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# The Effectiveness of Next-Generation Sequencing-Based Preimplantation Genetic Testing for Balanced Translocation Couples

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**Keywords**
Robertsonian translocation · Reciprocal translocation · Preimplantation genetic testing · Next-generation sequencing · Aneuploidy

**Abstract**
The purpose of this study was to evaluate the effectiveness of next-generation sequencing (NGS)-based preimplantation genetic testing (PGT) for balanced translocation carriers to identify normal/balanced blastocysts and to measure pregnancy outcomes following euploid embryo transfer. We enrolled 75 couples with a balanced translocation who underwent 83 PGT cycles (58 cycles for carriers with reciprocal translocations and 25 cycles for carriers with Robertsonian translocations) and 388 blastocysts were diagnosed. Moreover, we transferred single euploid blastocysts through frozen embryo transfer and calculated the biochemical pregnancy, clinical pregnancy, miscarriage, and ongoing pregnancy rates per embryo transfer cycle. Despite a mean maternal age of 29.8 years and mean of 4.34 embryos biopsied, there was a 32.8% chance of recording no chromosomally normal/balanced embryos for reciprocal translocation carriers. The proportion of normal/balanced embryos was significantly higher (44.1 vs. 27.8%) in Robertsonian translocation carriers than in reciprocal translocation carriers. Female carriers had a significantly lower (23.3 vs. 42.4%, 34.7 vs. 54.7%, respectively) percentage of normal/balanced embryos than male carriers, regardless of the translocation. After transfering single blastocysts, we obtained a 64.4% clinical pregnancy rate per transfer, and the clinical miscarriage rate was 5.7%. Amniocentesis results showed that all karyotypes of the fetuses were consistent with PGT results. The clinical outcomes are probably not influenced by the type of translocation, maternal age, and blastocyst morphology following the transfer of euploid blastocysts. Therefore, we conclude that NGS-based PGT is an efficient method for analyzing balanced translocation carriers, and aneuploidy screening had good clinical outcomes. 

## Introduction

Balanced translocations are common in humans, with an incidence rate of 0.1–0.2% in newborns and approximately 0.4% in prenatal samples [Ogilvie et al., 2001; Alfarawati et al., 2012]. Although most carriers of Robert-



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sonian translocations (RT) and reciprocal translocations (rcp) are phenotypically normal, they have an increased risk of producing gametes with chromosomal imbalances. This often leads to an increased risk of fertility problems, such as infertility, repeated miscarriages, repeated failure, or newborns with congenital anomalies [Simopoulou et al., 2003].

In rcp carriers, the quadrivalent complex segregates by the alternate and non-alternate (adjacent-1, adjacent-2, 3:1 and 4:0 for rcp carriers) mode to theoretically produce 18 different gametes. Only gametes produced by the alternate segregation mode are normal or balanced. Gametes derived from a non-alternate segregation pattern have unbalanced karyotypes [Ye et al., 2012]. Depending on the segregation patterns in meiotic divisions of RT carriers, theoretically only 1/6 normal and 6/1 balanced gametes produced by alternate segregation can form phenotypically normal embryos. Gametes derived from non-alternate (adjacent and 3:1 for RT carriers) segregation are unbalanced [Jin et al., 2010]. It has been suggested that for an abnormal embryo arising from balanced translocation carriers, beside the unbalance on the translocated chromosomes, there may also be an abnormity in the non-translocated chromosomes during meiosis [Alfarawati et al., 2012]. These abnormal embryos increase the risk of miscarriage, especially for first trimester abortions. Balanced translocation carriers have a high risk of miscarriage, reaching approximately 50% for RT carriers and 83.38% for rcp carriers [Stephenson and Sierra, 2006; Huang et al., 2019].

Preimplantation genetic testing for structural rearrangements (PGT-SR) is an effective method for translocation carriers that allows selection of balanced/normal euploid embryos for embryo transfer. It can improve pregnancy outcomes in these couples with balanced translocations, reducing the time to achieve a healthy live birth from 4–6 years to less than 4 months and decreasing the incidence of miscarriages from about 90% to less than 15% in rcp carriers [Munne et al., 2000; Verlinsky et al., 2005; Fischer et al., 2010]. In the past decades, FISH has been widely used for PGT-SR to distinguish balanced embryos from unbalanced ones. However, FISH is only applicable for specific chromosomes involved in the translocation and cannot be used to evaluate all 24 chromosomes simultaneously. FISH-based PGT is usually applied in the context of the biopsy of single blastomeres from cleavage-stage embryos. Recently, blastocyst biopsy was prefered to blastomere biopsy in PGT, owing to the higher amount of available DNA for genetic testing and the fact that it does not affect embryo viability or implantation potential [Scott et al., 2013]. Furthermore, FISH has some other limitations, including observation of split signals, cross-hybridization, chromosome polymorphisms, poor fixation quality, and loss of micronuclei or chromosomes during fixation [Velilla et al., 2002; DeUgarte et al., 2008]. A large number of prospective, randomized controlled trials have consistently failed to show any improvement in delivery rates using FISH-based PGS at cleavage stages [Mastenbroek et al., 2011]. As a consequence of these limitations, this methodology has become largely obsolete.

Therefore, an accurate and comprehensive determination of embryo ploidy is essential. In recent years, the focus in PGT-SR has shifted from day 3 single blastomere biopsy to day 5/6 trophectoderm biopsy and the use of comprehensive chromosome screening technologies in order to provide a more accurate assessment of the genetic potential of the embryos. A new generation of methods for detecting abnormalities in embryos include comparative genomic hybridization (CGH), microarrays (aCGH and single nucleotide polymorphism arrays), and NGS. Recent advances in NGS have stimulated an increasing interest in its application in the field of reproductive medicine. It is a precise, comprehensive, and high-throughput genetic technique that has been used to screen all 24 chromosome. NGS-based PGT-SR allows the calculation of the total aneuploidy rate (unbalanced translocation and/or sporadic aneuploidy) in the biopsied embryos. Previous studies on blastocysts demonstrated that NGS is an accurate technique to detect aneuploidy and unbalanced rearrangements [Kung et al., 2015; Rubio et al., 2019]. Although this approach offers exciting and potentially important advances toward improved PGT, its possible clinical effectiveness in PGT-SR still remains less studied.

Therefore, we aimed at assessing the ability of NGS-based PGT-SR in detecting chromosomal abnormalities in balanced translocation carriers. In addition, we sought to determine whether NGS can benefit pregnancy outcomes for couples with balanced translocations.

## Material and Methods

*Study Population*

A total of 75 balanced translocation patients who underwent 83 PGT-SR cycles were enrolled in our study from May 2018 to October 2019. These couples usually had a history of primary infertility, recurrent spontaneous abortions, or pregnancies with chromosome anomalies. In all couples only 1 member had rcp or RT, and patients with compound translocations were excluded.

*Controlled Ovarian Stimulation and Blastocyst Culture*

Ovarian stimulation was performed according to the clinical routine of the Center for Reproductive Medicine, The First Hospital of Lanzhou University. After oocyte aspiration, ICSI was performed on metaphase II oocytes, and zygotes were cultured until the blastocyst stage. Blastocysts were graded according to the Gardner blastocyst morphologic scoring system [Gardner et al., 2000]. Good-quality blastocysts were defined as ≥3BB (3, 4, 5, 6, AA, AB, BA, and BB), and poor-quality blastocysts were defined as C grade. Day 5/6 blastocysts were drilled, and 5–10 trophectoderm cells were biopsied.

*Whole Genome Amplification and NGS Procedure*

Ion ReproSeq TM PGS Kit (Thermo Fisher Scientific, USA) was used for whole genome amplificataion and NGS according to the manufacturer's protocol with the Ion Chef TM and Ion S5 System instruments (Thermo Fisher Scientific). Sequencing data were analyzed with Ion Reporter software 5.4, which aligns the readings with the human genome (hg19) and uses the bioinformatic tool ReproSeq Low-pass whole-genome aneuploidy workflow v1.0 with low coverage (minimum 0.01×). The effective resolution is optimal at 4 Mb. Chromosome fragments that differed from the expected ploidy were treated as aneuploid. Positive and negative controls were sequenced along with the samples.

*Single Embryo Transfer*

For single embryo transfer, the vitrified-warmed euploid blastocyst was transferred into the uterine cavity with an endometrium thickness of >7 mm. The serum hCG level was measured 14 days after blastocyst transfer. Clinical pregnancy was defined by the detection of a gestational sac and a fetal heartbeat via sonography 4 weeks after frozen embryo transfer (FET). Any pregnancy that went beyond 20 weeks of gestation was considered an ongoing pregnancy. Amniocentesis was performed at 20-22 weeks of gestation in order to confirm the chromosomal status of the fetus.

*Statistical Analysis*

Categorical variables are presented as absolute values and percentage. The differences between rates were tested by $\chi^2$ or Fisher's exact test, if appropriate. Normally distributed continuous data are expressed as means ± standard deviation and were compared between groups using a two-sample Student's *t* test. Data with skewed distribution are expressed as medians and quartile range and were compared with the Mann-Whitney U test.

All statistical calculations were performed using SPSS version 17.0 software, and $p < 0.05$ was considered to indicate statistically significant differences.

## Results

*General Characteristics of the Study Subjects*

Among 83 completed PGT cycles in couples with balanced translocations, 58 were rcp and 25 were RT carriers. Overall, we retrieved 1,363 oocytes and 1,212 MII oocytes; 79.1% (959/1,212) of MII oocytes successfully developed into normally fertilized oocytes with 2 pronuclei, and 865 day 3 embryos were developed, of which 45.8% (395/865) were successfully cultured to blastocysts for biopsy. We then diagnosed 388 (98.2%) blastocysts successfully and obtained 130 (33.5%) normal/balanced blastocysts. Specifically, there was no difference in the mean maternal age in the rcp and RT groups ($p > 0.05$). Among other relevant factors including mean retrieved oocytes, MII oocytes, 2-pronuclei zygotes, day 3 embryos, biopsied blastocysts, and diagnosed blastocysts per PGT cycle, no significant differences were recorded between the RT and rcp carrier group. However, the RT group had more normal/transferable embryos per cycle compared to the rcp group ($p < 0.05$). Thirty-nine (67.2%) PGT cycles for rcp resulted in 1 or more euploid embryos, and 22 (88.0%) RT cycles had transferable embryos. Rcp carriers had a higher percentage of cycles without transferrable blastocysts compared to RT carriers ($p < 0.05$) (Table 1).

Twenty-two of 75 couples were primary infertile before the PGT cycle, the other 53 couples had a pregnancy history. Among them, 14 had 1 miscarriage, 25 had 2 miscarriages, and 14 had 3 or more miscarriages. There was no significant difference in pregnancy history between the rcp and the RT group (Table 1). Eighty-three pregnancies occurred in 51 rcp couples before PGT. The details include 74 spontaneous abortions, 7 induced terminations because of fetal abnormality, and 2 normal/balanced offspring. Thirty pregnancies occurred in 24 RT couples before PGT. The details include 24 spontaneous abortions, 2 induced terminations because of fetal abnormality, and 4 healthy children.

*Distribution of Abnormal Embryos in rcp and RT Carriers*

We calculated the euploid versus aneuploid (unbalanced, sporadic, combined) rates of the embryos. In the rcp group, 27.8% (70/252) blastocysts were normal/balanced, 33.3% (84/252) blastocysts were unbalanced for the translocated chromosomes, 21.8% (55/252) of diagnosed blastocysts were aneuploid for non-translocated chromosomes (sporadic aneuploidy), and 17.1% (43/252) had a combined abnormality, unbalanced for the translocated chromosomes and sporadic aneuploidy simultaneously. In the RT group, 44.1% (60/136) were normal/balanced embryos, 16.9% (23/136) were unbalanced, 30.1% were sporadic aneuploid, and 8.8% (12/252) were combined abnormal. Compared with RT couples, the rcp couples had a lower percentage of normal/balanced blastocysts (27.8 vs. 44.1%; $p = 0.001$), a higher percentage of unbalanced embryos (33.3 vs. 16.9%; $p = 0.001$), and combined abnormal embryos (17.1 vs. 8.8%; $p = 0.026$) (Table 2).

**Table 1.** General clinical parameters and PGT results of balanced translocation carriers

| Parameter | Reciprocal translocation | Robertsonian translocation | Total |
|---|---|---|---|
| No. of cycles | 58 | 25 | 83 |
| Female/male carriers cycles | 45/13 | 12/13 | 57/26 |
| No. of patients | 51 | 24 | 75 |
| Female age, years | 29.80±3.48 | 30.28±3.76 | 29.92±3.58 |
| Male age, years | 31.45±4.14 | 30.96±3.68 | 31.30±4.01 |
| Number of previous miscarriages | | | |
| 0 | 13 (25.5%) | 9 (37.5%) | 22 (29.3%) |
| 1 | 10 (19.6%) | 4 (16.7%) | 14 (18.7%) |
| 2 | 17 (33.3%) | 8 (33.4%) | 25 (33.3%) |
| ≥3 | 11 (21.6%) | 3 (12.5%) | 14 (18.7%) |
| Retrieved oocytes | 956 (16.49±5.95) | 407 (16.30±5.29) | 1,363 (16.42±5.77) |
| MII oocytes | 850 (14.66±4.92) | 362 (14.47±5.05) | 1,212 (14.60±4.95) |
| 2-pronuclei zygotes | 676 (11.64±4.65) | 283 (11.32±5.03) | 959 (11.55±4.69) |
| Day 3 embryos | 614 (10.59±4.42) | 251 (10.05±4.86) | 865 (10.42±4.51) |
| Biopsied blastocysts | 255 (4.40±1.89) | 141 (5.64±3.06) | 396 (4.77±2.34) |
| Diagnosed blastocysts | 252 (4.34±1.91) | 136 (5.44±3.11) | 388 (4.67±2.36) |
| Normal/balanced blastocysts | 70 (27.8%)[a] | 60 (44.1%)[a] | 130 (33.5%) |
| Cycles without transferrable blastocysts | 19 (32.8%)[b] | 3 (12.0%)[b] | 22 (26.5%) |
| Embryo transfer cycles | 36 | 18 | 54 |
| Transferred blastocysts | 36 | 18 | 54 |
| Biochemical pregnancies | 24 (66.7%) | 14 (77.8%) | 38 (70.4%) |
| Clinical pregnancies | 22 (61.1%) | 13 (72.2%) | 35 (64.8%) |
| Clinical miscarriages | 2 (9.1%) | 0 (0%) | 2 (5.7%) |
| Ongoing pregnancies | 20 (55.6%) | 13 (72.2%) | 33 (61.1%) |
| Deliveries | 14 (38.9%) | 8 (44.4%) | 22 (40.7%) |
| Continued pregnancies | 6 (16.7%) | 5 (27.8%) | 11 (20.4%) |

Normally distributed data are expressed as mean ± SD, and data with skewed distribution are expressed as median (quartile). [a] $p = 0.001$; significant difference between reciprocal translocation group and Robertsonian translocation ($\chi^2$ test). [b] $p = 0.041$; significant difference between reciprocal translocation group and Robertsonian translocation ($\chi^2$ test).

It is well-known that the abnormality of embryos produced by rcp or RT carriers can result from 2 categories: abnormality in translocated chromosomes which derived from non-alternate segregation and abnormality in non-translocated chromosomes. So, we calculated the "unbalanced + combined abnormality" rate which was equal to non-alternate segregation, and "sporadic aneuploidy + combined abnormality" rate which was equal to abnormality in non-translocated chromosomes. The results showed that rcp carriers had a significantly higher percentage of non-alternate segregation compared to RT carriers (50.4 vs. 25.7%; $p = 0.000$) and the same proportion of abnormality in non-translocated chromosome (38.9 vs. 38.9%) (Table 2).

*Stratified Analysis of Abnormal Embryo Distribution according to the Carriers' Gender*

We compared the differences in the distribution of abnormal embryos with respect to the gender of the carriers. In the rcp group, male carrier couples had a higher normal/balanced rate than female carriers (42.4 vs. 23.3%; $p = 0.004$). Similarly, male RT carriers had a higher percentage of normal/balanced embryos than female carriers (54.7 vs. 34.7%; $p = 0.019$). Whether it is rcp or RT, there were no significant differences in maternal age between female and male carriers (Table 3).

To explore which kind of abnormality caused the distribution difference, we compared the rate of non-alternate segregation and abnormality in non-translocated chromosomes between male and female carriers. Regardless of the group, female carriers had a significantly high-

**Table 2.** The distribution of abnormal embryos in reciprocal translocation and Robertsonian translocation carriers

| Category | Total | Reciprocal translocation | Robertsonian translocation | *p* value |
|---|---|---|---|---|
| Overall | 388 | 252 | 136 | |
| Unbalanced | 107 | 84 (33.3%) | 23 (16.9%) | **0.001** |
| Sporadic aneuploidy | 96 | 55 (21.8%) | 41 (30.1%) | 0.070 |
| Combined abnormality | 55 | 43 (17.1%) | 12 (8.8%) | **0.026** |
| Total abnormality | 258 | 182 (72.2%) | 76 (55.9%) | **0.001** |
| Normal/balanced | 130 | 70 (27.8%) | 60 (44.1%) | – |
| Unbalanced + combined abnormality | 162 | 127 (50.4%) | 35 (25.7%) | **0.000** |
| Sporadic aneuploidy + combined abnormality | 151 | 98 (38.9%) | 53 (38.9%) | 0.987 |

$\chi^2$ test was used to compare the differences between the frequencies of chromosome abnormality. *p* values in bold show significant differences.

**Table 3.** Stratification analysis of chromosome abnormality according to the gender of the carriers

| Category | Total | Female carriers | Male carriers | *p* value |
|---|---|---|---|---|
| *Reciprocal translocation* | 252 | 193 | 59 | |
| Unbalanced | 84 | 68 (35.2%) | 16 (27.1%) | 0.247 |
| Sporadic aneuploidy | 55 | 43 (22.3%) | 12 (20.3%) | 0.752 |
| Combined abnormality | 43 | 37 (19.2%) | 6 (10.2%) | 0.108 |
| Total abnormality | 182 | 148 (76.7%) | 34 (57.6%) | **0.004** |
| Normal/balanced | 70 | 45 (23.3%) | 25 (42.4%) | – |
| Unbalanced + combined abnormality | 127 | 105 (54.4%) | 22 (37.3%) | **0.021** |
| Sporadic aneuploidy + combined abnormality | 98 | 80 (41.5%) | 18 (30.5%) | 0.131 |
| *Robertsonian translocation* | 136 | 72 | 64 | |
| Unbalanced | 23 | 15 (20.8%) | 8 (12.5%) | 0.196 |
| Sporadic aneuploidy | 41 | 20 (27.8%) | 21 (32.8%) | 0.523 |
| Combined abnormality | 12 | 12 (16.7%) | 0 (0%) | **0.001** |
| Total abnormality | 76 | 47 (65.3%) | 29 (45.3%) | **0.019** |
| Normal/balanced | 60 | 25 (34.7%) | 35 (54.7%) | – |
| Unbalanced + combined abnormality | 35 | 27 (37.5%) | 8 (12.5%) | **0.001** |
| Sporadic aneuploidy + combined abnormality | 53 | 32 (44.4%) | 21 (32.8%) | 0.165 |

There were no statistic differences in maternal age between female and male carriers. $\chi^2$ was used to compare the differences between the frequencies of chromosome abnormality. *p* values in bold show significant differences.

er proportion of non-alternate segregation than male carriers (54.4 vs. 37.3%, $p = 0.021$; 37.5 vs. 12.5%, $p = 0.001$). There was no difference in abnormality because of non-translocated chromosomes (Table 3).

*Pregnancy Outcome*

We analyzed 54 FET cycles from May 2018 to October 2019. Single blastocysts were transferred in each FET cycle. Overall, 38 couples were positive for β-hCG 14 days after blastocyst transfer, and we confirmed a clinical pregnancy in 36 women. The clinical pregnancy rate per embryo transfer cycle was 64.8%. There were 2 cases of spontaneous miscarriage in early pregnancy, and the clinical miscarriage rate was 5.7%. Finally, 33 women (61.1%) had an ongoing pregnancy for more than 20 weeks (Table 4). During the study period, 22 healthy babies were born,

**Table 4.** Embryo transfer and pregnancy outcome

| Category | Frozen embryo transfer cycles | Biochemical pregnancy | Clinical pregnancy | Ongoing pregnancy |
|---|---|---|---|---|
| Total | 54 | 38 (70.4%) | 35 (64.8%) | 33 (61.1%) |
| Reciprocal translocation | 36 | 24 (66.7%) | 22 (61.1%) | 20 (55.6%) |
| Robertsonian translocation | 18 | 14 (77.8%) | 13 (72.2%) | 13 (72.2%) |
| Maternal carrier | 35 | 21 (60.0%)[a] | 19 (54.3%)[b] | 19 (54.3%) |
| Paternal carrier | 19 | 17 (89.5%)[a] | 16 (84.2%)[b] | 14 (73.7%) |
| Good-quality embryos | 40 | 30 (75.0%) | 27 (67.5%) | 25 (62.5%) |
| Poor-quality embryos | 14 | 8 (57.1%) | 8 (57.1%) | 8 (57.1%) |
| Maternal age ≥35 years | 11 | 6 (54.5%) | 5 (45.5%) | 5 (45.5%) |
| Maternal age <35 years | 43 | 32 (74.4%) | 30 (69.8%) | 28 (65.1%) |

[a] Significant differences ($p < 0.05$) within rate of biochemical pregnancy between maternal carriers and paternal carriers ($\chi^2$ Test). [b] Significant differences ($p < 0.05$) within rate of clinical pregnancy between maternal carriers and paternal carriers ($\chi^2$ Test).

while 11 couples had a continued pregnancy (>20 weeks) (Table 1). The results of amniocentesis in the second trimester showed that all karyotypes of the fetuses were consistent with NGS-PGT.

To explore the factors affecting pregnancy outcomes when euploid embryos were transferred, we compared the rates of biochemical pregnancy, clinical pregnancy, and ongoing pregnancy based on the type of translocation, gender of the carrier, classification of embryo morphology, and maternal age (Table 4). Couples with RT had better clinical outcomes than rcp carriers in terms of biochemical pregnancy, clinical pregnancy, and ongoing pregnancy. Good-quality embryos had better pregnancy outcomes than poor-quality embryos. Balanced translocation couples with a maternal age <35 years also had higher rates of biochemical pregnancy, clinical pregnancy, and ongoing pregnancy than those with a maternal age of 35 years or older. Nevertheless, there was no statistical difference among these factors ($p > 0.05$). Male carriers of balanced translocations had higher rates of biochemical and clinical pregnancy than female carriers ($p < 0.05$) (Table 4). In order to avoid interference of potential confounders, a bivariate logistic regression model was used to assess the relationship among biochemical pregnancy (clinical pregnancy or ongoing pregnancy) and type of translocation, gender of the carrier, classification of embryo morphology, and maternal age. As the results of logistic regression analysis were similar to the results of the hierarchical analysis shown in Table 4, we did not discuss these data here.

## Discussion

Patients who carry balanced translocations are faced with multiple treatment options, such as expectant management, gamete donation, and IVF + PGT-SR, but there is little data to guide their decisions. In this study, we evaluated the effectiveness of NGS-based PGT-SR in detecting balanced translocations and the clinical outcome of the following FET cycle in order to provide data support for future genetic counseling of balanced translocation carriers.

The use of NGS-based PGT allows for testing of each embryo biopsy sample for the parental translocation in addition to simultaneous 24 chromosome aneuploidy screening. In our study, the incidence of normal/balanced embryos (27.8%) derived from rcp carriers was consistent with previous studies (27.3–30.6%), which were also NGS-based PGT for rcp [Zhang et al., 2016; Cai et al., 2019]. The transferable embryo rate was 20.8% among day 3 blastomeres analyzed, which was lower than ours [Keymolen et al., 2012]. The normal/balanced embryo rate was higher among blastocysts than blastomeres, which might reflect a strong natural selection process occurring between day 3 and day 5 that increased the proportion of transferable embryos.

Both rcp and RT carriers had a higher percentage of normal/balanced blastocysts than the theoretical value of synaptonemal complex segregation (1/9 vs. 27.8%, 1/3 vs. 44.1%). The results might also a reflect natural selection process in gametogenesis and embryogenesis. Analysis of the transferable embryo rates between dif-

ferent types of translocation showed that RT carriers had a significantly higher rate of normal/balanced blastocysts compared to rcp carriers, which is in line with a previous study [Mateu-Brull et al., 2019]. This finding was because the trivalent complex of RT had a higher percentage of alternate segregation compared to the quadrivalent complex of rcp. Moreover, rcp couples had a higher probability of cycles without transferrable blastocysts than RT carriers (32.8 vs. 12.0%). Once rcp carriers obtained a normal/balanced blastocyst, they would have a 61.1% clinical pregnancy rate. Similarly, RT carriers had a 72.2% clinical pregnancy rate after transfer of a normal/balanced embryo. In our study, the rcp couples obtained a 41.1% (67.2% × 61.1%) clinical pregnancy rate per oocyte retrieval, and the RT carriers rate was 63.5% (88.0% × 72.2%). Since 27.8% blastocysts of rcp couples were normal/balanced, theoretically 1 of the 4 obtained blastocysts was transferable. For RT carriers, at least 1 of 3 blastocysts was theoretically transferable. More oocytes and more embryos, to a certain extent, means a higher success rate. Therefore, patients with a different ovarian reserve status may have different clinical outcomes. It is important to convey this information during genetic counseling before PGT-SR.

We compared the proportion of different types of embryos with respect to the influence of the carrier's gender. Male carriers had a higher percentage of normal/balanced embryos, no matter the rcp or RT group. They also had a lower proportion of non-alternate segregation which produced embryos with translocation-related imbalances. Several studies about meiotic segregation analysis of embryos derived from rcp or RT carriers demonstrated that male carriers had a higher percentage of alternate segregation and lower non-alternate segregation [Ye et al., 2012; Zhang et al., 2019a, b]. Our results were consistent with findings in these cited studies and confirmed that the differences in the proportion of abnormal embryos resulted mainly from the difference in rearrangements of chromosomal segregation during meiosis.

In this study, we implemented NGS-based PGT for translocation testing as a valuable alternative for FISH-based testing aiming to increase the sensitivity and specificity of PGT and to improve pregnancy outcomes in couples with balanced translocations. Blastocyst trophectoderm biopsy provids more genetic material for subsequent analysis, and cryopreservation of biopsied blastocysts allows for enough time for genetic testing and a more acceptable embryo-endometrial synchronization cycle for transfer [Palini et al., 2015]. Fifty-four normal/balanced blastocysts were transferred, and the overall clinical pregnancy rate was 64.8% in our study. This was higher than the FISH-based PGT-SR with a clinical pregnancy rate of about 40% in a previous study [Harper et al., 2012] and similar to another NGS-based PGT-SR study with a clinical pregnancy rate of 65.38% [Cai et al., 2019]. These results confirmed that NGS-based PGT-SR based on blastocyst trophectoderm biopsy on day 5/6, massively parallel and high throughput DNA sequencing (NGS), vitrification, and thawed single embryo transfer were more efficient than FISH-based PGT-SR. NGS screening reliably distinguished all types of aneuploidy in human blastocysts and provided a higher resolution (>4 Mb in our study) for chromosome segmental changes. Given these advantages, NGS was applicable for couples with chromosome abnormalities.

Pregnancy outcome indicators for the transfer of euploid embryos are likely to at least partially drive patient decision-making processes. Therefore, we verified the influence of factors such as type of translocation, gender of the carrier, classification of embryo morphology, and maternal age on the clinical outcome. It was confirmed that the gender of the carrier could affect a biochemical and clinical pregnancy. Male carriers had significantly higher biochemical and clinical pregnancy rates than female carriers. No statistic difference was found among other factors. Simon et al. [2018] compared clinical pregnancy rates, miscarriage rates, and live birth rates among different maternal age groups after euploid embryo transfer using of 24 chromosome SNP-based PGT, and the results were consistent with ours. Idowu et al. [2015] confirmed that there were no significant differences in pregnancy outcome between different translocation carriers and their gender. However, this study calculated the pregnancy rate per biopsy cycle. In short, once an euploid blastocyst was transferred, advanced maternal age or young maternal age, rcp or RT group, and excellent or poor embryo did not seem to significantly influence the clinical pregnancy outcome, but male carriers of a balanced translocation may had a significantly better clinical outcome than female carriers.

Our study has some limitations. Firstly, the research is limited by its retrospective nature and small sample size. A larger number of embryo transfer cycles conducted with NGS-based PGT-SR is desirable for future prospective studies, especially to provide more solid evidence indicating the clinical outcomes. Secondly, we did not list the mosaic embryos separately but they were contained in the abnormal embryos for the convenience

in analyzing data. Thirdly, as the data of natural pregnancy outcomes after ascertainment of the carrier status had not been obtained, we can not compare PGT pregnancy outcomes with natural pregnancies in this study.

In conclusion, we demonstrated the effectiveness of NGS-based PGT for balanced translocation carriers with a high detection rate of aneuploid or unbalanced blastocysts. Subsequent euploid embryo transfer leads to high clinical pregnancy rates and low miscarriage rates. The normal/balanced embryo rates and clinical pregnancy rates after the transfer of euploid embryos is dependent on the carriers' gender. We hope our study can provide a more comprehensive understanding of the technology of NGS-based PGT-SR and help clinicians to better apply it.


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## Statement of Ethics

PGT was conducted at the Reproductive Medicine Hospital the First Hospital of Lanzhou University, with approval from the Ethics Committee of the First Hospital of Lanzhou University (LDYYLL2017–07). All patients provided written informed consent for IVF and PGT.

## Conflict of Interest Statement

The authors have no conflicts of interest to declare.


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## Author Contributions

H.L. and X.M. designed the study and wrote the manuscript. H.L., B.M., and X.X. performed the genetic analysis and data analysis. L.L. carried out the NGS experiments. X.M. and X.Z. collected all of the clinical data and revised the manuscript. All authors approved the final manuscript.

## References


Alfarawati S, Fragouli E, Colls P, Wells D. Embryos of Robertsonian translocation carriers exhibit a mitotic interchromosomal effect that enhances genetic instability during early development. PLoS Genet. 2012;8(10):e1003025.

Cai Y, Ding M, Lin F, Diao Z, Zhang N, Sun H, et al. Evaluation of preimplantation genetic testing based on next-generation sequencing for balanced reciprocal translocation carriers. Reprod Biomed Online. 2019;38(5):669–75.

DeUgarte CM, Li M, Surrey M, Danzer H, Hill D, DeCherney AH. Accuracy of FISH analysis in predicting chromosomal status in patients undergoing preimplantation genetic diagnosis. Fertil Steril. 2008;90(4):1049–54.

Fischer J, Colls P, Escudero T, Munné S. Preimplantation genetic diagnosis (PGD) improves pregnancy outcome for translocation carriers with a history of recurrent losses. Fertil Steril. 2010;94(1):283–9.

Gardner DK, Lane M, Stevens J, Schlenker T, Schoolcraft WB. Blastocyst score affects implantation and pregnancy outcome: towards a single blastocyst transfer. Fertil Steril. 2000;73(6):1155–8.

Harper JC, Wilton L, Traeger-Synodinos J, Goossens V, Moutou C, SenGupta SB, et al. The ESHRE PGD Consortium: 10 years of data collection. Hum Reprod Update. 2012;18(3):234–47.

Huang C, Jiang W, Zhu Y, Li H, Lu J, Yan J, et al. Pregnancy outcomes of reciprocal translocation carriers with two or more unfavorable pregnancy histories: before and after preimplantation genetic testing. J Assist Reprod Genet. 2019;36(11):2325–31.

Idowu D, Merrion K, Wemmer N, Mash JG, Pettersen B, Kijacic D, et al. Pregnancy outcomes following 24-chromosome preimplantation genetic diagnosis in couples with balanced reciprocal or Robertsonian translocations. Fertil Steril. 2015;103(4):1037–42.

Jin H, Ping L, Jie Q, Ying L, Yongjian C. Translocation chromosome karyotypes of the Robertsonian translocation carriers' embryos. Fertil Steril. 2010;93(4):1061–5.

Keymolen K, Staessen C, Verpoest W, Liebaers I, Bonduelle M. Preimplantation genetic diagnosis in female and male carriers of reciprocal translocations: clinical outcome until delivery of 312 cycles. Eur J Hum Genet. 2012;20(4):376–80.

Kung A, Munné S, Bankowski B, Coates A, Wells D. Validation of next-generation sequencing for comprehensive chromosome screening of embryos. Reprod Biomed Online. 2015;31(6):760–9.

Mastenbroek S, Twisk M, van der Veen F, Repping S. Preimplantation genetic screening: a systematic review and meta-analysis of RCTs. Hum Reprod Update. 2011;17(4):454–66.

Mateu-Brull E, Rodrigo L, Peinado V, Mercader A, Campos-Galindo I, Bronet F, et al. Interchromosomal effect in carriers of translocations and inversions assessed by preimplantation genetic testing for structural rearrangements (PGT-SR). J Assist Reprod Genet. 2019;36(12):2547–55.

Munne S, Sandalinas M, Escudero T, Fung J, Gianaroli L, Cohen J. Outcome of preimplantation genetic diagnosis of translocations. Fertil Steril. 2000;73:1209–18.

Ogilvie CM, Braude P, Scriven PN. Successful pregnancy outcomes after preimplantation genetic diagnosis (PGD) for carriers of chromosome translocations. Hum Fertil (Camb). 2001;4(3):168–71.

Palini S, De Stefani S, Primiterra M, Galluzzi L. Pre-implantation genetic diagnosis and screening: now and the future. Gynecol Endocrinol. 2015;31(10):755–9.

Rubio C, Rodrigo L, Garcia-Pascual C, Peinado V, Campos-Galindo I, Garcia-Herrero S, et al. Clinical application of embryo aneuploidy testing by next-generation sequencing. Biol Reprod. 2019;101(6):1083–90.

Scott RT, Jr., Upham KM, Forman EJ, Zhao T, Treff NR. Cleavage-stage biopsy significantly impairs human embryonic implantation potential while blastocyst biopsy does not: a randomized and paired clinical trial. Fertil Steril. 2013;100(3):624–30.

Simon AL, Kiehl M, Fischer E, Proctor JG, Bush MR, Givens C, et al. Pregnancy outcomes from more than 1,800 in vitro fertilization cycles with the use of 24-chromosome single-nucleotide polymorphism-based preimplantation genetic testing for aneuploidy. Fertil Steril. 2018;110(1):113–21.

Simopoulou M, Harper JC, Fragouli E, Mantzouratou A, Speyer BE, Serhal P, et al. Preimplantation genetic diagnosis of chromosome abnormalities: implications from the outcome for couples with chromosomal rearrangements. Prenat Diagn. 2003;23(8):652–62.

Stephenson MD, Sierra S. Reproductive outcomes in recurrent pregnancy loss associated with a parental carrier of a structural chromosome rearrangement. Hum Reprod. 2006;21(4):1076–82.

Velilla E, Escudero T, Munné S. Blastomere fixation techniques and risk of misdiagnosis for preimplantation genetic diagnosis of aneuploidy. Reprod Biomed Online. 2002;4(3):210–7.

Verlinsky Y, Tur-Kaspa I, Cieslak J, Bernal A, Morris R, Taranissi M, et al. Preimplantation testing for chromosomal disorders improves reproductive outcome of poor-prognosis patients. Reprod Biomed Online. 2005;11(2):219–25.

Ye Y, Qian Y, Xu C, Jin F. Meiotic segregation analysis of embryos from reciprocal translocation carriers in PGD cycles. Reprod Biomed Online. 2012;24(1):83–90.

Zhang L, Jiang W, Zhu Y, Chen H, Yan J, Chen ZJ. Effects of a carrier's sex and age on the segregation patterns of the trivalent of Robertsonian translocations. J Assist Reprod Genet. 2019a;36(9):1963–9.

Zhang L, Wei D, Zhu Y, Jiang W, Xia M, Li J, et al. Interaction of acrocentric chromosome involved in translocation and sex of the carrier influences the proportion of alternate segregation in autosomal reciprocal translocations. Hum Reprod. 2019b;34(2):380–7.

Zhang W, Liu Y, Wang L, Wang H, Ma M, Xu M, et al. Clinical application of next-generation sequencing in preimplantation genetic diagnosis cycles for Robertsonian and reciprocal translocations. J Assist Reprod Genet. 2016;33(7):899–906.