.12)	2.65(0.68–3.96)	0.567
FSH (IU/L)	7.66 ± 3.99	6.67 ± 4.49	0.575
Paternal Age (years)	38.5(35.25–40.25)	35(33–39)	0.261
Paternal Education level			0.656
≤Senior high school	2(20 %)	2(13.3 %)	
>Senior high school	8(80 %)	13(86.7 %)	
Total sperm count	182.28(151.76–207.10)	231(77.49–492.12)	0.723
Prog motility (%)	41.90(35.68–66.43)	35.32(17.5–46.95)	0.099
Normal. Morphology (%)	4.5(3.7–5.28)	3.8(2.19–5.33)	0.403
DFI	7.26(5.53–11.85)	10.18(7.7–14.78)	0.2
COH Protocol			0.247
long GnRH agonist	2(20 %)	6(40 %)	
antagonist	2(20 %)	5(33.3 %)	
PPOS	6(60 %)	4(26.7 %)	
Gonadotropin dosage	2962.5(2336.25–3375)	2512.5(1868.75–3187.5)	0.508
Number of retrieved oocytes	10.2 ± 4.29	10.93 ± 8.43	0.784
Number of MII oocytes	7.8 ± 3.26	8.79 ± 6.8	0.642
Number of 2 PN embryos	6.5 ± 2.6	7.71 ± 5.53	0.481
Type of endometrial preparation protocol			0.181
NC (%)	1	10	
HRT (%)	9	5	
Endometrial thickness at transfer(mm)	9.35 ± 1.73	9.73 ± 2.23	0.643
Endometrial type			0.622
A-B	5(50 %)	9(60 %)	
B	5(50 %)	6(40 %)	
Embryo of development days (%)			<0.001
D3	0	15(100 %)	
D5	5(50 %)	0	
D6	5(50 %)	0	
Number of embryo transfer per cycle			<0.001
1	10(100 %)	8(53.3 %)	0.011
2	0	7(46.7 %)	
HCG-positive rate (%)	7(70 %)	9(60 %)	0.610
Clinical pregnancy rate (%)	7(70 %)	9(60 %)	0.610
Persistent pregnancy rate (%)	5(50 %)	6(40 %)	0.622
Pregnancy loss rate (%)	2(28.6 %)	3(33.3 %)	0.838

Abbreviations: HCG, human chorionic gonadotropin; BMI, body mass index; DFI, DNA fragment index; COH, controlled ovarian hyperstimulation; PPOS, progestin primed ovarian stimulation; NC, natural cycle; HRT, hormone replacement cycle.

group. However, it should be noted the maternal age in the PGT-A group was relatively higher compared to the non-PGT-A group. Given that advanced maternal age is a crucial factor which hinder successful euploid embryo implantation[20], we can speculate that PGT-A may potentially enhance reproductive outcomes in RIF sufferers with advanced maternal age. In previous literature, the effects of PGT-A in RIF sufferers presented contrasting results. Sato's study [21] revealed a significant increased live birth rate (62.5 *vs* 31.7 %, $p = 0.016$) per embryo transfer and a reduced biochemical pregnancy loss rate (10.5 *vs* 40.9 %, $p = 0.04$) in RIF PGT-A group compared to those who did not have PGT-A (62.5 *vs* 31.7 %, $p = 0.016$). Fodina's retrospective study [22] showed that the PGT-A group exhibited statistically significant higher rates of both biochemical (17.9 % vs 5.6 %, p = 0.001) and clinical pregnancy (49.3 % vs 44.4 %, p = 0.049) in RIF sufferers. However, Rao's findings contradicted these trends, indicating that the implantation rate was similar between the PGT-A group and the control group (47 vs. 42 %) in RIF sufferers [23]. Similarly, Kato's study revealed no significant differences in the live birth rate per embryo transfer (90.0 % *vs* 69.2 %, $p = 0.2313$) and pregnancy loss rate (0 % *vs* 10.0 %, $p = 0.3297$) between the PGT-A and non-PGT-A groups in RIF women aged 35–42 years undergoing minimal ovarian stimulation cycle [24]. These inconsistent results may be due to the intrinsic characteristics of the studied populations, the specific PGT-A detection methods utilized, or the relatively small sample sizes. It is worth mentioning that the indications for ICSI differed between the PGT-A group and the non-PGT-A group in both uRPL and uRIF sufferers. Specifically, the PGT-A group underwent ICSI primarily to prevent contamination, while the non-PGT-A group underwent ICSI primarily due to sperm-related factors or valuable oocytes. Given that sperm quality can significantly impact reproductive outcomes [25], this difference in ICSI indications could potentially influence the results. However, despite the fact that 69.6 % uRPL and 66.7 % uRIF sufferers had ICSI due to sperm factor in the non-PGT-A group, the abnormal sperm ($\geq$ one abnormal sperm parameter) rates in the PGT-A group were also up to 40.8 % and 40 % respectively. Additionally, the sperm parameters such as total sperm count, progressive motility, normal morphology, and sperm DNA fragment index were comparable between PGT-A group and non-PGT-A group in both uRPL and uRIF sufferers. These findings suggest that the difference in ICSI indications between the two groups on reproductive outcomes may be limited in our study.

It should be recognized that PGT-A is a technique used to identify embryo aneuploidy instead of normal karyotype. For example, PGT-A could not distinguish embryos with balanced chromosomal aberrations which are technically euploid but not normal karyotype [26]. Additionally, PGT-A cannot detect all genetic abnormalities or developmental defects, such as imbalances in mitochondrial copy number to nuclear DNA and certain de novo deletions or duplications [27]. Moreover, when embryos fail to reach the blastocyst stage, PGT-A can potentially delay the timing of embryo transfer. Therefore, it is crucial for RPL or RIF sufferers to undergo a comprehensive evaluation to identify all potential causes and devise a personalized treatment strategy.

Our study had several limitations. Firstly, the small sample size diminishes the statistical power and reliability of our findings. Secondly, the retrospective nature of our research and the subjective choices made by physicians and patients regarding PGT-A introduce inevitable biases. Thirdly, a notable difference exists in the embryo transfer stage, that the PGT-A group had transferred blastocysts, while the non-PGT-A group had transferred blastocysts or cleaved blastocysts. Fourthly, our study is lack of the long-term obstetric and neonatal outcomes which were recommended to follow-up [28]. In previous studies, Alteri reported that an increased risk of hypertensive disorder complicating pregnancy was observed for cleaved and blastocyst stages biopsy methods [29]. Ginod's research found a greater risk of preterm birth and birth defects following trophectoderm biopsy and frozen embryo transfer (FET) [30]. A registry-based analysis revealed that children conceived through PGT faced a higher risk of preterm birth, placenta praevia, and caesarean delivery than those born spontaneously. However, no significant differences in perinatal outcomes, birth defects, or maternal health were observed in comparison to IVF/ICSI cycles [31]. The potential side effects of PGT could be attributed to the reduction of trophoblast cells, which may hinder placental development, thereby increasing the likelihood of adverse outcomes linked to placental dysfunction.

In conclusion, NGS-based PGT-A was beneficial for uRPL sufferers. While its advantages were less evident in uRIF sufferers, individuals with advanced maternal age may still derive some benefit. To confirm these findings, well-designed, randomized controlled trials with a substantial sample size are warranted.

Data availability statement

All raw data would be provided on reasonable requests.

CRediT authorship contribution statement

Youwen Mei: Writing – review & editing, Writing – original draft, Methodology, Data curation, Conceptualization. **Yacong Wang:** Writing – original draft, Methodology, Data curation. **Lin He:** Data curation. **Jiafeng Zheng:** Data curation. **Yonghong Lin:** Writing – review & editing, Conceptualization. **Fang Wang:** Writing – review & editing, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Abbreviations

PGT-A	preimplantation genetic testing for aneuploidy
NGS	next-generation sequencing
RRF	recurrent reproductive failure
RPL	recurrent pregnancy loss
RIF	repeated implantation failure
ICSI	intracytoplasmic sperm injection
IVF	in vitro fertilization
FET	frozen-thawed embryo transfer
EET	euploid embryo transfer
BMI	body mass index
HCG	human chorionic gonadotropin
DFI	DNA fragment index
COH	controlled ovarian hyperstimulation

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