Reproductive outcome and fetal karyotype of couples with recurrent miscarriages

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Summary

Purpose: The purpose of this study was to evaluate the relationship between fetal karyotype and parental chromosomal abnormalities, and assess the long-term reproductive outcomes in couples with recurrent miscarriages (RM). *Materials and Methods:* The reproductive outcomes of 34 couples with abnormal karyotypes and RM were investigated. Ultrasound examinations were performed during pregnancy, fetal karyotypes were determined following miscarriages, and successful pregnancy outcomes were recorded. *Results:* Of the 34 couples, 20 individuals presented with chromosomal abnormalities, specifically in nine females and 11 males (45% vs 55%, $\chi^2 = 0.2833$, $p > 0.05$). Fifteen couples (44.1%) possessed karyotype polymorphisms, of which the most common variant was a long Y chromosome in males. The reproductive outcomes of subsequent pregnancies consisted of 25 live births of phenotypically normal infants (73.5%), one infant with multiple malformations (2.9%), and eight RM (23.6%). With regards to karyotypes, 69.2% (9/13) of couples had inversions and 73.3% (11/15) had karyotype polymorphisms that resulted in live births of phenotypically normal babies. Fetal karyotyping was performed in a total of 29 cases. Normal karyotypes were present in 48.3% (14/29) of cases, whereas 41.4% (12/29) had abnormalities (either numerical or structural), and 10.3% (3/29) has a karyotype polymorphism. *Conclusions:* There is a positive correlation between chromosomal abnormalities and spontaneous miscarriages. A complete evaluation and special treatment should be provided to couples with a history of recurrent miscarriage(s) during a subsequent pregnancy, particularly when one partner is a carrier of chromosome abnormalities (i.e., inversions of chromosome 9 and long Y chromosome in males). Prenatal diagnosis is necessary in carrier couples suffering from more than two miscarriages.

Key words: Fetal karyotype; Parental karyotype; Recurrent miscarriage; Reproductive outcome.

Introduction

Spontaneous abortion is one of the most common complications of pregnancy, which can be classified as either sporadic or recurrent. Recurrent miscarriage (RM) is defined as two or more consecutive pregnancy losses before 20 weeks of gestation, according to the guidelines of the American Society of Reproductive Medicine, and affects about one to three percent of the child-bearing population [1-5]. RM causes significant psychosocial morbidity and also presents couples with a challenge of having a family successfully. The causes of repeated pregnancy loss is multifactorial, including antiphospholipid syndrome, thrombophilias, infections, endocrine disorders, uterine structural abnormalities, autoimmune-related disorders, and genetic abnormalities [6]. Moreover, the etiology in 50% of RM cases is unknown. It has been confirmed that there is a relationship between chromosomal abnormalities and RM. Chromosomal abnormalities are identified in more than half of all miscarriages, and these mainly consist of reciprocal translocation, Robertsonian translocations, and inversions [7-9]. Furthermore, in couples with RM, structural chromosomal abnormalities range from 3% to 6% [10-12]. There have been already many reports on chromosomal analyses in couples with RM and the karyotypes of the abortuses. However, in most studies, there is a lack of detailed information on the long-term reproductive outcomes in carrier couples with RM. Furthermore, no previous studies have investigated the relationship between fetal karyotypes and pregnancy outcomes. In this study, the authors prospectively investigated the karyotypes of abortuses and live-born infants of RM couples that underwent appropriate therapy. Additionally, data on the growth and development performance of babies were collected. They aimed to assess the long-term reproductive outcomes of couples with chromosomal abnormalities and RM, as well as determine the relationship between fetal and parental karyotypes.

Materials and Methods

Patient characteristics

The subjects comprised of 34 couples that visited the Department of Obstetrics and Gynecology, Sun Yat-sen Memorial Hospital, Sun Yat-sen University, between September 2007 and August 2010. These couples ranged in age between 24 and 49 years, and all of them met the following criteria: 1) all the couples had a history of more than two consecutive RM, 2) abnor-

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mal chromosome karyotypes were detected in either of each couples, including numerical abnormalities, structural chromosomal abnormalities, and karyotypic polymorphisms, 3) all couples were non-consanguineous, and the women were not pregnant when they visited the hospital, and 4) systematic examination and appropriate comprehensive treatment had been provided to all 34 couples. All of the couples that participated in this study signed an informed consent form.

Chromosomal analysis

In this study, the authors obtained chromosome preparations from routine peripheral blood lymphocyte, villi, amniotic fluid, and cord blood. Analysis of G-banded metaphase chromosomes was performed on cultured tissue samples using standard procedures. Karyotypes were described according to the 2005 guidelines of the International System for Human Cytogenetic Nomenclature.

Assessment of pregnancy outcomes

Subsequent pregnancy outcomes were followed up prospectively. The following items were recorded in detail, including fetal karyotype, gestational age at the time of pregnancy termination, and primary pregnancy outcome. A successful outcome was defined as the birth of at least one phenotypically normal child. The other reproductive outcomes comprised of miscarriages, stillbirths, and viable offspring with chromosomal abnormalities.

Ultrasound examination

The gestational age was calculated from the last menstrual period (LMP) and confirmed by measuring crown–rump length in transvaginal ultrasound examination. A careful examination for fetal abnormalities was performed in all cases.

Statistical analysis

Statistical analysis was performed using SPSS (version 16.0.) A $p < 0.05$ was considered statistically significant. The t-test, Chi-square test (χ^2), and Fisher Exact test were used, where appropriate.

Results

Chromosomal abnormalities in couples

A total of 34 couples were included in the final analysis. The mean age of the females was 31.76 ± 4.87 years and the mean age of the males was 34.65 ± 4.88 years. The median number of clinically proven miscarriages was 2.56 ± 0.93. After karyotyping, 20 abnormalities were identified, more specifically in nine females and 11 males (45% vs 55%, $\chi^2 = 0.2833$, $p > 0.05$). There was one couple with abnormalities in both the female and male, whereas only one partner was found to carry a chromosomal abnormality in the other 18 cases. These abnormalities included both numerical and structural abnormalities, which consisted of translocations and inversions. In particular, there were four (20%) females with the mosaic Turner syndrome, three (15%) individuals with translocations (i.e., two male and one female), and 13 (65%) individuals with inversions in chromosome 9, where there were no significant differences between males and females (53.8% vs 46.2%, $\chi^2 = 0.0951$, $p > 0.05$).

Table 1. — *Reproductive outcomes of couples with chromosomal abnormities and recurrent miscarriages.*

Reproductive outcome	n	Karyotype abnormality
Healthy birth	25	46, XX, inv(21)(p12q21)1qh+
		46, XY, inv(9)
		46, XY, inv(9)(p11q13)
		46, XX, t(11;12)(p13q13)
		45, XX, t(13q;14q)
		45, X[3]/46, XX[95]/47, XXX[2]
		45, X[4]/46, XX[94]/47, XXX[2]
		45, X[5]/46, XX[100]/47, XXX[5]
		46, XY, inv(2)(p11q13)
		46, XY, inv(9)(p11q13)
		46, XY, small Y chromosome
		46, XX, inv(9)(p11q13)
		46, XY, t(4;6)(q27q21)
		46, XX, inv(9)(p11q13)
		46, XY, inv(9)(p12q13)
		46, XY, inv(9)(p13q21)
		46, XX, 22pstk+
		46, XY, 13pss
		46, XY, Y>18
		46, XY, Y>18
		46, XY, Y>18
		46, XY, Y>18
		46, XY, 9qh+
		46, XY, 1qh+
		46, XY, Y>18
		46, XY, Y>18
Miscarriage	8	46, XY, 13pstk+
		46, XY, inv(9)(p11q13)
		45, XY, t(13;14)(p11q11)
		46, XX, 22pss
		46, XX, inv(9)
		46, XX, inv(9)(p11q13)
		46, XX, inv(9)(p11q13)
		46, XX, 1qh+
Induced labor (multiple malformations)	1	46, XY, small Y chromosome

Chromosomal polymorphisms in couples

Fifteen out of 34 couples (44.1%) presented with a karyotype polymorphism, which was more prevalent in males than females (80% vs 20%, $\chi^2 = 6.9283$, $p < 0.05$). Furthermore, the most common variant in males was the long Y chromosome, which accounted for 40% (6/15) of all polymorphisms.

Reproductive outcomes

After a systematic examination and appropriate treatment, all of the 34 couples became pregnant. Of the 34 pregnancies, 26 resulted in a live birth, where 25 (73.5%) of the offspring were phenotypically normal and one (2.9%) infant had multiple malformations (induced labor). The other eight (23.6%) pregnancies were RM. With regards to karyotypes, 69.2% (9/13) of the couples that had

Table 2. — *Reproductive outcomes according to fetal karyotype.*

Reproductive outcome	n	Offspring karyotype
Healthy birth	20	46, XX
		46, XY
		46, XX
		46, XY
		46, XY
		46, XX
		46, XY
		46, XY
		46, XY, inv(2)(p11q13)
		46, XY, inv(9)(p11q13)
		46, XY, small Y chromosome
		46, XX, inv(9)(p11q13)
		46, XX, inv(9)(p11q13)
		46, XY, inv(9)(p11q13)
		46, XY, 1qh+
		46, XX
		46, XY
		46, XX, dup(1)(q12q21)
		46, XY, inv(9)(p11q13)
		46, XX, 13pstk+
Loss of pregnancy	9	46, XY
		46, XX
		46, XY
		47, XX, +16
		47, XY, +19[78]/ 47, XY, +19,inv(4)[22]
		69, XXX
		46, XX, inv(9)(p11q13)[80] / 92, XXYY, inv(9)(p11q13)[20]
		46, XX, inv(9)(p11q13)
		46, XX

an inversion of chromosome 9 gave birth to healthy children, and two of the three couples that had translocations also had successful deliveries after 38 weeks of gestation. Four children were born to Turner syndrome females. It is worthwhile to note that 73.3% (11/15) of the cases with karyotype polymorphisms resulted in live and phenotypically normal births, including 54.5% (6/11) of cases with a long Y chromosome. The karyotypes of the RM couples and their pregnancy outcomes are presented in Table 1.

Fetal karyotypes

In five out 34 pregnancies, karyotyping was not performed because four of these couples decided not to undergo genetic testing and in one case the karyotype was uncertain due to culture failure. A total of 29 fetal karyotypes were included in this analysis. A normal karyotype was found in 14 out of the 29 cases analyzed (48.3%).

Among the 14 pregnancies with a normal fetal karyotype, ten (71.4%) of the cases ended in the birth of healthy infants, whereas one case resulted in induced labor due to multiple malformations and three pregnancies were terminated via abortions. The remaining 15 karyotypes were abnormal. Of these, 41.4% of the karyotypes (12/29) had abnormalities and 10.3% (3/29) displayed a karyotype polymorphism.

The fetal karyotype abnormalities included both numerical and structural chromosomal abnormalities. Four cases of numerical chromosomal abnormalities were observed in this study and all of the embryos terminated in the first trimester. Seven healthy children were born with structural karyotype abnormalities, and the most frequent of these were inversions, with only one abortus detected with a karyotype abnormality. Moreover, three children with a karyotype polymorphism was born phenotypically normal. The pregnancy outcomes according to fetal karyotype are listed in Table 2.

Discussion

Approximately ten to 15 percent of clinically diagnosed pregnancies end via spontaneous miscarriages [13]. Unfortunately, in many couples suffering from RM, the causes of this condition are unknown, partly because they differ between cases. While an increasing number of studies suggest that most RM are due to the presence of parental karyotype aberrations, according to literature, the incidence of chromosome abnormalities in couples that experience a spontaneous abortion is three to 11 percent [14-17]. In the present study, the proportion of parental chromosomal abnormalities among couples was 55.9% (19/34), which is much greater than that reported by others. The authors believe that the higher percentage may be because their subjects were selected from RM couples that had chromosomal aberrations. Further, the incidence of abnormal karyotypes was not significantly different between males and females ($p > 0.05$). With respect to the reproductive outcome, 73.5% of these couples had a live birth of a healthy infant in subsequent pregnancies. Thus, the authors suggest that karyotype analysis should be an integral part of diagnostics in both spouses with RM.

With regards to karyotype abnormalities, in the present study there were 20% of cases with numerical abnormalities, and 80% of cases with structural rearrangements. The results showed the majority of chromosomal anomalies in cases of structural rearrangements were inversions involving chromosome 9 inversions, which is a rearrangement of a segment that is reversed end to end. Recently, there has been some evidence indicating that an inversion of chromosome 9 leads to an increased risk of miscarriage in about 30% of affected couples [18-22]. In the present study, the authors identified 13 cases with an inversion involving chromosome 9, accounted for 19.1% of the examined patients. The incidence is much higher than that found in the general population, which was reported to be about one percent to 1.65%. It may be that inversions of chromosome 9 are one of the major chromosomal aberrations leading to RM. Furthermore, after a comprehensive evaluation and specific treatment paradigm, nine of these couples with

chromosomal inversion gave birth to healthy children. The authors suggest that chromosomal examination, as well as close monitoring and supportive care, should be provided to couples with chromosomal inversions.

Chromosomal polymorphisms refer to microscopically visible minor differences in chromosome morphology among human groups. Via traditional genetic points-of-view, it is believed that karyotype polymorphisms have no obvious clinical phenotypic or pathological significance. However, a growing number of reports have shown that chromosomal polymorphisms may produce clinical effects and lead to various adverse reproductive outcomes, for instance, infertility, recurrent spontaneous miscarriages, fetal malformations, etc [20]. In this study, the authors found that 15 out of 34 couples had a chromosomal polymorphism, more specifically, in 12 males and six females, which was significantly different. Moreover, chromosomal polymorphisms were primarily of the long Y chromosome type, which accounted for 40% of the cases. The long Y chromosome is a common type of chromosomal polymorphisms, which refers to an increase in the distal sites of the long arm of the Y chromosome. It has been reported that this variation is related to a repeated duplication of heterochromatin or changes in the extent of chromosomal spiralization. According to previous statistics, the long Y chromosome accounts for 2.18% to 30.20% of reproductive abnormalities in male patients [23, 24]. Rodriguez *et al.* suggested that the variability in the length of the Y chromosome is a polymorphism in human males that is unassociated with reproductive problems. Furthermore, Verma *et al.* observed that a long Y chromosome in fathers may be unrelated to fetal loss [24-26]. Conversely, Yan *et al.* suggested that an increasing in the number of DNA repeats in the Y chromosome may influence the development of the nervous system during the early stages of fetal development, and leads to a stillbirth or abortion [23]. According to our observations, all of the female partners of the six males with a long Y chromosome had successful deliveries after 38 weeks of gestation. The authors suggest that a comprehensive etiological screening and a specific treatment paradigm should be performed in all couples with a karyotype polymorphism and RM. Additionally, owing to the risk of abnormal fetal development, they advise that such couples accept a prenatal diagnosis in subsequent pregnancy.

Aside from parental chromosomal aberrations, fetal chromosomal abnormalities are also a major cause of RM. Goddijn *et al.* suggested that fetal chromosomal abnormalities account for about 50% of first-trimester pregnancy losses [22]. Similarly, Carp *et al.* observed that embryonic chromosomal aberrations have been found in 29% to 60% of RM. Furthermore, most of these abnormalities were numerical abnormalities (86%) and a low proportion were caused by structural abnormalities (6%) or other genetic mechanisms [22, 27-29]. According to our data, 51.7% (15/29) of fetal karyotypes were abnormal, which is in accordance with previous research. Among these, numerical abnormalities accounted for one-third of the karyotype abnormalities, including trisomy, triploid, and tetraploid, and all of these embryos did not grow beyond the first trimester. In the current study, inversions turned out to be due to abnormalities in fetal karyotypes, which accounted for 66.7% of the cases. The authors believe the higher incidence may be attributable to the small sample size and effective treatment, which increased survival.

It is well known that the causes leading to offspring karyotype abnormalities are very complicated, and may be due to various causes, such as an error in mitosis during early pregnancy, maternal age, various environmental exposures, etc. Furthermore, half of the structural abnormalities may be inherited from a parent carrying a balanced chromosome translocation or inversion. Goddijn *et al.* reported that a subsequent pregnancy may result in a child with an unbalanced structural chromosomal abnormality due to a parental structural chromosomal abnormality [22]. Consequently, this child may have multiple congenital malformations and/or a mental handicap. Couples with RM are more likely to produce chromosomally abnormal embryos than those without RM. While parental karyotyping is part of the standard management of RM, it is rarely a measure of the fetal karyotype. Hence, in the authors' opinion, fetal karyotyping should be determined directly via an embryonic biopsy or pre-gestational diagnosis.

In conclusion, there is a positive correlation between chromosomal abnormalities and spontaneous miscarriages. The clinical effects of chromosomal polymorphisms need to be recognized better, in particular, inversions of chromosome 9 and the long Y chromosome in males. Prenatal diagnosis should also be implemented in carrier couples suffering more than two miscarriages. Moreover, the prognosis for a subsequent pregnancy may be affected by other factors, such as antiphospholipid syndrome, thrombophilias, infections, endocrine disorders. Hence, in couples with a history of RM, where one of the partners is a carrier of chromosomal abnormalities, an evaluation, special treatment, supportive care, and close monitoring is associated with a marked improvement in subsequent live birth rates.

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