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Research Article
Application of High-Throughput Ligation-Dependent Probe Amplification (HLPA) Detection in Karyotype Analysis of Spontaneous Abortion Pregnancy Tissues

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Abstract

Background and Objective: Spontaneous abortion is one of the most common pregnancy diseases and seriously affects women's reproductive health. To investigate the relationship between spontaneous abortion and chromosomal abnormality of pregnancy tissues, the karyotype of aborted pregnancy tissues was analyzed by using high-throughput ligation-dependent probe amplification (HLPA). **Materials and Methods:** About 159 patients with spontaneous abortion were used as research subjects. The villi of all patients were detected by fluorescence *in situ* hybridization (FISH) and the distribution of normal and abnormal chromosomes was confirmed. Chromosome examination was performed by HLPA technique and karyotype analysis. The sensitivity and specificity of HLPA and karyotype analysis were compared and the karyotype of aborted pregnancy tissues was analyzed by the HLPA technique. **Results:** The abnormal rate of chromosomes detected by HLPA was higher than that by karyotype analysis (54.09 vs. 38.36%) and the difference was statistically significant ($p<0.05$). The sensitivity and specificity of HLPA detection were better than those of karyotype analysis (sensitivity: 84.95 vs 77.42%, specificity: 89.39 vs. 86.36%). The abnormal rate of chromosome number, structure and single diploid detected by HLPA was 88.37, 10.47 and 1.16%, respectively. Among the abnormal chromosome numbers, trisomy 16 (27.63%), triploid (21.05%) and 45XO (13.16%) are the top three. The older the age, the higher the abnormal rate of embryo chromosome, The greater the gestational age of abortion, the lower the abnormal rate of embryo chromosome. **Conclusion:** HLPA has high flux, sensitivity, specificity and accuracy in karyotype analysis of spontaneous abortion pregnancy, which is worthy of popularization and application in clinics.

Key words: Spontaneous abortion, probe, chromosomal, karyotype analysis, clinical application, HLPA, pregnancy

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Spontaneous abortion is one of the most common reproductive diseases and has seriously affected the reproductive health of women. It is defined as patients with natural termination because of having less than 28 weeks of gestation and less than 1000 g of fetal weight[1]. The incidence of spontaneous abortion is about 10 20% of clinical pregnancies, 80% of these are early spontaneous abortion[2]. Based on current clinical findings, the known causes of spontaneous abortion include embryonic, maternal, immunologic and environmental factors and embryo chromosome abnormality accounts for more than 50%[3]. Existing studies have confirmed that chromosomal abnormalities in embryos are the most important cause of early spontaneous abortion. In spontaneous abortion in the first trimester, chromosomal abnormalities in embryos account for about 50 70%, among them, abnormal chromosome number (chromosome aneuploidies) is the most important cause of early abortion. The main chromosomal number abnormalities were trisomy (60%), polyploid (15%) and X monomer (13%)[4]. Therefore, chromosome detection is very important for early spontaneous abortion embryos.

High-throughput ligation-dependent probe amplification (HLPA) is a kind of multiplex fluorescence PCR amplification technology based on traditional multiplexed probe amplification technology (MLPA), It is mainly used to detect the change of copy number of nucleotide sequence, A maximum of 6 copies of variation can be detected by this detection technique With traditional karyotype analysis techniques, Such as G generation karyotype analysis, FISH PCR and other technologies, HLPA has the advantages of high throughput, high accuracy, high resolution, high repeatability and fast detection speed[5]. In this study, a copy number analysis of 159 spontaneous abortion pregnancies patients using HLPA, discuss the relationship between spontaneous abortion and chromosomal abnormality of pregnancy tissues and the application value of HLPA technique in karyotype analysis of abortion pregnancy tissues.

MATERIALS AND METHODS

Study area: The study was carried out at the Department of Center of Clinical Reproductive Medicine, Second Affiliated Hospital of Soochow University Suzhou, Jiangsu, China from May, 2014-July, 2020.

Clinical information: From May, 2014-July, 2020, a total of 159 pregnant women who were diagnosed by ultrasound to stop the development of the embryo required an abortion pregnancy tissues embryo chromosome examination, Informed consent was signed before inspection. All pregnant women were detected by fluorescence *in situ* hybridization (FISH) with 66 normal chromosome cases and abnormal 93 cases of them. The average age of patients was 30±3.68 years (23~42 years), the average gestational week of abortion was 8.72±2.39 w (6~25 weeks), there were 57 cases of recurrent abortion and 102 cases of incidental abortion, there were 77 cases in ≤29 years old, 62 cases in 30~34 years old and 20 cases in ≥35 years old.

Villi specimen pretreatment: Made sure the embryo stop and explained the condition to the patients, then performed uterine cleaning after patients signed the consent form. Professionals selected 50 200 mg villi from the abortion tissue obtained by the operation and immediately rinsed it with sterile saline, then removed the pregnant woman's blood and decidual tissue until the villi tissue became clear. In the end, put it into a 2 mL sterile centrifuge tube containing 1 mL preservation solution (75% alcohol) and put the label into a sample box as well as send it to test immediately.

Abortion villi detection (HLPA detection) and maternal blood contamination identification (STR detection): To detect the DNA of abortion villi: (1) Sample DNA extraction: The DNA of villi was extracted by Kaijie's DNA extraction kit (model: Qiagen51304) (to ensure that the extracted DNA concentration was not less than 20 ng μL^{-1}), (2) Multiple-specific double junction reaction: DNA samples of 150 ng were added to each reaction tube, added sterilized distilled water and diluted to 8 μL and added 8 μL of elution solution to the negative control. Preparation of premixed solution (according to the instructions provided by the manufacturer): After mixing, each tube was divided into 12 μL to the reaction hole for subsequent PCR (ABI2720PCR instrument). Reaction process: 98°C 2 min, 95°C 30 sec and 60°C 3 hrs for 5 cycles, It took a total of 15 hrs for the connection reaction to be placed in a warm bath at 60°C, after the end of the reaction, 10 μL of "connection reaction termination liquid" was added to terminate the connection reaction, (3) Multiple fluorescence PCR amplification of ligands: For the linked product, multiplex fluorescence PCR amplification. Preparation of premixed liquid (according to the instructions provided by the manufacturer): after mixing, each reaction tube was divided into 19 μL, respectively PCR amplification after adding 1 μL of junction products, PCR program was designed as follows: 95°C for 5 min, 5×(94°C for 20 sec, 6~l°C/cycle for 40 sec, 72°C for 1.5 min), 94°C for 20 sec, 57°C for 30 sec, 72°C for 1.5 min,

27 cycles, 68°C for 1 hr, storing at 4, (4) Fluorescence capillary electrophoresis separation of amplification products: 1 μL PCR products was diluted 5 times with sterilized purified water, then the diluent of 1 μL amplification product was mixed with 0.1 μL IZ500 and 8.9 μL Hi-DI and denaturated at 95°C for 5 min with upper ABI gene analyzer (PRISM 3130XL). Data collection used data collection® software, data gene mapper ID 4.0 (applied Biosystems), CNVplex Converter (GeneSky) and CNV Reader 1.00 (GeneSky) software automatic analysis, (5) Copy count, (6) Data quality assessment for detection values between 0~0.1, 0.8~1.2, 1.75~2.25, 2.7~3.3, 3.6~4.4, we set the quality level as 1, others as 2, the probe pair ratio with a quality level of 1 could be used as a quality assessment parameter for the overall data, If the probe pair ratio was less than 80%, the sample is recommended to re-experiment, (7) Human aneuploidy of 24 chromosomes: (1) If the integer copy values of more than 3 consecutive target areas deviate from the normal copy value 2 and there are at least 2 detection values with quality level 1, the areas detected by these probes are judged to be missing or duplicate, (2) If the integer copy value deviates from the normal copy value only occurs in more than two consecutive target areas and the quality level of at least one detection value is 1, then the target areas determined by these two pairs of probes are judged as possible missing or duplicates, it is recommended to repeat the experiment to determine, (3) If there are more than three consecutive copy number of target zone values deviate from 0.8~1.2 (for X or Y chromosome probe pair) or 1.75~2.25 (for autosomal chromosome probe pair) but not in the above two cases and these three values the variation coefficient is less than 10%, so this 3 probe to determine the copy number of target zone is defined as the candidate of abnormal (absent or repeated) area, under the circumstances, you need to repeat the experiment to determine after the test sample is considered to be suspicious or chimera pollution sample. Identification of maternal blood contamination (STR test): (1) 17 detection sites in chromosomes were selected, including 10 commonly used D13S317, D7S820, D18S51, D8S1179, D2S1338, D16S539, vWA, TH01, D8S588, D5S818 individual identification locus and one gender locus, in addition, six additional loci with high heterozygosity G4S0001, G2S0002, G15S0001, G7S0005, G10S0001 and G5S0001 were added to enhance individual recognition rate, (2) During the detection, 17 STR loci were detected in maternal blood and villi tissue samples, capillary electrophoresis was used to analyze 17 multiplex PCR products and the source of the two sites was compared to determine whether the villi were contaminated with maternal blood.

Karyotype analysis: After routine villus cell culture, analysis were conducted on the Karyotypes by MetaSystems chromosome automatic scan analysis system.

Statistical analysis technique: Using SPSS 13.0 software to analysis of statistical analysis, the Chi-square test was used for inter-group comparison of counting data. Measurement data were expressed as mean standard deviation "$\bar{x}\pm s$" and comparisons between the two groups were performed by independent sample t-test. Enumeration data were presented in the form of cases (percentage) and the chi-square test was used. p 0.05 indicated that the difference was statistically significant.

RESULTS

Baseline data of different spontaneous abortions were compared: There was no statistically significant difference in age, Number of births and abortion gestational age between 1 time and $\geq$2 times spontaneous abortions ($p>0.05$). However, the difference between the number of spontaneous abortions and the number of adverse pregnancies was statistically significant ($p<0.05$) (Table 1).

Detection of villous specimens from spontaneous abortion pregnant women compared the two detection methods: All 159 spontaneous abortion villi samples were successfully detected by two detection methods. According to the HLPA test, 73 cases were normal and 86 cases were abnormal, with an abnormal rate of 54.09%. According to karyotype analysis, 98 cases were normal, 61 cases were abnormal and the abnormal rate was 38.36%. The chromosome abnormality rate detected by HLPA was higher than that detected by karyotype analysis and the difference was statistically significant ($p<0.05$) (Table 2).

Comparison of detection efficiency between two detection methods: The sensitivity and specificity of HLPA detection were 84.95 and 89.39%, respectively. The sensitivity and specificity of karyotype analysis were 77.42 and 86.36%, respectively. The comprehensive analysis shows that the sensitivity and specificity of HLPA detection are better than that of nuclear type detection (Table 3).

HLPA detection results: Among the 86 abnormal specimens, 76 cases (88.37%) had abnormal chromosome numbers, 9 cases (10.47%) had abnormal structure and 1 case (1.16%) had a single diploid (Table 4 and Fig. 1a). Among the

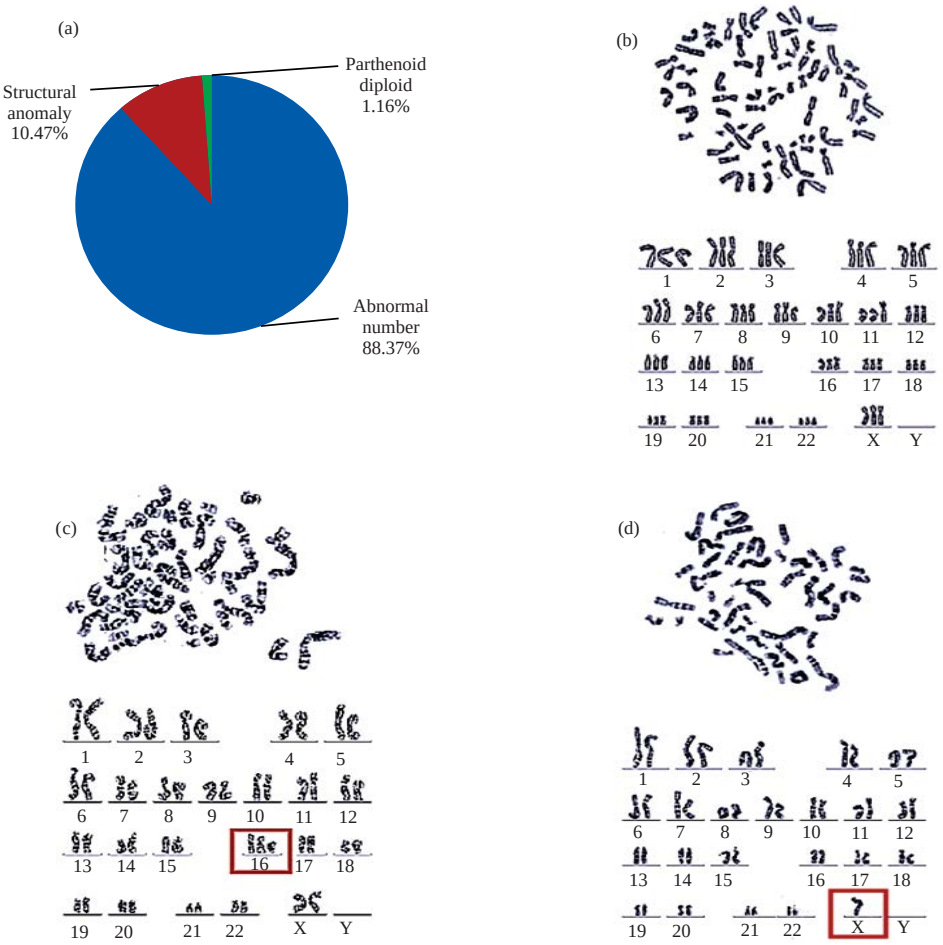

Fig. 1: HLPA detection results, (a) Analysis of chromosomal abnormalities in 86 cases, (b) Morphology of Triploid, (c) Trisomy 47, XX, +16 and (d) X monomer

Table 1: Baseline data of different spontaneous abortions were compared

Number of spontaneous abortions	Number of case (N)	Age (year)	Number of pregnancies (time)	Number of births (time)	Adverse pregnancy (time)	Abortion gestational age (week)
1 time	20	30.35±3.43	1.30±0.57	0.10±0.31	1.0	9.20±2.82
≥2 times	139	30.12±3.75	3.16±1.16	0.19±0.40	2.61±0.88	8.67±2.33
t		0.259	7.035	0.964	8.160	0.926
p-value		0.796	<0.001	0.336	<0.001	0.356

Table 2: Results of the detection of villous specimens from spontaneous abortion pregnant women were compared between the two detection methods

Detection method	Number of case (N)	Chromosomal normal	Chromosome abnormality
HLPA	159	73 (45.91)	86 (54.09)
Karyotype analysis	159	98 (61.64)	61 (38.36)
χ^2			7.907
p-value			0.005

Table 3: Comparison of detection efficiency between two detection methods

	HLPA detection results				Karyotype analysis results		
Actual results	Abnormality	Normal	Total	Actual results	Abnormality	Normal	Total
Abnormality	79	14	93	Abnormality	72	21	93
Normal	7	59	66	Normal	9	57	66
Total	86	73	159	Total	61	98	159

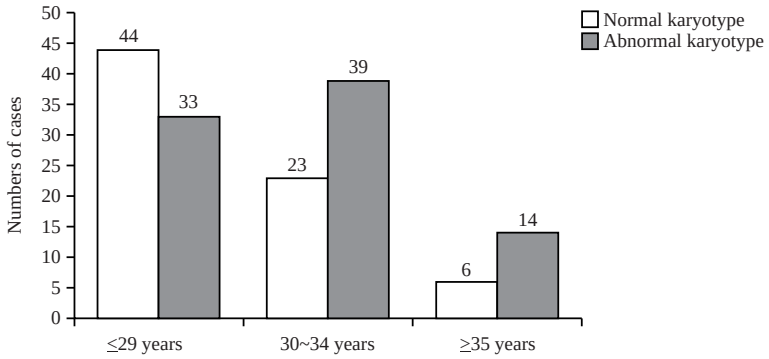

Fig. 2: Relationship between different age groups and karyotype of the embryo

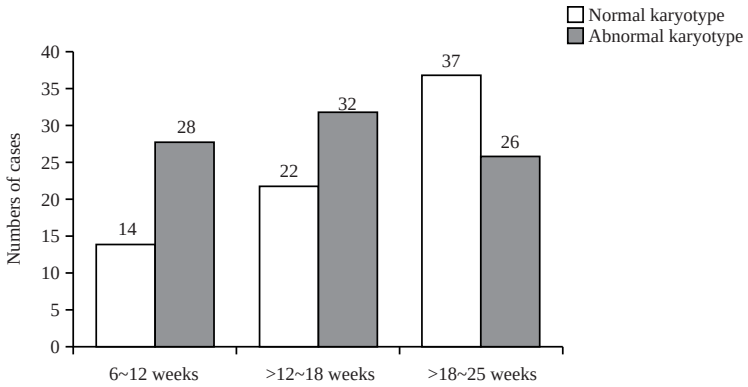

Fig. 3: Relationship between different gestational ages of abortion and karyotype of the embryo

chromosome number abnormalities, 21 cases (21/76, 27.63%), 16 cases (16/76, 21.05%) and 10 cases (10/76, 13.16%) with trisomy 16 were the top three, respectively. Examples of karyotypes are shown in Fig. 1b-d.

Relationship between different age groups and karyotype of the embryo: There were 77 cases of abortion in ≤29 years old, among which 33 cases were of abnormal karyotype, accounting for 42.9%. There were 62 cases of abortion in 30~40 years old, among which 39 cases were of abnormal karyotype, accounting for 62.9%. There were 20 cases of abortion ≥35 years old, including 14 cases of abnormal karyotype, accounting for 70%. The proportion of patients with abnormal karyotypes in the three groups showed an increasing trend and the difference between the three groups was statistically significant ($p<0.05$) (Table 5 and Fig. 2).

Relationship between different gestational ages of abortion and karyotype of the embryo: There were 42 patients with 6~12 weeks abortions, among whom 28 patients had abnormal karyotype, accounting for 66.67%. There were 54 cases of >12~18 weeks abortion, including 32 cases of abnormal karyotype, accounting for 59.26%. There were 63 patients with >abortion (18~25 weeks), among whom 26 were of abnormal karyotype (41.27%). The proportion of patients with abnormal karyotypes in the three groups showed a decreasing trend and the difference between the three groups was statistically significant ($p<0.05$) (Table 6 and Fig. 3).

Incidence of chromosomal abnormalities in both husband and wife and their relationship with chromosomal abnormalities in embryos: Among 159 couples with spontaneous abortion, 126 of them underwent chromosome testing in peripheral blood of both couples and 33 of them did not. 8 of the 126 cases were abnormal, with an abnormal rate of 6.35%. Among them, 6 patients were abnormal in females, 1 in males and 1 in both males and females. The chromosome abnormality rate of embryos in the 8 patients was 100%. Among them, female and embryo chromosome abnormalities accounted for 6.98%, male and embryo chromosome abnormalities for 1.16%, both male and female chromosome abnormalities for 1.16% and both male and female normal but embryo chromosome abnormalities for 90.70% (Table 7).

Table 4: HLPA detection results

Type of chromosomal abnormalities	Chromosomal abnormal karyotype	Number of case (N)	Proportion (%)
Abnormal chromosome number			
Trisomy 45 cases (52.33%)	47, +16	21	24.42
	47, +22	4	4.65
	47, +2	3	3.49
	47, +13	3	3.49
	47, +3	2	2.33
	47, +8	2	2.33
	47, +14	2	2.33
	47, +15	2	2.33
	47, +21	2	2.33
	47, +4	1	1.16
	47, +6	1	1.16
	47, +11	1	1.16
	47, +20	1	1.16
Double trisomy 4 cases (4.65%)			
	48, +7, +18	1	1.16
	48, +8, +8	1	1.16
	48, +10, +16	1	1.16
	48, +12, +14	1	1.16
Triploid	Triploid	16	18.61
X monomer	45, XO	10	11.63
Sex chromosome polybody	47, XXX	1	1.16
Abnormal structure	Microdeletion, microduplication	7	8.14
	Heterozygous absence	1	1.16
	Chimera	1	1.16
Single diploid	-	1	1.16
Total		86	100.00

Table 5: Relationship between different age groups and karyotype of the embryo

Age (years)	Number of cases (N)	Normal karyotype [(case)%]	Abnormal karyotype [(case)%]
≤29	77	44 (57.1)	33 (42.9)
30~34	62	23 (37.1)	39 (62.9)
≥35	20	6 (30.0)	14 (70.0)

Table 6: Relationship between different gestational ages of abortion and karyotype of the embryo

Embryo stop time (week)	Number of cases (N)	Normal karyotype [(case)%]	Abnormal karyotypes [(case)%]
6~12	42	14 (33.33)	28 (66.67)
>12~18	54	22 (40.74)	32 (59.26)
>18~25	63	37 (58.73)	26 (41.27)

Table 7: Result of chromosome abnormality in couples and chromosome abnormality in embryo

	A couple of peripheral blood chromosome	Embryonic chromosome
Female	Robertson translocation	47, +3
Female	Inv (9) (p12q13)	Triploid
Female	t (3, 5) (p13, p14)	The p-terminal portion of chromosome 3 was duplicated and the p-terminal portion of chromosome 5 was missing
Female	Inv (9) (p12q13)	48, +8, +8
Female	Inv (14) (p13q13)	45, X0
Female	Inv (9) (p12q13)*2	47, +13
Male	Inv (9) (p12q13)	45, -21(Suspected chimeric)
Both male and female	46,xx,9qh+/46,xy,14ps+	47, +14

DISCUSSION

This study showed that the chromosome abnormality rate detected by HLPA was higher than that detected by karyotype analysis (54.09 vs. 38.36%) and the sensitivity and specificity of HLPA detection were both superior. The advantage of HLPA detection technology lies in that the high-throughput ligation-dependent probe amplification (HLPA) could detect the copy number of the target region. A highly specific ligase was used to connect the hybridized target region, then through the

patented technology, the tag sequence with different lengths was introduced into the end of the 3 'end connection probe to obtain the target probe connection products with different lengths. PCR amplification was performed on the ligated products using fluorescent labelled universal primers, furthermore, the amplified PCR products were separated and detected by fluorescence capillary electrophoresis. Finally, The peak height of each PCR amplification product was obtained by analyzing the electrophoretic map, after that the data software provided by the manufacturer was automatically analyzed to determine the copy number of the sample destination area[6,7]. HLPA probes cover 24 chromosomes and can detect the deletion and duplication of all chromosome aneuploidy, chromosome arm and terminal and partial microdeletion and microduplication. Combined with the individual identification kit, it can also detect polyploids and monoploids. Besides, hints are also given for chimaeras with chimerism rates greater than 30%.

One fifty nine cases of spontaneous abortion pregnancy tissues were detected by HLPA. Among them, the proportion of abnormal karyotypes was 54.09% and the abnormal number of chromosomes in abnormal karyotypes (88.37%) was the most common, mainly trisomy (59.21%), triploid (21.05%), 45, XO (13.16%), which was consistent with literature reports[8]. Trisomy 16 was the most common autosomal trisomy (46.67%). The abnormal trisomy 16 is usually fatal to the development of human embryos, which is clinically manifested as pregnancy abortion. Pregnancy abortion is mainly caused by the process of germ cell meiosis or the non-separation of chromosomes during early cleavage after the zygote. Trisomic abnormalities are mostly the result of random mutations and natural selection, rather than due to the environment in which a woman is conceived or inadequate precautions taken. The triploid abnormality was second only to autosomal trisomy (21.05%). The occurrence of triploid is mostly caused by a spontaneous mutation and abortion is caused by serious genetic defects of the fetus, which is a result of natural selection[9]. In the second pregnancy, a woman preparing normally for pregnancy is ok but pay attention to monitoring and having a regular B-scan during pregnancy. At the same time, the woman still needs a close follow-up clinic. When triploid syndrome is highly suspected, it is suggested that the chromosome karyotype analysis of amniotic fluid cells be carried out promptly to confirm the diagnosis and terminate the pregnancy as soon as possible. In this study, the X-haplotype was 45, XO (13.16%). The 45, XO sex chromosome has only one X chromosome, which is a member of Turner syndrome (X Monosomy syndrome), also known as congenital ovarian dysplasia. 99% of patients with Turner syndrome have spontaneous abortions during pregnancy and about 15% of early spontaneous abortions. The reason for abortion is that the neutral chromosomes do not separate during meiosis[10]. However, about 70% of sex chromosome non-segregation occurs on the paternal side[11]. Most of these chromosomal abnormalities are newly occurring random mutations with a low risk of recurrence. Therefore, women only need to prepare for pregnancy again. In this study, there were 9 cases of abnormal chromosome structure in embryos. Among them, 8 couples had normal chromosomes, only 1 case had p-terminal partial duplication of 46, XY and chromosome 3. The maternal karyotype of a fetus with a partial p-terminal deletion of chromosome 5 was t (3,5) (p13, P14), however, the paternal karyotype is normal. It suggests that chromosome abnormalities in embryos are inherited from their mothers[12]. Balanced translocations are the most common form of chromosomal structural abnormalities. Since there is generally no increase or decrease in genetic material, if one of the spouses carries a balanced translocation, there is usually no phenotypic abnormality of its own. Yet during the process of germ cell meiosis, a variety of unbalanced rearrangements of gametes can be produced and thus further cause abortion, stillbirth, stillbirth, birth of chromosomal abnormalities multiple teratomas. In this way, if partial deletion and partial duplication are found in the flow product detection, it is highly suspected that there is a balanced translocation in both spouses. If the parents are not balanced translocation carriers, it is suggested that the abortion is a random mutation and the result of natural selection, so the woman just needs to adjust the state of normal pregnancy. Older women have a higher risk of aneuploidy pregnancy. In the experimental study in this paper, the chromosome abnormality rates of embryos in the groups $\leq$29 years, 30~34 years and $\geq$35 years were 43.6, 62.9 and 70%, respectively.

The results showed that the chromosome abnormality rates of embryos in the three age groups from high to low were 70, 62.9 and 42.9%, respectively. According to literature reports, the risk of abortion will increase with the gradual increase of the age of the woman due to chromosomal abnormalities in the embryo[13]. With the growth of age, the egg cells gradually senescence and degeneration and the spindle in the fertilized egg cells ages, resulting in functional insufficiency or loss and the non-separation of chromosomes in germ cells or fertilized egg during cell slowing division or mitosis, leading to the generation of fetuses with abnormal chromosome number or structure[14]. Therefore, advanced age is a high-risk factor for chromosomal abnormalities in embryos. The results also showed that the larger the gestational week of abortion, the lower the chromosome

abnormality rate of embryos. It indicated that the earlier abortion occurs, the higher the probability of chromosome abnormality in embryos. More than 50% of early abortion patients with the gestational age of 6~8 weeks are caused by chromosome abnormalities[15]. Accordingly, the couple that has a birth plan should make a chromosomal check as early as possible when the woman or her mother, both brothers and sisters have ≥2 abortion history, discover the pregnancy with chromosomal abnormalities to terminate the pregnancy immediately, reduce the harm caused by abortion to the woman.

The chromosome abnormality rate in the general population of China is 0.5 1.0%, while the chromosome abnormality rate in patients with adverse pregnancy history is 2~10%[16]. In this study, 126 cases of peripheral blood chromosomes of couples were examined and 8 cases were found to be abnormal, with an abnormal rate of 6.35%, which was consistent with literature reports. However, the results of chromosome karyotype examination showed that the chromosomal abnormalities in embryos were not completely consistent with the chromosomal structural abnormalities in parents. And thus the results do not suggest the parental chromosome abnormality, the progeny chromosome abnormality. Instead, it can only prove that chromosomal abnormalities in one or both spouses are high-risk factors for developing chromosomal abnormalities in embryos.

In addition, some scholars have proved that the abnormal rate of the embryo chromosome is 54.09%, while the abnormal rate of chromosome in peripheral blood of one or both couples with abnormal embryo chromosome is only 9.30% (8/86). It showed that embryos with abnormal chromosomes had more normal parents. Therefore, vertical inheritance is not the main cause of chromosomal abnormalities but abnormalities in gametes of one or both parents or chromosomal mutations in the formation and development of embryos are the main causes[17,18].

Similar to previous studies, this study believed that chromosomal abnormalities in embryos were the main cause of early spontaneous abortion. Therefore, when looking for the cause of spontaneous abortion, it is very necessary to carry out chromosome testing on the aborted embryo or villi first, especially for the patients with more than 2 times of spontaneous abortion. It can help explain the causes of abortion scientifically, reduce the unnecessary psychological burden of patients, provide genetic guidance for the next pregnancy or choose the right auxiliary treatment. This way can help women avoid unnecessary testing and receiving the wrong treatment regimens. In the meantime, it provides clinicians with the correct direction in the screening of recurrent abortion. However, the sample size of the data included in this study is small, so further analysis will not be carried out at present and further research will be needed to expand the sample size at a later stage.

CONCLUSION

HLPA can not only cover all 24 chromosomes but also detect subtle aberrations that cannot be detected by traditional detection techniques. This detection method is cost-effective for the detection of flow products and has high clinical application value.

SIGNIFICANCE STATEMENT

This study showed high-throughput ligation-dependent probe amplification detection in karyotype analysis of spontaneous abortion pregnancy tissues which can be helpful in the improvement of current detection of spontaneous abortion during early pregnancy in women. This result will help the researchers to discover the critical function of HLPA technology that many researchers were not able to explore.

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