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# Analysis results of 169 cases of chorionic villus samples of missed abortion using high throughput sequencing

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**Abstract. – OBJECTIVE: This study aimed to evaluate the use of high-throughput sequencing (HTS) technology to detect chromosomes in chorionic villus samples of missed abortion embryos and investigate its utility in the genetic diagnosis of missed abortion.**

**PATIENTS AND METHODS: HTS was used to assess chorionic villus samples obtained from 169 patients with missed abortions from August 2020 to March 2022, at the Second Affiliated Hospital of Guangxi Medical University. The test results were statistically analyzed. To investigate the impact of advanced age on the incidence of chromosomal abnormalities, the patients were divided into two groups: elderly (≥35 years) and nonelderly pregnant women (<35 years).**

**RESULTS: (1) Among the samples of 169 patients, 100 (59.17%) cases of chromosomal abnormalities were detected. Among these 100, 90 (90%) had chromosomal numerical abnormalities and 10 (10%) had chromosomal structural abnormalities. (2) Chromosomal numerical abnormality was abnormalities mainly included aneuploidy (92.22%, 83/90), with trisomy (62.22%, 56/90) and monosomy (22.22%, 20/90) accounting for the majority. The top three numerical abnormalities included 18 cases of Turner syndrome (monosomy X; 20%, 18/90), 10 cases of trisomy 16 (11.11%, 10/90), and 10 cases of trisomy 22 (11.11%, 10/90). (3) Villous chromosomal abnormalities were found in 48 (70.59%) elderly pregnant women, and 52 (51.48%) nonelderly pregnant women, with statistically significant differences ($p < 0.05$).**

**CONCLUSIONS: (1) Chromosomal abnormality is an important cause of missed abortion, it majorly includes chromosomal numerical abnormality, of which most cases are of aneuploidy. (2) Advanced age may increase the risk of embryonic chromosomal abnormalities. (3) Villus chromosome detection using HTS has a positive value and can be used for analyzing and determining the causes of missed abortion.**

*Key Words:*
Missed abortion, High-throughput sequencing, Chorionic villus sample, Chromosome abnormality.

## Introduction

Missed abortion is a type of spontaneous abortion in which although the embryo or fetus is dead, it is still retained in the womb without natural birth. Recently, an increase in the occurrence of missed abortions has been noted. Studies have shown that approximately 12%-15% of missed abortions occur during the first 20 weeks of pregnancy[1,2]. Missed abortions have taken a significant negative impact; therefore, determining their cause and preparing for the next pregnancy is an urgent need for many women and families. However, the cause is complex and diverse; thus, the mental burden on patients is high, especially on females in whom missed abortions

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occur repeatedly. However, the exact cause and pathophysiological mechanism of missed abortion remain unknown. Most researchers considered the single action or interaction of genetic, environmental, maternal, infectious, endocrine, and immune factors as the underlying cause[3,4]. However, increasing evidence suggests that embryonic chromosomal abnormality is the major cause of early abortion, accounting for 50%-60% of cases[5-7], with chromosome aneuploidy being the most common cause[8]. Changes in chromosomal numbers increase genetic defects in embryos, and major genetic additions or deletions destroy gene balance thereby affecting normal cell proliferation and eventually leading to abortion. When missed abortion occurs, chromosome detection in the abortion sample is critical for diagnosing the cause of the abortion and guiding fertility treatments in the future[9]. Presently, the common methods of genetic testing include cell culture, fluorescence *in situ* hybridization (FISH), and high-throughput sequencing (HTS). Because specimen used for karyotype analysis of pregnancy products is tissue, maternal tissue contamination and examination failure are possible, and using the villus culture method for karyotype analysis is not recommended[10]. FISH can only detect an abnormal number of chromosomes but not detect the abnormal chromosomal structure and small variations. HTS, also known as next-generation sequencing, is a whole genome sequencing method that is commonly used to detect chromosome copy number variants (CNVs) in pregnancy products. HTS shows high sensitivity and specificity for screening common aneuploidy abnormalities in chromosomal diseases. It can simultaneously sequence hundreds of thousands to millions of deoxyribonucleic acid (DNA) molecules Further, it can not only perform uniform coverage scanning of the whole genome but also detect unreported chromosome CNVs, thereby exhibiting the noteworthy advantages of high flux, fast detection speed, and low cost of analyzing reported chromosome CNVs of >100 kb with high precision and accuracy[11-13]. Presently, HTS has attracted much attention and is being used in various fields, particularly in the study of genetic diseases. In this study, HTS was used to detect chromosomes in chorionic villus samples from 174 cases of missed abortion and determine the genetic factors of missed abortion and for guiding further clinical applications.

## Patients and Methods

### Patients

The research subjects were 169 patients with missed abortions from whom HTS of chorionic villus samples was performed at the Second Affiliated Hospital of Guangxi Medical University from August 2020 to March 2022. They had no complications and were singleton pregnancies. Complete information was available for all included patients. The patients had an average age of 32.46 ± 5.11 years, ranging from 20 to 46 years. Among the 169 patients, 68 were elderly pregnant women (≥35 years) and 101 were nonelderly pregnant women (<35 years old). Their gestational periods ranged from 5 to 14 weeks, with an average of 9.10 ± 1.88 weeks.

### Diagnostic Criteria

The diagnostic criteria for missed abortion were as follows[14,15]: (1) an ultrasonic examination revealing that the head hip length is > 7 mm without a fetal heartbeat; (2) the average diameter of the gestational sac in the uterine cavity is > 25 mm, without an embryo; (3) after 2 weeks of intrauterine pregnancy, there is no yolk sac, no embryo, and no fetal heartbeat; and (4) yolk sac can be seen, but without fetal heartbeat after 11 days in intrauterine pregnancy.

### Methods

#### Sample collection

In an aseptic environment, 5-10 g of clear white or milky white chorionic villus samples without blood filaments in abortion tissues that were in a good growth condition were selected and repeatedly washed with 0.9% sodium chloride injection before being submitted for examination. Simultaneously, 2-3 ml of pregnant women's peripheral blood was drawn for testing to determine maternal contamination in the chorionic villus samples.

#### Detection steps of HTS

Under bacteria-free conditions, DNA was extracted from chorionic villus samples and then cut into small fragments. The interrupted genomic DNA was used as the starting template, and after preparation using the PCR-free database building method, it was used to construct a DNA fragment library. Subsequently, the quality-controlled DNA library was sequenced on the Illumina NextseqCN500 sequencing platform using the amount of data required for chromosomal abnormality analysis. Finally, the detection results were analyzed by searching public databases, such as the data-

base of genetic variation, the database of chromosomal imbalance and phenotype in humans using ensemble resources, and the Online Mendelian Inheritance in Man database.

### Statistical Analysis

The proportion of chromosomal abnormalities was calculated by directly counting their frequency. The enumeration data were tested using $\chi^2$ test and statistically analyzed using Statistical Package for the Social Sciences version 25.0 software (SPSS Inc., IBM, Armonk, NY, USA). Differences were considered statistically significant for $p$-values of $<0.05$.

## Results

### Test Results of Chorionic Villus Samples of Missed Abortion

The chorionic villus samples of 169 cases of missed abortion were all successfully tested using HTS, with 68 (40.24%) chorionic villus samples having normal chromosomes. With chromosomal abnormality detection rate of 59.17% (100/169), 100 cases of chorionic villus samples with abnormal chromosomes were detected, including 90 (90%, 90/100) cases of chromosomal numerical abnormalities and 10 (10.00%, 10/100) cases of chromosome structural abnormalities.

### Chromosomal Numerical Abnormality

The 90 cases of chromosomal numerical abnormality were mostly of aneuploidy (92.22, 83/90), with trisomy (62.22%, 56/90) and monosomy (22.22%, 20/90) being the most common. The top three numerical abnormalities included 18 cases of Turner syndrome (monosomy X; 20%, 18/90), 10 cases of trisomy 16 (11.11%, 10/90), and 10 cases of trisomy 22 (11.11%, 10/90). There were also 2 cases of chromosome 21 monosomy (2.22%, 2/90), and 4 cases of double trisomy (8 + 13, 9 + 16, 13 + 16, and 15 + 16) (4.44%, 4/90). Moreover, as shown in Table I, 7 cases of euploid numerical abnormality (7.77%, 8/90), including 3 cases of triploidy (3.33%, 3/90) and 4 cases of polyploidy (4.44%, 4/90), were detected.

### Chromosomal Structural Abnormality

Table II shows that of the 10 cases of chromosomal structural abnormalities, 5 were microdeletions (50%, 5/10), 1 was microrepetition (10%, 1/10), and 4 were microdeletions and microrepetitions (40%, 4/10).

### Relationship Between Chorionic Chromosomal Abnormalities and Maternal Age

Among the 68 elderly pregnant women, 48 (70.59%) had villous chromosomal abnormalities. In contrast, among the 101 nonelderly pregnant

**Table I.** Chromosomal numerical abnormality in chorionic villus samples of patients with missed abortion.

| Type of Chromosomal Abnormality | n | Percentage (%) |
|---|---|---|
| Trisomy 2 | 1 | 1.11 |
| Trisomy 3 | 3 | 3.33 |
| Trisomy 4 | 3 | 3.33 |
| Trisomy 5 | 1 | 1.11 |
| Trisomy 6 | 2 | 2.22 |
| Trisomy 7 | 3 | 3.33 |
| Trisomy 8 | 2 | 2.22 |
| Trisomy 9 | 1 | 1.11 |
| Trisomy 10 | 1 | 1.11 |
| Trisomy 11 | 1 | 1.11 |
| Trisomy 13 | 6 | 6.66 |
| Trisomy 14 | 2 | 2.22 |
| Trisomy 15 | 6 | 6.66 |
| Trisomy 16 | 10 | 11.11 |
| Trisomy 18 | 1 | 1.11 |
| Trisomy 20 | 3 | 3.33 |
| Trisomy 21 | 3 | 3.33 |
| Monosomy 21 | 2 | 2.22 |
| Trisomy 22 | 10 | 11.11 |
| Monosomy X (Turner syndrome) | 18 | 20.00 |
| Double trisomy (8 + 13, 9 + 16, 13 + 16 and 15 + 16) | 4 | 4.44 |
| Triploidy | 3 | 3.33 |
| Polyploidy | 4 | 4.44 |
| Total | 90 | 100.00 |

**Table II.** Chromosomal structural abnormality in chorionic villus samples of patients with missed abortion.

| Type of Chromosomal Abnormality | Chromosomal Abnormality | Size (Mb) | Pathogenic | n | Percentage (%) |
|---|---|---|---|---|---|
| Short arm deletion and repetition of chromosome 1 | del(1) (p36.23p36.33):820000~8160000 | 7.34 | Yes | 1 | 10.00 |
| | dup(1) (p36.22p36.23):8160000~12340000 | 4.18 | Yes | | |
| Long arm deletion of chromosome 2 | del(2)(q37. 3q37.3):241160000~243020000 | 1.86 | Unknown | 1 | 10.00 |
| Long arm repetition of chromosome 6 | dup(6) (q21q27):111040000~170920000 | 59.88 | Yes | 1 | 10.00 |
| Short arm deletion and long arm repetition of chromosome 8 | del(8) (p23.3p21.2):160000~25960000 | 25.80 | Yes | 1 | 10.00 |
| | dup(8) (q21.13q22.1):81400000~98500000 | 17.1 | Yes | | |
| Short arm deletion of chromosome 11 | del(11)(p11.12p11.12) :50140000~51580000 | 1.44 | Unknown | 1 | 10.00 |
| Long arm deletion of chromosome 12 | del(12)(q23.1q23.1) :100240000~100360000 | 0.12 | Unknown | 1 | 10.00 |
| Long arm deletion of chromosome 14 | del(14)(q13.2q13.2) :35420000~35660000 | 0.24 | Unknown | 1 | 10.00 |
| Long arm deletion of chromosome 16 | del(16) (q24.3q24.3):89480000~89680000 | 0.20 | Maybe | 1 | 10.00 |
| Long arm deletion of chromosome 4 and long arm repetition of chromosome 18 | del(4)(q32. 3q35.2):167680000~190940000 | 23.26 | Yes | 1 | 10.00 |
| | dup(18) (q11.2q23):23200000~78020000 | 54.82 | Yes | | |
| Long arm repetition of chromosome 7 and long arm deletion of chromosome 13 | dup(7)(q31.3 2q36.3):121180000~159138663 | 37.96 | Yes | 1 | 10.00 |
| | del(13) (q21.33q34):69020000~115100000 | 46.08 | Yes | | |
| Total | | | | 10 | 100.00 |

women, 52 (51.48%) had villous chromosomal abnormalities. Table III shows that there were statistically significant differences between these two groups ($p < 0.05$).

## Discussion

### Results of Chromosome Detection in Chorionic Villus Samples of Missed Abortion

In this study, HTS was used for the genetic assessment of chorionic villus samples of patients with missed abortions. Among the included samples, 59.17% had chromosomal abnormalities, 90% of which were chromosomal numerical abnormalities, with aneuploidy accounting for 92.22%. The most common aneuploidy anomalies were trisomy and monosomy, with the top three being Turner syndrome (monosomy X chromosome monomer; 20%), trisomy 16 (11.11%), and trisomy 22 (11.11%). In most cases of Turner syndrome miscarriage occurs during the embryonic stage, and trisomies 16 and 22 usually lead to embryonic development discontinuation in early pregnancy. It is a common autosomal abnormality in case of spontaneous abortion in early pregnancy. This study corroborates the findings of previous reports[16-18]. Herein, we found 10 cases of chromosomal structural abnormalities, which were mainly chromosomal microdeletion, microrepetition, and concomitant microdeletion and

**Table III.** Relationship between chorionic chromosomal abnormalities and maternal age.

| Groups | n | Chromosomal Abnormalities [n (%)] |
|---|---|---|
| Elderly pregnant women | 68 | 48 (70.59) |
| Non-elderly pregnant women | 101 | 52 (51.48) |
| $X^2$ value | | 6.14 |
| *p*-value | | 0.01 |

microrepetition. In five of these cases, abnormal chromosome fragments with pathogenicity were detected. According to our findings, chromosomal abnormality remained the leading cause of missed abortions, with chromosomal numerical abnormality being the most important factor. Aneuploidy abnormalities were the main form, with Turner syndrome (monosomy X), trisomy 16, and trisomy 22 being the most common. Among chromosomal structural abnormalities, HTS can detect microdeletions and microduplications of chromosome fragments, thereby improving the detection rate of chromosomal abnormalities. Therefore, chromosome analysis of chorionic villus samples of patients with missed abortions can help to clarify the etiology of this condition, guide the next pregnancy, improve the success rate of pregnancy, reduce the birth rate of fetuses with chromosomal abnormalities, and reduce physical injury and psychological burden in patients. With a high detection rate, HTS shows a high sensitivity for diagnosing chromosomal structural abnormalities, making it suitable for chromosome analysis of chorionic villus samples of patients with missed abortions.

### Relationship Between Age and Chromosomal Abnormalities

Advanced maternal age (≥35 years old) during conception is related to an increased risk of poor maternal, perinatal, and neonatal outcomes, in addition to being a high-risk factor for chromosomal abnormalities in the embryo[19]. With the increase in pregnant women's age, their ovarian function decreased and the incidence of embryonic chromosomal abnormalities increased, thereby increasing the incidence of embryonic aneuploidy and the probability of abortion. Advanced age is a high-risk factor for early embryonic development discontinuation[20]. In this study, the incidence of chorionic chromosomal abnormalities in elderly pregnant women was 70.59%, which was higher than in nonelderly pregnant women (51.48%), indicating a correlation between advanced age and the increased incidence of embryonic chromosomal abnormality. Therefore, women should have children at a younger age to reduce the incidence of missed abortion and avoid its negative effects.

## Conclusions

Embryonic chromosomal abnormality is a major cause of missed abortions in early pregnancy, and advanced age may increase the risk of embryonic chromosomal abnormality. Chromosome analysis of chorionic villus samples after abortion using HTS is helpful to clarify the etiology of missed abortions and has a positive application value in providing reasonable eugenic genetic counseling and guiding the next pregnancy. HTS can also help in accumulating relevant data for online databases, guide genetic counseling for patients with missed abortions, and aid in elucidating the causes of missed abortions.

### Conflicts of Interest

The authors declare no conflicts of interest.

### Ethical Approval

This study was approved by the Second Affiliated Hospital of Guangxi Medical University Ethics Committee on 24/09/2021.

### Informed Consent

All patients in the study signed an informed consent.

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### Authors' Contributions

Li Deng performed the experiment and wrote the manuscript; Junyou Su performed the experiment and contributed to the paper revision and submission; Yan Huang performed the data arrangement and analyses; Baoheng Gui and Xianda Wei performed the experiment; Junru Tong, Yan Chen, Ping Xu, Jian Cai, Aihua Xia, Lingfang Tang, Xixi Li, Jingyou Lan and Yuanli Wang contributed to the specimen collection.

### Data Availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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