Reproductive BioMedicine Online (2016) **32**, 414–419

www.sciencedirect.com
www.rbmonline.com

ARTICLE

Chromosomal analyses of 1510 couples who have experienced recurrent spontaneous abortions

Erdal Tunç, Nilgün Tanrıverdi, Osman Demirhan *, Dilara Süleymanova, Nesrin Çetinel

Medical Biology and Genetics Department, Çukurova University, 01330, Adana, Turkey
* Corresponding author. *E-mail address:* osdemir@cu.edu.tr (O Demirhan).

Professor Osman Demirhan was born in Konya, Turkey, in 1958. After completing elementary and middle school in the same town, he attended Hacettepe University Biology Department (Faculty of Science) and graduated in 1982. He began working at Çukurova University, Medical School, Department of Medical Biology, as a research assistant in 1983. He received his MSC degree in 1987, PhD degree in 1991 and was appointed Professor in the same department in 2004. He received professor degree at 2004, is still working at the same deparment. He also studied at the Department of Medical Genetics, Antwerp University, Belgium, in 1995.

Abstract In this retrospective study, karyotype results of 1510 couples with a history of recurrent spontaneous abortion were evaluated. The study was conducted at Balcalı Hospital in Adana region of Turkey. For all cases, peripheral blood lymphocytes were cultured for chromosome study using the standard method. Chromosome aberrations were detected in 62 couples (4.1% of all couples). At an individual level, chromosome aberrations were found in a total of 65 cases (41 females and 24 male cases), with structural chromosomal aberrations in 58 cases including balanced translocations in 30 cases, Robertsonian translocations in 12 cases, deletions in seven cases, inversions in nine cases and numerical chromosome aberrations in seven cases. The results of the study indicated that structural aberrations, particularly translocations, were the most common type of aberration observed among couples who had experienced recurrent spontaneous abortions and that these couples might benefit from cytogenetic analyses.

KEYWORDS: aberrations, chromosome, lymphocytes, recurrent miscarriages, translocations

Introduction

Recurrent spontaneous abortions that occur before the 24th week of gestation are observed among 1–3% of couples trying to have children. Various different definitions for recurrent spontaneous abortion have been proposed; certain studies defining recurrent spontaneos abortion as three or more losses of pregnancy (Coulam, 1991; Dubey et al., 2005; Flynn et al., 2014; Garrido-Gimenez and Alijotas-Reig, 2015; Grande et al., 2012; Mozdarani et al., 2008; Stirrat, 1990), whereas others

http://dx.doi.org/10.1016/j.rbmo.2016.01.006
1472-6483/

define it as two or more failed pregnancies (Kochhar and Ghosh, 2013; Sugiura-Ogasawara, 2014; Van den Berg et al., 2012); these losses might be consecutive or non-consecutive. Nearly 15% of all clinically identified pregnancies end in spontaneous abortion (El-Dahtory, 2011; Exalto, 2005; Niroumanesh et al., 2011). Recurrent spontaneous abotions can be caused by a variety of factors, generally related to implantation, anatomic uterine defects, infections, autoimmunity, allo-immunity, endocrine abnormalities and genetics (Dubey et al., 2005). In 50% of couples, however, the exact cause of spontaneous abortion cannot be identified; in such cases, the spontaneous abortion is considered as idiopathic, or referred to as an unexplained spontaneous abotion (Flynn et al., 2014). A considerable proportion of couples who experience recurrent spontaneous abortions also exhibit chromosome aberrations. Numerous studies have been conducted to investigate the proportion of chromosome aberrations among couples experiencing recurrent spontaneous abortions, with some indicating that between 2.7–6.7% of couples experiencing recurrent spontaneous abortions are chromosome aberration carriers (Dutta et al., 2011; Goud et al., 2009; Stephenson and Sierra, 2006). Other studies have reported that, among couples who have experienced recurrent spontaneous abortions, the frequency of chromosome aberrations varies between 4 and 8% (El-Dahtory, 2011; Kavalier, 2005). Both numerical and structural chromosome aberrations have been observed, although the former type of aberration tends to be less common than the latter. Frequently observed chromosome aberrations include balanced translocations, Robertsonian translocations and inversions. The chromosome aberrations mentioned above might not necessarily result in the gain or loss of genetic material; such chromosome aberrations are defined as balanced rearrangements, which do not affect the viability or life of the individual.

During the segregation of chromosomes in meiosis, however, the presence of balanced rearrangements may lead to an unbalanced karyotype in the carrier's gametes, which in turn can result in spontaneous abortion, still birth or neonate congenital defects (Flynn et al., 2014).

The purpose of our study was to investigate 1510 couples who experienced recurrent spontaneous abortions, and to evaluate their karyotypes. We also aimed to obtain and identify significant statistical data that would contribute to research and help guide clinicians and genetic counsellors in counselling couples who have experienced recurrent spontaneous abortion. We believe that, owing to its evaluation of a large number of cases over a 28-year period, the present study and its findings are of considerable importance.

Materials and methods

The study was conducted retrospectively on couples who had experienced recurrent spontaneous abortion who were admitted to our institution between January 1982 and December 2010. The study was conducted at the cytogenetics laboratory of Çukurova University Balcalı Hospital's Medical Biology and Genetics Department, located in Adana, Turkey. All 1510 couples with a minimum of two pregnancy losses were referred by gynaecologists of the same hospital for chromosome analysis. After all other possible causes of spontaneous abortion were excluded, i.e. anatomic uterine defects, infections, autoimmunity, allo-immunity, and endocrine abnormalities, the couples were directed to chromosome analyses. Couples presenting diverse pregnancy loss histories were included in the study. As such, couples participating in the present study included couples who only experienced recurrent spontaneous abortions, couples whose recurrent spontaneous abortions were preceded by abnormal children or by stillbirths, and couples who had healthy children despite recurrent spontaneous abortions. A total of 2 ml heparinized (Vasparin, Defarma, Ankara, Turkey) venous blood was collected from each study participant. Slides were prepared using standard culture, swelling, and fixation procedures, and then stained according to the banding using the Trypsin and Giemsa GTG method. Chromosome analysis was conducted using CytoVision software. At least 20 metaphases were analysed for each case, whereas a minimum of 30 metaphases or 50 metaphases were evaluated in abnormal and mosaic cases, respectively. Solid staining was used for a few cases, specifically for cases who had applied to our laboratory during the early 1980s. When reporting the study result, the international system for human cytogenetic nomenclature (ISCN 1981 and 1985) at 500–550 band resolution was primarily used. Definition of any aberration at 500–550 band resolution paves the way for using molecular cytogenetic techniques, other molecular techniques, or both, and describing the aberrations at a molecular level. The chromosome aberrations were classified as either numerical or structural aberrations. The latter were further sub-divided as balanced translocations, Robertsonian translocations, inversions and deletions.

When planning the study, the necessary consultations were made with the ethics committee of the Çukurova University Medical Faculty.

Results

A total of 1510 couples were included in the study; 62 (4.1%) of the couples were identified as having chromosome aberrations and, in three of the couples, both the male and female were chromosome aberration carriers. As such, the total number of cases with chromosome aberrations was 65 (41 women and 24 men) (**Table 1**). The mean age of these cases was 29 years for women and 33.5 for men. At an individual level, a total of 3020 cases (1510 women and 1510 men) were evaluated. Among these cases, the mean age was 28.7 years for 1486 women/mothers, and 32.6 for 1474 men/fathers; records indicating age were absent for 24 women and 36 men. The proportions of structural and numerical aberrations were determined as 89% (58 cases) and 11% (seven cases), respectively. In addition, the proportions of balanced translocations, Robertsonian translocations, deletions and inversions were determined as 46%, 18%, 11% and 14%, respectively (**Figure 1**).

On the basis of the available medical records, the number of spontaneous abortions could be identified for 763 couples. As gynaecologists refer any couple with a minimum of two spontaneous abortions for chromosome analysis, the exact number of spontaneous abortions for the other 747 couples could not be determined, despite knowing that they had at least two spontaneous abortions. These couples were mainly those who had applied to our laboratory before 2000. The total numbers of spontaneous abortions varied between two and nine, with

Table 1 Chromosome aberrations in couples who have experienced recurrent spontaneous abortions.

Couple number	*Females, karyotype*	*Age (Years)*	*Males, karyotype*	*Age (years)*
Structural aberrations				
Balanced translocations				
CN-1	46, XX, t(4;5)(q31;p15)	25	46, XY	33
CN-2	46, XX, t(9q;10q)	24	46, XY	33
CN-3	46, XX, t(15;4)(q25;p16)	29	46, XY	30
CN-4	46, XX, t(10;11)(q25;q25)	21	46, XY	26
CN-5	46, XX, t(3;12)(p25;q23)	26	46, XY	30
CN-6	46, XX, t(6p;14q)	24	46, XY	29
CN-7	46, XX, t(3p;7q)	26	46, XY	30
CN-8	46, XX, t(7;14)(p22;q22)	39	46, XY	44
CN-9	46, XX, t(5p+;7q-)	26	46, XY	28
CN-10	46, XX, t(2;5)(p25;q13)	32	46, XY	29
CN-11	46, XX, t(10;21)(p15;q21)	26	46, XY	29
CN-12	46, XX, t(1p;17q)	27	46, XY	36
CN-13	46, XX, t(4;15)(q35;q15)	29	46, XY	32
CN-14	46, XX, t(5;6)(q31;p23)	35	46, XY	35
CN-15	46, XX, t(1;4)(q25;q35)	29	46, XY	34
CN-16	46, XX, t(11;18)(q11;q11)	35	46, XY	44
CN-17	46, XX, t(6;15)(q23;qter)	28	46, XY	40
CN-18	46, XX, t(1;10)(q42;q24)	34	46, XY	40
CN-19	46, XX, t(4;7)(q25;qter)	27	46, XY, t(7;14)(pter;q22)	31
CN-20	46, XX	34	46,XY/46,XY,t(10;11)(p11;q13)(%50)	35
CN-21	46, XX	34	46, XY, t(8;10)(q24;q24)	38
CN-22	46, XX	24	46, XY, t(2p;15q)	32
CN-23	46, XX	30	46, XY, t(1;4)	31
CN-24	46, XX	23	46, XY, t(6;9)	29
CN-25	46, XX	29	46, XY, t(2p;22q)	34
CN-26	45, XX rob t(13;14)	23	46, XY, t(14;19)(p32;q12)	28
CN-27	46, XX	29	46, XY, t(2;3)(q21;p21)	32
CN-28	46, XX	-	46, XY, t(7;14)(q36;q11)	-
CN-29	46, XX	27	46, XY, t(15;14)(q32;q11)	28
Robertsonian translocations				
CN-30	45, XX, robt(14;22)	29	46, XY	26
CN-31	45, XX, robt(13;14)	23	46, XY	29
CN-32	45, XX, robt(13;14)	23	46, XY	28
CN-33	45, XX, robt(14;21)	26	46, XY	28
CN-34	45, XX, robt(13;14)	29	46, XY	36
CN-35	45, XX, robt(13;14)	25	46, XY	30
CN-36	45, XX, rob t(14;15)	30	46, XY	-
CN-37	45, XX, robt(14;15)	41	46, XY	36
CN-38	45, XX, robt(13;14)	30	45, XY, robt(13;14)	-
CN-39	46, XX	32	45, XY, robt(21;21)	35
Deletions				
CN-40	46, XX, del(15)(p11-pter)	21	46, XY	28
CN-41	46, XX, del(9)(q11;q13)	28	46, XY	30
CN-42 46, XX, del(18)(p13,2-pter)		29	46, XY	31
CN-43	46, XX, del(1q32)	25	46, XY	27
CN-44	46, XX, del(4p)	25	46, XY	35
CN-45	46, XX	39	46, XY, del(1q32)	47
CN-46	46, XX	-	46,XY/46,XY, del(8q12)(%40)	-
Inversions				
CN-47	46, XX, inv (5)(q12;q25)	24	46,XY	30
CN-48	46, XX, inv(18)(q21;q23)	34	46, XY	43
CN-49	46, XX, inv(1)(q23;p22)	31	46, XY	33
CN-50	46, XX, inv(18)(q11;q23)	29	46, XY	29
CN-51	46, XX	32	46, XY, inv(7)(q11;q36)	37
CN-52	46, XX	31	46, XY, inv(X)(p22;p21)	29
CN-53	46, XX	30	46, XY, inv(Yq)	35
CN-54	46, XX	32	46, XY, inv(Yq)	38
CN-55	46, XX	29	46, XY, inv(12)(q13;p11)	32
Numerical aberrations				
CN-56	46, XX/47, XXX(%50)	30	46, XY	30
CN-57	47,XX, + mar	42	46, XY	50
CN-58	46,XX/47,XXX(%30)/46,X,i(Xp)(%50)	34	46, XY	39
CN-59	46, XX	30	47, XX, + mar	-
CN-60	46, XX	23	47, XY, + mar	25
CN-61	46, XX	37	46, XY/47, XY, + mar(%70)	44
CN-62	46, XX	39	46, XY/47, XYY(%50)	50
Mean		29		33,5

CN, couple number.

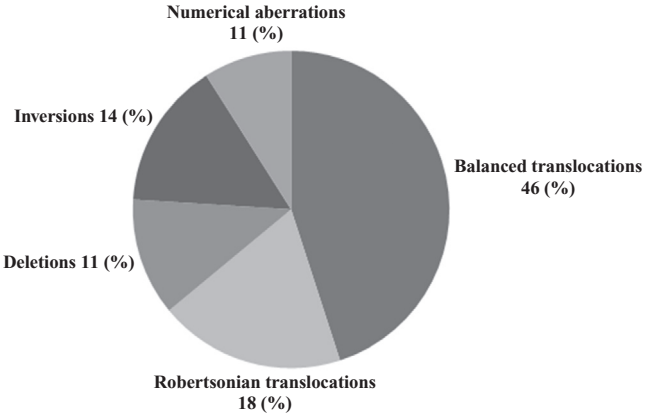

Figure 1 The proportions of chromosome aberration categories.

Table 2 Couples were stratified according to number of spontaneous abortions.

Number of spontaneous abortions	*Number of cases*	*Percentages (%)*
2	420	55
3	211	28
4	73	10
5 and higher	59	8
Total	763	

most couples (420 couples) having two spontaneous abortions, and only a single couple having had nine spontaneous abortions (**Table 2**). A negative relationship was identified between the number of spontaneous abortions and the number of cases. Three per cent (13 out of 420), 3% (7 out of 211), 7% (5 out of 73) and 7% (4 out of 59) of cases showed aberrations in couples who had experienced two, three, four, five or more spontaneous abortions, respectively. Statistical evaluations were conducted to determine whether an increase in the number of spontaneous abortions was associated with an increase in the frequency of aberrations. A Pearson's chi-squared test indicated that an increase in the number of spontaneous abortions did not result in a statistically significant increase in aberration frequency. Among the 1510 couples, 174 (11.5%) were consanguineous.

Balanced translocations

The balanced translocations in the female and male cases, together with their chromosomal locations are shown in **Table 1**. Balanced translocations were observed in only 29 couples; in two of these couples (CN-19 and CN-26), both the male and female were translocation carriers. In total, balanced translocations existed among 19 women and 11 men (the proportion of women and men for each aberrations type is shown in **Figure 2**). Balanced translocations were observed on chromosome 7 in six cases (four women and two men); on chromosome 10 in six cases (four women and two men); and on chromosome 5 in four cases (all of them women). No balanced translocations were identified on chromosomes X and Y.

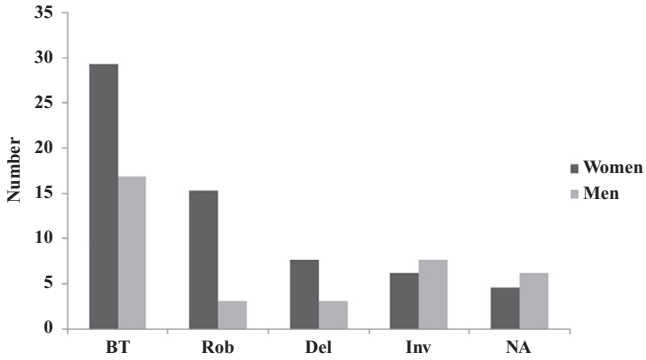

Figure 2 Incidence and gender distribution of various chromosomal aberrations in couples who have experienced recurrent spontaneous abortions. BT, balanced translocations; Rob, Robertsonian translocations; Del, deletions; Inv, inversion; NA, numerical aberrations.

Balanced translocations were observed on all autosomes other than chromosomes 13, 16 and 20, although their break points and frequencies varied from one autosome to another (**Table 1**).

Robertsonian translocations

Robertsonian translocations were identified in a total of 12 individuals (11 couples), including 10 women and two men. In one of these couples (CN-38), both the man and woman were Robertsonian translocation carriers (**Table 1**). In CN-26, the woman was a Robertsonian translocation carrier, whereas the man was a balanced translocation carrier (**Table 1**). Robertsonian translocations were mainly observed between chromosome 13 and 14, with seven cases (six female and one male) having Robertsonian translocations between these autosomes. On the other hand, Robertsonian translocations between chromosomes 14 and 15 were identified in only two cases. Robertsonian translocations between chromosomes 14 and 22, between chromosomes 14 and 21, and between chromosomes 21 and 21 were each observed in a single case. Chromosome 14 mainly translocated with chromosomes 13, 15, 21 and 22. Only a single case with a 45, XY, robt(21;21) karyotype had a Robertsonian translocation that did not involve chromosome 14.

Deletions and inversions

Deletions were identified in seven couples, with five women and two men showing deletions in chromosomes 1, 4, 8, 9, 15 or 18. A 1q32 deletion was observed in two cases (one woman, and a man). Nine couples were identified as having inversion, with four female cases and five male cases having various inversions in chromosomes 1, 5, 7, 12, 18, X or Y at different break points. Two men were observed to have the same inversion type of the Y chromosome's q arm.

Numerical aberrations

Numerical chromosome aberrations (aneuplodies) were identified in seven couples, including three women and four men.

An additional marker chromosome was identified in one woman and three men. One woman and one man had an extra X and Y chromosomes in mosaic form, exhibiting the karyotypes 46,XX/47,XXX(%50) and 46,XY/47,XYY(%50), respectively. A woman exhibited the karyotype 46,XX/47,XXX(%30)/46,X,i(Xp)(%50), which indicates mosaicism in three cell lines.

Discussion

A total of 1510 couples were studied, corresponding to a total of 3020 cases. In 62 (4.1%) of these couples, at least one partner was a chromosome aberration carrier, whereas, in three couples, both the man and woman were chromosome aberration carriers. In this study, the proportion of cases with chromosome aberrations (4.1%) was in agreement with other studies reporting a chromosome aberration frequency of 3–4% (Flynn et al., 2014; Iyer et al., 2007; Saxena et al., 2012). Other studies have reported a chromosome aberration frequency of 6–8% (Dutta et al., 2011; El-Dahtory, 2011; Fuente-Cortés et al., 2009), which is higher than the proportion observed in our study. Studies conducted around the world indicate considerable differences in the reported chromosome aberration frequency, ranging from 2.76% to 18.75% (Dutta et al., 2011). The variation observed in chromosome aberration frequency possibly stemmed from the differences in the sample sizes and sample selection (Iyer et al., 2007).

In the present study, the proportion of structural and numerical aberrations was 89% and 11%, respectively. Therefore, the proportion of structural aberrations was eight times higher than that of the numerical aberrations. Although the proportion of structural and numerical aberrations tends to vary from one study to another (Amudha et al., 2005; Dubey et al., 2005; Rajangam et al., 2007), all the studies showed that structural abnormalities have a higher proportion than numerical aberrations. In this study, the most common structural aberrations were translocations, and the proportions of balanced and Robertsonian translocations were 46% and 18%, respectively. These proportions were in agreement with the previously published findings (Dubey et al., 2005; El-Dahtory, 2011; Fuente-Cortés et al., 2009; Goud et al., 2009; Iyer et al., 2007; Saxena et al., 2012). Parents who are balanced and Robertsonian translocation carriers might have normal phenotypes. During meiosis in these carriers, however, the segregation of chromosomes might lead to unbalanced karyotypes in gametes. As a consequence of this, a zygote (and eventually embryo) with an unbalanced karyotype might result in a spontaneous abortion. As with other types of aberrations, translocations were generally observed in women; this observation was also compatible with the previously published findings (Fuente-Cortés et al., 2009; Goud et al., 2009; Iyer et al., 2007).

Both partners were determined to have karyotype aberrations in three of the non-consanguineous couples (CN-19, CN-26, and CN-38). The age range of these cases was 23–31 years. We considered it interesting that, in all three couples, aberrations were present in at least one common chromosome. Furthermore, we observed that five of the six aberrations in these couples involved chromosome 14. Although these results are certainly noteworthy, further studies and cases are required before they can be properly interpreted. Two other published studies have described similar couples, with each study reporting a single couple where both partners had karyotype aberrations (Makino et al., 1990; Saxena et al., 2012). We also noted that three out of six cases constituting couples CN-19, CN-26, and CN-38 exhibited the same robt(13;14), which was not surprising given the relatively high prevalence of this type of Robertsonian translocation.

Seven deletions were identified in seven cases, including five women and two men, accounted for 11% of aberrations observed in the study. Different studies have reported deletion proportions ranging from 3 to 6.7% (Dubey et al., 2005; Dutta et al., 2011; Iyer et al., 2007; Saxena et al., 2012). In this study, the proportion of deletions was higher than those reported previously. Deletions are essentially unbalanced alterations, as they involve the loss of genetic material. In certain cases, specifically depending on the region and size of the deletion, deletions might be tolerated and not affect viability of the embryo. Nevertheless, such deletions may still lead to a broad range of phenotypic anomalies, such as congenital heart defects, renal abnormalities, gastrointestinal abnormalities, vision problems, delayed and poor development, short stature, hearing loss, abnormal behavior, intellectual impairment, distinctive facial features, orofacial clefting and brain anomalies (Burnside, 2015; Imataka et al., 2015; Jordan et al., 2015; Nguyen et al., 2015). On the other hand, most deletions will still have severe detrimental effects that influence the viability of the embryo. For this reason, such deletions might result in spontaneous abortion at any gestational stage. In our study, we identified deletions on chromosomes 1, 4, 8, 9, 15 and 18.

Inversions were identified in nine cases, including four women and five men, and accounted for 14% of the aberrations observed in our study. These inversions involved chromosomes 1, 5, 7, 12, 18, X and Y. Studies described the presence of chromosome 1, 5, 7, 18, and Y inversions in women who have experienced recurrent spontaneous abortion (Goud et al., 2009; Iyer et al., 2007; Niroumanesh et al., 2011; Saxena et al., 2012). Although the break points of our cases were different from the previously described break points, we nevertheless think that the common chromosomes observed in our study can be considered as dynamic chromosomes, which tend to be prone to inversions. It is well documented that familial pericentric inversions are associated with an increased likelihood of early spotnaneous abortion (Rao et al., 2005; Wenger and Steele, 1981). Of the nine cases with inversions in our study, two had pericentric inversions, whereas the other seven had paracentric inversions. It was determined that paracentric inversions were more frequent than pericentric inversions, which is noteworthy since a previous study described paracentric inversions as having more detrimental effects compared with pericentric inversions (Niroumanesh et al., 2011).

In this study, additional marker chromosomes were identified in four cases, including one woman and three men. Most published studies describe additional X and Y chromosomes (including karyotypes) instead of additional marker chromosomes (Dubey et al., 2005; Goud et al., 2009; Saxena et al., 2012).

In conclusion, the results of our study were generally in agreement with other published studies. The study findings indicated that structural chromosome aberrations (translocations in particular) are a common type of chromosome aberrations in couples experiencing recurrent spontaneous abortion. The study also identified numerical aberrations

among couples with history of recurrent spontaneous abortion. Our study findings also showed that the effective clinical management of couples who have experienced recurrent spontaneous abortion also requires chromosome analysis. After the identification of a chromosome aberration in a couple, they could be advised to receive genetic counselling and to undergo prenatal assessments for future pregnancies. In addition, couples with chromosome translocations should be provided with IVF and pre-implantation genetic evaluation options. Apart from chromosome analysis, couples who have experienced recurrent spontaneous abortion should also be evaluated with molecular techniques (such as fluorescence in-situ hybridization); with microarrays evaluating specific chromosome regions that harbour genes presumed to be involved in recurrent spontaneous abortion and with comparative genomic hybridization techniques.

Acknowledgements

We would like to express our thanks to the routine cytogenetic laboratory personnel (such as technicians) for their assistance and contribution.

References

Amudha, S., Aruna, N., Rajangam, S., 2005. Consanguinity and chromosomal abnormality. Indian J. Hum. Genet. 11, 108–110.

Burnside, R.D., 2015. 22q11.21 deletion syndromes: a review of proximal, central, and distal deletions and their associated features. Cytogenet. Genome Res. 146, 89–99.

Coulam, C.B., 1991. Epidemiology of recurrent spontaneous abortion. Am. J. Reprod. Immunol. 26, 23–27.

Dubey, S., Chowdhury, M.R., Prahlad, B., Kumar, V., Mathur, R., Hamilton, S., Kabra, M., Menon, P.S.N., Verma, I.C., 2005. Cytogenetic causes for recurrent spontaneous abortions-an experience of 742 couples (1484 cases). Indian J. Hum. Genet. 11, 94–98.

Dutta, U.R., Rajitha, P., Pidugu, V.K., Dalal, A.B., 2011. Cytogenetic abnormalities in 1162 couples with recurrent miscarriages in southern region of India. J. Assist. Reprod. Genet. 28, 145–149. doi:10.1007/s10815-010-9492-6.

El-Dahtory, F.A.M., 2011. Chromosomal abnormalities as a cause of recurrent abortions in Egypt. Indian J. Hum. Genet. 17, 82–84.

Exalto, N., 2005. Recurrent miscarriage. Int. Congr. Ser. 1279, 247–250.

Flynn, H., Yan, J., Saravelos, S.H., Li, T.C., 2014. Comparison of reproductive outcome, including the pattern of loss, between couples with chromosomal abnormalities and those with unexplained repeated miscarriages. J. Obstet. Gynaecol. Res. 40, 109–116.

Fuente-Cortés, B.E.D.L., Cerda-Flores, R.M., Dávila-Rodríguez, M.I., García-Vielma, C., Alvarado, R.M.D.L.R., Cortés-Gutiérrez, E.I., 2009. Chromosomal abnormalities and polymorphic variants in couples with repeated miscarriage in Mexico. Reprod. Biomed. Online 18, 543–548.

Garrido-Gimenez, C., Alijotas-Reig, J., 2015. Recurrent miscarriage: causes, evaluation and management. Postgrad. Med. J. 91, 151–162.

Goud, T.M., Harassi, S.M.A., Salmani, K.K.A., Busaidy, S.M.A., Rajab, A., 2009. Cytogenetic studies in couples with recurrent miscarriage in the Sultanate of Oman. Reprod. Biomed. Online 18, 424–429.

Grande, M., Borrell, A., Garcia-Posada, R., Borobio, V., Muñoz, M., Creus, M., Soler, A., Sanchez, A., Balasch, J., 2012. The effect of maternal age on chromosomal anomaly rate and spectrum in recurrent miscarriage. Hum. Reprod. 27, 3109–3117.

Imataka, G., Ohwada, Y., Shimura, N., Yoshihara, S., Arisaka, O., 2015. Del(18)(q12.2q21.1) syndrome: a case report and clinical review of the literature. Eur. Rev. Med. Pharmacol. Sci. 19, 3241–3245.

Iyer, P., Wani, L., Joshi, S., Lakshmi, J., Dalvi, R., Chavan, D., Das, B.R., Mandava, S., 2007. Cytogenetic investigations in couples with repeated miscarriages and malformed children report of a novel insertion. Reprod. Biomed. Online 14, 314–321.

Jordan, V.K., Zaveri, H.P., Scott, D.A., 2015. 1p36 deletion syndrome: an update. Appl. Clin. Genet. 8, 189–200.

Kavalier, F., 2005. Investigation of recurrent miscarriages. BMJ 331, 121–122.

Kochhar, P.K., Ghosh, P., 2013. Reproductive outcome of couples with recurrent miscarriage and balanced chromosomal abnormalities. J. Obstet. Gynaecol. Res. 39, 113–120.

Makino, T., Tabuchi, T., Nakada, K., Iwasaki, K., Tamura, S., Iizuka, R., 1990. Chromosomal analysis in Japanese couples with repeated spontaneous abortions. Int. J. Fertil. 35, 266–270.

Mozdarani, H., Meybodi, A.M., Moradi, S.Z., 2008. A cytogenetic study of couples with recurrent spontaneous abortions and infertile patients with recurrent IVF/ICSI failure. Indian J. Hum. Genet. 14, 1–6.

Nguyen, J.M., Qualmann, K.J., Okashah, R., Reilly, A., Alexeyev, M.F., Campbell, D.J., 2015. 5p deletions: current knowledge and future directions. Am. J. Med. Genet. C Semin. Med. Genet. 169, 224–238.

Niroumanesh, S., Mehdipour, P., Farajpour, A., Darvish, S., 2011. A cytogenetic study of couples with repeated spontaneous abortions. Ann. Saudi Med. 31, 77–79.

Rajangam, S., Tilak, P., Aruna, N., 2007. Rema devi: karyotyping and counseling in bad obstetric history and infertility. Iranian J. Reprod. Med. 5, 7–12.

Rao, L., Murthy, K., Babu, A., Venkata, P., Deenadayal, M., Singh, L., 2005. Chromosome inversions and a novel chromosome insertion associated with recurrent miscarriages in South India. Arch. Gynecol. Obstet. 272, 273–277.

Saxena, S.G., Desai, K., Shewale, L., Ranjan, P., Saranath, D., 2012. Chromosomal aberrations in 2000 couples of indian ethnicity with reproductive failure. Reprod. Biomed. Online 25, 209–218.

Stephenson, M.D., Sierra, S., 2006. Reproductive outcomes in recurrent pregnancy loss associated with a parental carrier of a structural chromosome rearrangement. Hum. Reprod. 21, 1076–1082.

Stirrat, G.M., 1990. Recurrent miscarriage. Lancet 336, 673–675.

Sugiura-Ogasawara, M., 2014. Recurrent pregnancy loss and obesity. Best Pract. Res. Clin. Obstet. Gynaecol. 29, 489–497. pii: S1521-6934(14)00257-0.

Van den Berg, M.M., van Maarle, M.C., van Wely, M., Goddijn, M., 2012. Genetics of early miscarriage. Biochim. Biophys. Acta 1822, 1951–1959.

Wenger, S.L., Steele, M.W., 1981. Meiotic consequences of pericentric inversions of chromosome 13. Am. J. Med. Genet. 9, 275–283.

Declaration: The authors report no financial or commercial conflicts of interest.

Received 12 August 2015; refereed 14 January 2016; accepted 19 January 2016.