

# Cytogenetic Analysis of 570 Couples with Recurrent Pregnancy Loss: Reporting 11 Years of Experience

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ABSTRACT

**Objectives:** Recurrent pregnancy loss (RPL) is a serious complication of pregnancies, affecting almost 2%–5% of couples. Among numerous underlying causes, chromosomal anomalies in one of the partners are regarded as important issues, with varying frequencies among different populations. In this study, we aimed to determine the prevalence of chromosomal abnormalities and chromosome polymorphisms in couples with a history of RPL from Kermanshah province, west of Iran. **Materials and Methods:** In this 11-year retrospective study, a total of 1140 cases with two or more spontaneous abortions were recruited and studied according to standard cytogenetic analysis. **Results:** From a total of 1140 reviewed blood samples, 1011 people (88.5%) had a normal karyotype and 129 people (11.5%) had chromosomal aberrations. These aberrations were found in 62 females and 67 males. The prevalence of chromosomal abnormalities was as follows: 18 (1.5%) structural aberrations, 1 numerical anomaly and 110 (9.6%) apparently normal polymorphic variants. **Conclusions:** Our findings could determine the underlying cause of RPL in 1.5% of the population while the majority still remained unexplained. This emphasizes the importance of searching for other genetic and nongenetic causes of RPL in apparently idiopathic cases of RPL.

**KEYWORDS:** Chromosomal abnormality, cytogenetics, recurrent pregnancy loss

## INTRODUCTION

Recurrent pregnancy loss (RPL) is defined as three or more consecutive loss of pregnancies before 20–22 weeks of gestation,<sup>[1-3]</sup> however, in recent years, even two consecutive miscarriages are considered as RPL.<sup>[4]</sup> Near 15% of all clinically recognized pregnancies may result in pregnancy failure, a subset of which remains unrecognized as occult abortion.<sup>[5]</sup>

The etiology of RPL can be only determined in 50% of cases, while the rest remain unexplained. To date, numerous factors have been proposed as key contributors of RPL, among which are genetic factors, luteal phase defect during pregnancy, congenital or structural uterine anomalies, antiphospholipid syndrome, thyroid dysfunction, maternal and paternal age, endocrine dysfunction, autoimmune disorders, infectious diseases, and environmental toxins.<sup>[6,7]</sup>

About 50% of all spontaneously aborted fetuses have chromosomal abnormalities,<sup>[8]</sup> These include numerical chromosomal abnormalities (e.g. aneuploidy, triploidy and tetraploidy) and structural chromosomal aberrations (e.g. inherited derivative chromosomes raised from chromosomal rearrangements). Apparently, balanced chromosomal rearrangements can result in unbalanced gametes, which subsequently lead to conditions such as RPL, stillbirth, and neonates with multiple congenital anomalies.<sup>[9]</sup>

Previous studies have reported varying frequency of 2%–8% of balanced chromosomal rearrangements in couples experiencing RPL.<sup>[10]</sup> This rate is much

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higher than the frequency observed in the general population (0.2%–0.55%). However, there was not an accurate estimate on the frequency of chromosomal rearrangements in couples with RPL in our population. Herein, in this retrospective study, we aimed to determine the frequency of chromosomal anomalies in couples with RPL.

## MATERIALS AND METHODS

### Study subjects

This retrospective study was carried out from 2008 to 2018. Couples experiencing at least two or more consecutive miscarriages were referred to our Cytogenetic Department for chromosomal analysis. Written informed consent was taken from all the study participants. Personal characteristics, clinical, and medical histories were taken. All participants met the inclusion criteria as follows: (i) two or more consecutive miscarriages; (ii) Normal reproductive tract anatomy; (iii) normal endocrine function (female) and semen (male) testing results; (iv) Negative results of anti-TORCH virus series, *Toxoplasma gondii*, anti-nuclear antigen, anti-cardiolipin antigen and anti-husband cytotoxicity; (v) absence of reproductive tract or systemic inflammatory response; (vi) absence of thrombotic disease or tendency. Couples experiencing any of the above-mentioned criteria were excluded from the study.

### Chromosome preparation and analysis

The metaphase chromosome preparation from whole blood was performed according to standard protocols; subsequently, the chromosomes were G-banded using trypsin–Giemsa banding preparations. At least 20 metaphases were then studied in all the patients; however, in the cases of abnormal findings, the number increases to 50. All chromosomal aberrations were reported according to the International System for Human Cytogenetic Nomenclature. The metaphases were visualized using an Axioscope microscope (Carl Zeiss Light Microscopy, Germany) and karyotyped using MetaSystems software (MetaSystems, Germany). Statistical analysis was performed using SPSS for Windows, Version 16.0. Chicago, SPSS Inc.

## RESULTS

A total of 570 couples (1140 individuals) with a history of RPL were included in this study. Of these, the number of miscarriages ranged from 2 to 8 (mean = 2.6) [Table 1], and the mean age of participants was 29.33 in women and 33.74 in men [Table 2]. Among all participants, 508 female (89%) and 503 males (88.2%) had a normal karyotype, while chromosomal aberrations were found in

62 (11%) of females and 67 (11.8%) of males. Detailed information on the frequency of chromosomal aberrations is provided in Table 3. Partial karyotypes of a subset of chromosome aberrations are depicted in Figure 1.

Pedigree analysis revealed a positive family history of RPL in 8.8% of all participants; Moreover, 9 and 11 couples with chromosomal abnormalities had a family history of RPL and mental retardation, respectively.

In this study, 129 of 1140 individuals experiencing RPL had chromosomal anomalies, including 18 cases (13.95%) with structural aberrations, 1 numerical abnormality (0.78%), and 110 cases (85.27%) with heteromorphic variants [Table 3].

The structural aberrations included reciprocal translocations detected in 10 females and 6 males and Robertsonian translocation in 2 cases. Chromosomes

**Table 1: Number of abortions in couples with recurrent miscarriages**

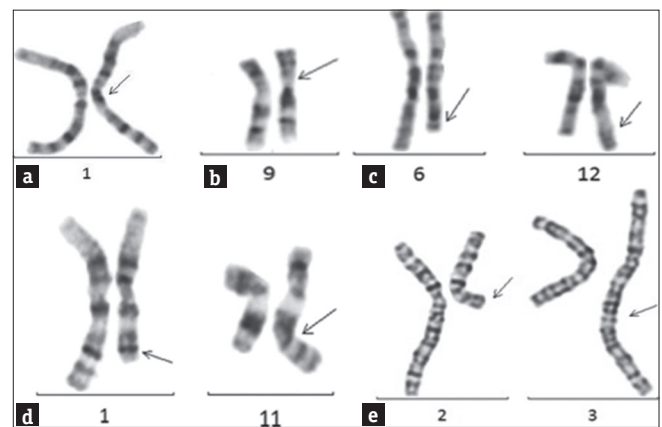
Number of abortions	Number of couples with RPL (%)
2	289 (50.74)
3	195 (34.21)
4	55 (9.64)
5	16 (2.8)
6	9 (1.57)
7	5 (0.87)
8	1 (0.17)
Total	570 (100)

RPL=Recurrent pregnancy loss

**Table 2: Distribution of age and number of abortions between male and female carriers**

	Male	Female	P
Mean age	33.74	29.33	<0.05
Number of abortion	2.53	2.54	NS

Data are expressed as mean±SD and evaluated by Student's t-test. SD=Standard deviation, NS=Not significant



**Figure 1: Partial Karyotypes Showing: (a) 1qh+, (b) inv(9), (c) t(6;12)(q24;q24), (d) t(1;11)(q31;q23), (e) t(2;3)(q12;q27)**

1, 2, 4, 7, and 9 were more frequently involved in translocations. The only numerical anomaly was 47, XYY. The incidence of structural anomalies in couples with RPL is shown in Table 4.

Heteromorphic variants comprise about 85% of total chromosomal aberrations observed. These polymorphisms included variations in the length of heterochromatin region, size of satellites, and length of stalks of acrocentric chromosomes. The most frequent heteromorphism observed was 1qh+ (12 male and 14 females) followed by 9qh+ (6 male and 8 females) and Yqh- [Table 5].

## DISCUSSION

RPL is already defined as two or more consecutive loss of pregnancies before 20–22 weeks of gestation; Determining the etiology of RPL can be an important step in managing future pregnancies and subsequent interventions.<sup>[11]</sup> The exact etiology of the human reproduction problems have not been definitely clarified yet for both patients and clinicians; however, as is clear RPL has a multifactorial etiology with several underlying factors including genetic makeup, uterine

abnormalities, hormonal imbalances, immunological disorders, and environmental factors.<sup>[12]</sup> Since the frequency of chromosomal abnormalities in couples with a history of RPL varies significantly among different populations, here we conducted a retrospective study to determine the frequency of chromosomal anomalies in individuals suffering from RPL.

In this study, the mean maternal age of individuals experiencing RPL was 29.63 years, and 20.8% of females were above the age of 35. We could also observe a significant correlation between maternal age and the number of abortions ( $P < 0.05$ ); this finding is in agreement with Elise de La Rochebrochard's report, which found an association between maternal age and increased number of miscarriages.<sup>[13]</sup> In another study, Hook and Cross found that the risk of chromosomal abnormalities was 1/476 at the age of 25 while the risk increased with advancing maternal age at delivery, which was 1/385, 1/196, 1/66 at 30, 35, and 40 years, respectively.<sup>[14]</sup>

The frequency of chromosomal abnormalities among couples with RPL varies in different studies, ranging

**Table 3: Classification of chromosome abnormalities among all referred patients with recurrent pregnancy loss**

Karyotype	Type of chromosomal aberration	Total number of cases	Male/female
Normal karyotype		1011	503/508
Chromosomal aberrations		129	67/62
	Numerical abnormality	1	1/0
	Structural abnormality	18	6/12
	Chromosome polymorphisms	110	61/59

**Table 4: Structural chromosomal aberrations in recurrent miscarriage cases**

Chromosomal abnormality	Karyotype	Number of cases	Age (years)/sex	Male/female	Number of abortions	Time of loss of pregnancy	
Translocation	46,XX,t(2;3)(q12;q27)	1	26/female		3	1 <sup>st</sup> trimester	
	46,XX,t(1;11)(q31;q23)	1	25/female		2	1 <sup>st</sup> trimester	
	46,XX,t(6;12)(q24;q24)	1	26/female		2	1 <sup>st</sup> trimester	
	46,XX,t(7;9)(q32;q24)	1	35/female		4	1 <sup>st</sup> trimester	
	46,XY,t(4;7)(q21;q11)	1	31/male		3	1 <sup>st</sup> trimester	
	46,XY,t(2;9)(q11;q32)	1	30/male		2	1 <sup>st</sup> trimester	
	46,XY,t(11;22)(q13;q25)	1	30/male		2	1 <sup>st</sup> trimester	
	46,XX,t(12;15)(q24.2;q24)	1	26/female		4	1 <sup>st</sup> trimester	
	46,XY,t(1;7)(q24;q32)	1	41/male		4	1 <sup>st</sup> trimester	
	46,XX,t(3;6)(q26.2;q26)	1	28/female		3	1 <sup>st</sup> trimester	
	46,XX,t(1;7)(q25;q11.2)	1	35/female		4	1 <sup>st</sup> trimester	
	46XX,t(2;18)(q11;q23)	1	39/female		3	1 <sup>st</sup> trimester	
	46,XX,t(1;11)(q32;q24)	1	31/female		3	2 <sup>nd</sup> trimester	
	46,XX,t(8;13)(q11;q11)	1	30/female		3	1 <sup>st</sup> trimester	
	46,XY,t(4;6)(q31.3;q15)	1	34/male		4	1 <sup>st</sup> trimester	
	46,XX,t(2;18)(q31.1;q23)	1	36/female		3	1 <sup>st</sup> trimester	
	Robertsonian translocation	45,XX,der(14;15)(q11.2;q11.2)	1	27/female		2	1 <sup>st</sup> trimester
		45,XY,der(13;13)(q10;q10)	1	25/male		5	1 <sup>st</sup> trimester

**Table 5: Distribution of chromosome polymorphisms in recurrent miscarriages cases**

Variant	Number of cases (male:female)
1qh+	26 (12:14)
9 qh+	14 (6:8)
9 qh-	2 (1:1)
16 qh+	5 (4:1)
13ps+	7 (3:4)
14 ps+	3 (2:1)
14 pstk+	5 (1:4)
15 ps+	6 (2:4)
21 ps+	9 (3:6)
21pstk+	1 (1:0)
22ps+	6 (5:1)
22pstk+	2 (0:2)
Yqh+	9
Yqh-	12
Inv(9)	3 (0:3)
Total	110

from zero<sup>[15]</sup> to as high as 21.4%.<sup>[16]</sup> In this study, the incidence of structural chromosomal aberrations among couples with two or more miscarriages was 3.15%, which is approximately similar to two previous studies from Canada and India<sup>[17,18]</sup> and significantly lower than the frequency reported in China and Egypt.<sup>[19,20]</sup> These differences might be partly attributed to different sample size, ethnicity and consanguinity, social, and other criteria.

In addition, the male to female ratio of structural chromosomal abnormality was 6:10, which is consistent with the majority of studies,<sup>[21,22]</sup> except two, which reported a high frequency of structural chromosomal abnormality in males compared to females.<sup>[23]</sup>

Reciprocal translocations are the most frequent structural chromosomal abnormalities in RPL<sup>[24-26]</sup> with frequencies ranging from 0% to 31%.<sup>[27,28]</sup> In our population study, the prevalence of structural chromosomal abnormality was as follows: 10 reciprocal and 1 Robertsonian translocation in affected females, and six reciprocal and 1 Robertsonian translocation in male affected partners [Table 4]. Accordingly, balanced reciprocal translocations account for the largest proportion of chromosomal abnormalities (16/18). These findings are again, in concordance with the previous finding that the proportion of reciprocal balanced translocations is higher than the Robertsonian ones.<sup>[25,29-31]</sup>

Numerical chromosomal aberrations usually appear as sex chromosomal aneuploidy in RPL with a low frequency of about 0.15%.<sup>[32]</sup> The only numerical aberration we observed here was a 47, XYY male who may not be associated with their history of RPL.

Chromosomal heteromorphisms are an expression of morphological variability of a chromosome in the amount of heterochromatin. It has been speculated that the presence of chromosomal variants and changes in the structural element of the centromere may have a key role in the synapsis of human homologous chromosomes and subsequently increase the risk of nondisjunction during chromosome segregation.<sup>[33-35]</sup> However, the role of polymorphic variants of chromosomes in reproductive failure remains controversial. There is a general agreement in favor of a lack of association between chromosomal heteromorphisms and reproductive issues. However, several studies have reported a significantly higher frequency of chromosome heteromorphisms in individuals suffering from reproductive failure compared with the normal population.<sup>[30,36,37]</sup> For example, Minocherhomji *et al.* reported a significant increase in the frequency chromosomal variation in infertile women (28.31% vs. 15.16%) and infertile men (58.68% vs. 32.55%).<sup>[38]</sup> The prevalence of chromosomal normal variants among the individuals with recurrent abortion referring to our cytogenetic department was 19.3%, and it was higher in males than females (59/51). However, the impact of chromosomal heteromorphisms on reproductive issues remains to be considered benign as long as there is strong evidence against it.

## CONCLUSIONS

We observed a total of 11.5% chromosomal abnormalities and variations in couples experiencing RPL with the following frequencies in our study: 1.5% structural aberrations, 1 numerical anomaly and 9.6% normal polymorphic variants. Accordingly, it is highly recommended to order cytogenetic analysis in couples with a history of repeated pregnancy loss in the very early stages of clinical evaluation. In addition, the high frequency of heteromorphic variants observed here and similar studies automatically suggest the high demand of clearly defining their role in the pathogenesis of RPL.

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## Conflicts of interest

There are no conflicts of interest.

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