Result and pedigree analysis of spontaneously abortion villus chromosome detecting by FISH

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ABSTRACT. The aim of this study was to evaluate the relationship between fetal karyotype and parental chromosomal abnormalities, and to provide a basis for clinical diagnosis and therapy in Northeast China. A total of 144 spontaneously aborted fetuses were analyzed by FISH to test for chromosome number and to recall couples for peripheral blood karyotype analysis. The rate of abnormal chorionic villus chromosomes was 35.42%. Villus chromosome abnormality rate of the first spontaneous abortion and repeated abortions were 40.54 and 33.64%, respectively ($P < 0.05$). The rate of chromosome abnormality in women with advanced maternal age and women younger than 35 years old were 46.43 and 32.76%, respectively ($P < 0.05$). In a recall of 112 couples for peripheral blood karyotype analysis, just 3 cases of 7 patients with peripheral blood chromosome abnormality showed abnormal FISH

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results in their abortion villi. Fetal chromosome number abnormality is a major cause of early abortion, and parental chromosomal abnormality is not the main factor in abnormal fetal karyotype. A complete evaluation and special treatment should be provided to couples with a history of recurrent miscarriage.

Key words: Chromosomal abnormality; Spontaneous abortion; Amniocentesis; Genetic counseling

INTRODUCTION

Almost 15-20% of all pregnancies end up in a spontaneous abortion (SA), in which the incidence of chromosomal abnormalities is as high as 70% (Usha et al., 2011). Recurrent miscarriage (RM) is defined as the occurrence of two or more consecutive pregnancy losses. RM occurs in 1 to 5% of all couples trying to conceive (Hogge et al., 2003). About 50 to 60% of all miscarriages are associated with cytogenetic abnormalities. Therefore, our study aimed to determine the frequency and distribution of fetal chromosomal abnormalities from miscarriages in couples with SA or RM and to see whether or not there was any difference in the frequency and distribution of chromosome abnormalities between the first pregnancy loss and two or more subsequent losses.

MATERIAL AND METHODS

This study involved a retrospective evaluation of fluorescence *in situ* hybridization (FISH) results of chorionic villus samples in 144 SA cases at the First Bethune Hospital of Jilin University between September 2012 and April 2014. The median age of the mothers was 31.0 (range 23-41 years). The gestational age (fetal demise at 6-12 weeks gestation) at the time of pregnancy loss was estimated by reviewing ultrasound. Written informed consent was obtained from all study subjects prior to enrollment in the study.

FISH was performed using commercially available whole-chromosome painting probes for chromosomes 13, 16, 18, 21, 22, X and Y. Chromosome denaturation, hybridization, and signal detection were done according to published protocols (Licher et al., 1988). Peripheral blood karyotyping was performed in parents. Chromosomal abnormalities were reported according to the current international standard nomenclature. In all tables, data are reported as number of cases and percentage. Comparisons between groups were conducted using the chi-square test, and $P < 0.05$ was considered to be statistically significant in all tests.

RESULTS

Among the 144 cases of SA, villus chromosome analysis revealed 51 cases of abnormal signals, indicating a 35.42% rate of chromosomal abnormality in the chorion villus samples. Among these 51 cases, there were 12 cases of trisomy 16 and monosomy X, 7 cases of trisomy 22, 5 cases of trisomy 13, and 4 cases of triploid and tetraploid (details are given in Table 1).

Table 1. Total chromosomal abnormalities in chorionic villus sample among 144 cases of spontaneous abortion.

Abnormality	Number (N)	Proportion in abnormal cases (%)	Proportion in total cases (%)
Trisomy 16	12	23.53	8.33
Monosomy X	12	25.49	9.03
Trisomy 22	7	13.73	4.86
Trisomy 13	5	9.80	3.47
triploid	4	7.84	2.78
tetraploid	4	7.84	2.78
Trisomy 18	2	3.92	1.39
Trisomy 21	2	3.92	1.39
Monosomy 21	1	1.96	0.69
Trisomy 16/22	1	1.96	0.69
Trisomy 21/22	1	1.96	0.69

In Table 2, data are divided into SA and RM groups. There were 37 cases in the RM group and 10 cases were abnormal, showing a rate of chromosomal abnormality of 40.54%. In the SA group, there were 41 cases of numerical chromosomal abnormality in a total of 107 cases, showing a rate of chromosomal abnormality of 33.64%. The SA and RM groups showed no statistically significant difference ($P < 0.05$).

Table 2. Outcome of FISH in chorionic villus chromosomes according to the frequency of miscarriages.

Group	Number (N)	Abnormal (N)	Rate (%)	χ^2	P
RM	37	15	40.54	0.57	0.45
SA	107	36	33.64		

$P < 0.05$.

The outcome of FISH in the SA or RM group with aneuploidy was analyzed according to maternal age (Table 3). There were 28 cases in the advanced maternal age (≥35 years old) group, of which 18 cases had a numerical abnormality (46.43%). The younger maternal age group (<35 years old) had 166 cases, of which 38 cases had a numerical abnormality (32.76%). The two age groups showed no statistical difference ($P < 0.05$) (Table 3).

Table 3. Outcome of FISH in chorionic villus chromosomes according to maternal age.

Group	Number (N)	Abnormal (N)	Rate (%)	χ^2	P
≥35	28	13	46.43	1.84	0.18
<35	116	38	32.76		

$P < 0.05$.

Among the 144 cases of abortuses, 70 were male, including 22 with numerical abnormality (31.43%). The frequency of female abortuses with chromosomal abnormality was 29/74 (39.19%). There was no statistical difference between the two groups (Table 4).

Table 4. Outcome of FISH in chorionic villus chromosomes according to gender.

Group	Number (N)	Abnormal (N)	Rate (%)	χ^2	P
Male	70	22	31.43	0.95	0.33
Female	74	29	39.19		

$P < 0.05$.

To determine whether fetal chromosomal abnormality was inherited or *de novo*, all parents were informed about the importance of testing their genetic material. We only collected 112 parents' peripheral blood lymphocytes for karyotype analysis, and 7 karyotypes were found to be abnormal (Table 5).

Table 5. Lymphocyte karyotype analysis of 7 couples.

Number	FISH	Cytogenetic diagnosis (female)	Cytogenetic diagnosis (male)
1	Normal	46,XX,inv(9)(p11q13)	46,XY
2	Normal	46,XX	46,XY,inv(9)(p11q13)
3	Normal	46,XX,15p+	46,XY
4	Normal	46,XX,t(4;11)(q21;q23)	46,XY
5	Trisomy 16	46,XX,1qh+	46,XY
6	Trisomy 16/22	46,XX	46,XY,16qh+
7	Trisomy 21/22	46,XX	46,XY,Yqh+

DISCUSSION

Recently, there has been a gradual increase in the incidence of SA in China. Approximately 31% of SA occur in embryo implantation, and among these, 80% are early abortion (Pisani et al., 2002). About 15% of all clinically recognized pregnancies are spontaneously aborted and 60-70% of these are attributable to detectable chromosome abnormalities (Philipp et al., 2003). Trisomies have been reported in all human chromosomes; the most common ones are in chromosomes 16, 21, 18, 13, 15, 22, and X, accounting for 90% of trisomies (Sullivan et al., 2004). Trisomy 16 accounts for approximately 1/3, and is associated with high mortality. We detected 30 cases of chromosome trisomy and 40% were trisomy 16. Polyploidy mainly originates from fertilization by polyspermy or postzygotic division error (Simpson, 2007).

In our population of RA patients, abortus aneuploidy occurred (40.54%) more than in sporadic miscarriages (33.64%), but there was no statistically significant difference between the two groups, illustrating that the incidence of embryo chromosomal abnormalities showed no obvious correlation with the frequency of SA. Therefore, clinically, we recommend testing chorionic villus chromosomes in patients with SA in time, not only for normal pregnancy further strive for the time, but also to reduce patient stress (especially in recurrent SA). Furthermore, the frequency of villus chromosomal abnormality was a little higher in advanced maternal age than in maternal age younger than 35, but there was no statistically significant difference between the two groups, showing that advanced maternal age may not be the main cause of embryonic chromosomal aneuploidy.

In addition, the frequency of male and female abortuses in spontaneous abortion was, respectively, 48.6 and 51.4%, and embryonic chromosomal aneuploidy was, respectively, 43.2 and 56.9%. The two groups showed no statistically significant difference. These results differed from those of Xiong et al. (2009), who found that in both SA and embryonic chromosomal aneuploidy, female embryos were significantly more frequent than male embryos. We believe that this discrepancy can be resolved with further studies with more patient samples.

In addition, we collected 112 parents' peripheral blood lymphocytes for karyotype analysis, and 7 karyotypes were abnormal, among which only 3 chorionic villus samples showed chromosomal abnormalities, indicating that vertical transmission was not the main cause of embryonic chromosomal aneuploidy. Nevertheless, errors in meiosis or mitosis in embryonic germ cells or generative cells leading to chromosome aberration are the main cause. However,

embryonic chromosomal aneuploidy may occur repeatedly in parents with abnormal chromosomes, ultimately leading to abortion. Therefore, these patients can be considered for preimplantation genetic diagnosis/screening to have a healthy baby.

In conclusion, embryonic chromosomal aneuploidy is the main cause of early spontaneous abortion, and a detailed embryoscopic examination of the dead embryo is likely to be useful in couples who have experienced recurrent abortion. In such cases, chromosome analysis is generally recommended (Wolf and Horger, 1995).

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