Chromosomal Analysis In Couples With Recurrent Spontaneous Abortion: A Case Control Study

Malamoni Dutta*, Jagadish Mahanta, Gokul Chandra Das***, Avdesh Kumar Rai******

*Associate Professor, Anatomy, Assam Medical College, Dibrugarh, ** Former Director R.M.RC(Dibrugarh) Cum Distinguished Scientist Chair,ICMR, New Delhi, ***Rtd. Professor And Ex Head Of The Department Of Obstetrics And Gynaecology, Gauhati Medical College, Ghy-32, **** Assistant Research Officer, DBT Centre For Molecular Biology And Cancer Research, B.B.C.I, Guwahati, India

Abstract: Background: Normal expected outcome of a couple is birth of a healthy child after completion of the period of pregnancy. About 50% of pregnancies end in fetal loss as abortion. Lower implantation and higher spontaneous abortion rate are closely associated with the chromosomal abnormalities of the parents. It has been recommended that the standard investigation of such cases should include karyotyping of both the parents for chromosomal aberrations. The aim of the study was to find out whether any specific chromosomal abnormalities exist in couples with recurrent spontaneous abortion. Method: 75 couples with history of recurrent spontaneous abortion as well as 75 fertile couples as control were investigated for chromosomal aberrations. Statistical analysis (Chi square test) was done to find out the association of recurrent spontaneous abortion with chromosomal abnormalities of the parents. Result: Out of 75 couples (150 subjects) with recurrent spontaneous abortion, 10 subjects(6.7%) were found to be having abnormal karyotypes. The statistical analysis ($p<0.05$) signifies that chromosomal abnormalities of the parents are associated with recurrent spontaneous abortion. Conclusion: Recurrent spontaneous abortion continues to be a challenging reproductive problem for the clinician. Therefore, identifying a cytogenetic cause may be of great significance for the management of such cases. [Malamoni D, Natl J Integr Res Med, 2018; 9(4):25-31]

Key Words: recurrent spontaneous abortion, chromosomal abnormalities, karyotyping, cytogenetic cause, miscarriage

Author for correspondence: Malamoni Dutta, Prithivi Priya Apartment, Namghar Path, Bye Lane:-1, Lakhiminagar, Hatigaon, Guwahati-781038, Kamrup, Assam, E-Mail: malamonid@yahoo.in M:9401969575

Introduction: Spontaneous abortion or Miscarriage is a common outcome of Pregnancy. Spontaneous miscarriage means abortion without any mechanical or medical means in order to evacuate uterus[1]. The classic definition of recurrent spontaneous abortion(RSA) is the loss of three or more clinically documented conceptions unexpectedly during early gestation. However, the modern definition refers to the spontaneous loss of two or more consecutive pregnancies before twenty weeks of gestation[2]. Recurrent miscarriage is an extremely stressful condition for both the couples and the physicians because it is extremely difficult to find a reason behind it.

These fetal losses are attributed to various causes like autoimmune disease, endocrinal disorders, genetic factors, anatomic factors, infections and environmental factors[3,4]. Chromosomal anomalies seem to cause about 40% to 50% of spontaneous abortions in those cases in which the conceptus has been recovered and examined[5]. Abnormal chromosome can be produced in the germ line of either parent through an error in meiosis or fertilization or can arise in the early embryo through an error in mitosis. Jacobs and Hassold (1988)[6] reported that approximately 95% of chromosomal abnormalities were due to maternal gametogenesis errors and 5% paternal errors.

Approximately 15% of all clinically recognized pregnancies are spontaneously aborted[7]. Parenteral chromosomal anomalies are one of the undisputed causes of spontaneous abortion. Spontaneous abortion of a developing embryo may be caused by the presence of rearrangements in parental chromosome set resulting in formation of gamates with unbalanced chromosomes, like duplications or deletions by unequal crossing over during meiosis[8]. The most common structural rearrangement involved in multiple abortions is chromosomal translocation[9]. Translocation involves exchange of genetic material between two or more non-homologous chromosomes. Robertsonian translocation shows the fusion of two acrocentric chromosomes, near the centromere region with loss of the short arms. Carriers of these rearrangements are likely to produce unbalanced gametes resulting in abnormal offspring with unbalanced karyotypes[10,11]. In approximately 4% of couples with recurrent miscarriages, at least one partner is a carrier for either a balanced reciprocal translocation or a Robertsonian translocation. Carriers of balanced translocations are phenotypically normal but their gametes are genetically unbalanced due to

meiotic errors[12]. It has been recommended that the standard investigation of such patients should include karyotyping of both parents for chromosomal aberrations[13].Northeast is a boiling pot of genetic pool where population migration over the years from South East Asia, Chinese region as well as main land of India made it very interesting for genetic study. Though karyotyping of both parents in recurrent abortion has been recommended as a standard investigation no such study has been done in the North east region of our country.

Keeping this view in mind the aim of the study was to evaluate the contribution of chromosomal anomalies of parents in recurrent spontaneous abortion and to analyze the relative occurrence of different anomalies in them.

Methods: This case control study was conducted at Gauhati Medical College and Hospital, Guwahati. On approval from Institutional Ethical Committee the study was conducted during the period from February 2015 to July 2017.

75 numbers of couples with history of 2 or more episodes of recurrent spontaneous abortion was selected for the study. Similarly 75 numbers of fertile couples without any history of spontaneous abortion was included in the study as control.

Inclusion criteria: Couples with history of two or more episodes of recurrent spontaneous abortion in absence of any apparent cause were included in the study.

Exclusion Criteria: Non-consenting couples and patients with known immunological, infectious and endocrinal disorders were excluded from the study.

Structured questionnaires were administered and information about age, religion, education, socioeconomic status etc. was obtained. Special emphasis was given in family history and consanguinity according to prescribed questionnaire.
Peripheral blood was collected from each of the partner of the couples. The karyotyping procedure was done at "DBT Centre for Molecular Biology & Cancer Research" of Dr. B. Borooah Cancer Institute, Guwahati. Karyotyping (lymphocyte culture method) was done by analysis of G-banded chromosomes using 5ml heparinized peripheral blood sample. Metaphase spreads were made from phytohemaglutinin stimulated peripheral lymphocytes using standard cytogenetic techniques. Cultures were harvested and karyotypes were prepared using G-banding technique with trypsin and Giemsastaining (GTG). For each subject, a minimum of 30 metaphases were examined under microscope. Chromosomes were analyzed with the help of Cyto-vision software(Applied Imaging, USA). The chromosome identification was done in accordance with the International System for Human Cytogenomic Nomenclature (ISCN) 2016[14].

Statistical Analysis: Software SPSSv20 was used for statistical analysis. Chi-square test was done for independence of attributes to find out whether there is any association between recurrent spontaneous abortion and chromosomal abnormalities of the parents. The level of $p<0.05$ was considered as significant.

Results: Out of 75 couples (150 subjects) with history of recurrent spontaneous abortion, normal karyotype was found in 140 cases. Abnormal karyotype was found in 10 cases(6.7%).

Normal karyotype was found in all 75 couples (150 subjects) who were enrolled as control.

Among the 10 affected individuals 7 were female and 3 were male. Structural abnormalities were found in 6 cases(60%); whereas 4 cases(40%) had numerical abnormalities(Table 1 and 2).

The mean maternal and paternal age of subjects carrying chromosomal anomalies were 30.4 years and 33.7 years respectively.

Among structural abnormalities(Table1), balanced reciprocal translocations were seen in 3 female patients. In the first case(Serial no.1) balanced reciprocal translocation was found between long arm of chromosome 6 and 18(Fig.1). In the second case(Serial no.3) reciprocal translocation was found between long arm of chromosome number 1 and 3(Fig.8). In the third case (Serial no.4), balanced reciprocal translocation was found between long arm of chromosome 18 and 22(Fig.6).
Robertsonian translocation was found in a male patient(Serial no.5) which involved chromosome 13 and 14(Fig.7).

Deletion of terminal portion of short arm of chromosome no 10(Serial no.2) was found in one male patient(Fig.9).

Inversion involving chromosome no 16(Serial no.6) was found in one female patient(Fig. 5).

Table 1: Cases with structural abnormalities

No.	Type of abnormality	Age	Sex
1	46XXt(6,18)(q27,q23)	22	Female
2	46XYdel(10) pter	30	Male
3	46XXt(1,3)(q43,q29)	30	Female
4	46XXt(18,22)(q21.1q12)	29	Female
5	45XY rob(13,14)	35	Male
6	46XXinv16(p13q22)	36	Female

Numerical abnormalities (Table.2) were found in four subjects. Out of these, two subjects were mosaics (Serial no.1 and 2) with monosomy 45XO along with normal cell line. Karyotype of one such case is shown in Fig.4.One subject (Serial no.3) had 47XXX karyotype with normal cell line (Fig.2).47XXY karyotype (Serial no.4) was observed in one male patient along with normal cell line(Fig 3).

Table2: Cases with numerical abnormalities

No.	Type of abnormality	Age	Sex
1	46XX/45XO	27	Female
2	46XX/45XO	34	Female
3	47XXX/46XX	35	Female
4	47XXY/46XY	36	Male

To find out if there is any relation between couples with Recurrent spontaneous abortion(RSA) and fertile couples on the basis of their chromosomal constitution of individual, chi-square test was carried out for independence of attributes. The outcome of the study can be represented in a 2x2 contingency table as follows-

Table 3: Couples with Repeated spontaneous abortion and Fertile couple Crosstabulation

	Condition		Total
	Normal	Abnormal	
RSA	140	10	150
Fertile Couple	150	0	150
Total	290	10	300

Table:4 Couples with Repeated spontaneous abortion and Fertile couple Crosstabulation with Expected Number of observations

		Condition		Total
		Normal	Abnormal	
Couples with RSA	Observed Count	140	10	150
	Expected Count	145.0	5.0	150.0
Fertile couples	Observed Count	150	0	150
	Expected Count	145.0	5.0	150.0
Total	Observed Count	290	10	300
	Expected Count	290.0	10.0	300.0

Table 5:-Chi-Square Tests

	Value	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	10.345[a]	.001		
Continuity Correction[b]	8.379	.004		
Likelihood Ratio	14.208	.000		
Fisher's Exact Test			.002	.001
Linear-by-Linear Association	10.310	.001		
N of Valid Cases	300			

H_0: Recurrent abortion is not associated with chromosomal abnormalities. For testing the null hypothesis, we are using chi-square test for independence of attributes.

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 5.00
b. Computed only for a 2x2 table

From the above table we see that the p value (0.001) for Pearson Chi-square test is less than 0.05 therefore we should reject our null hypothesis and conclude that there is association between recurrent spontaneous abortion and chromosomal abnormalities.

Fig 1: Karyotype showing translocation between chromosome 6 and 18.

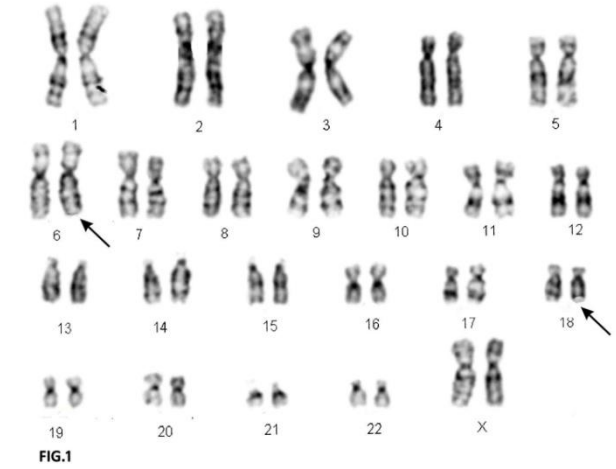

FIG.1

Fig 2: Karyotype showing 47XXX

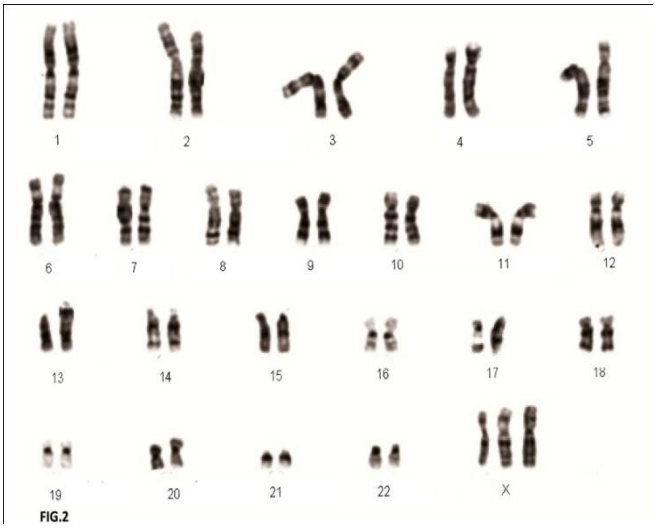

FIG.2

Fig.3: Karyotype showing 47XXY

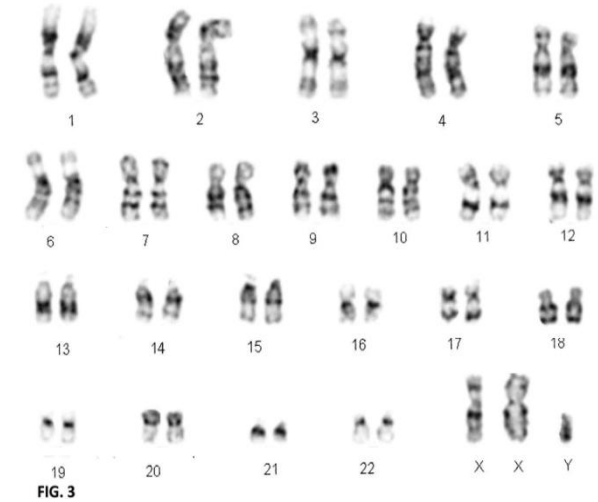

FIG. 3

Fig.4: Karyotype showing 45 XO

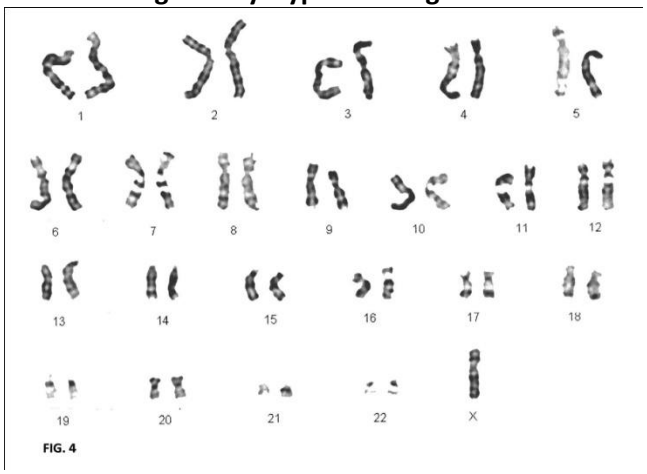

FIG. 4

Fig.5: Karyotype showing 46 XX inv (16)

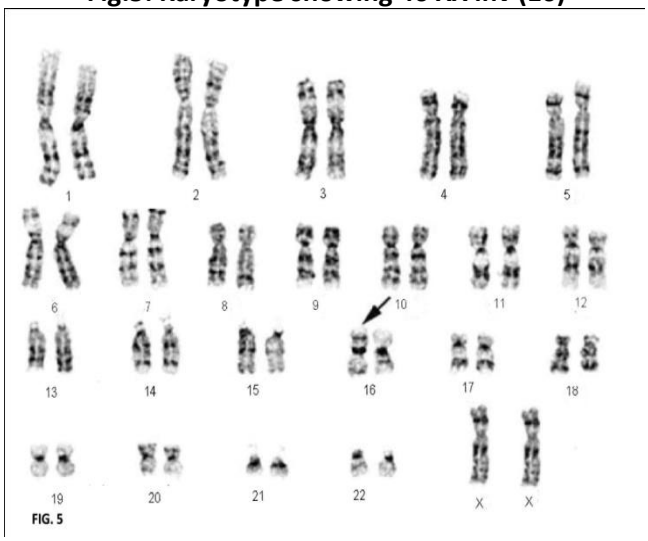

FIG. 5

Fig.6: Karyotype showing translocation between chromosome 18 and 22.

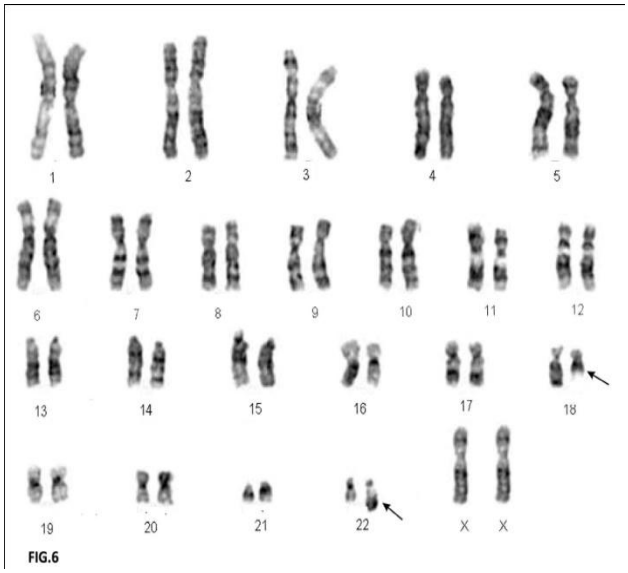

FIG.6

Fig. 7: Karyotype showing Robertsoniantranslocaton 45XY rob(13,14)

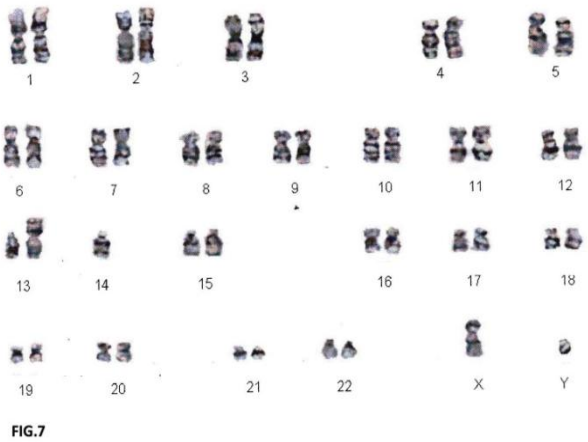

FIG.7

Fig. 8: Karyotype showing translocation between chromosome 1 and 3.

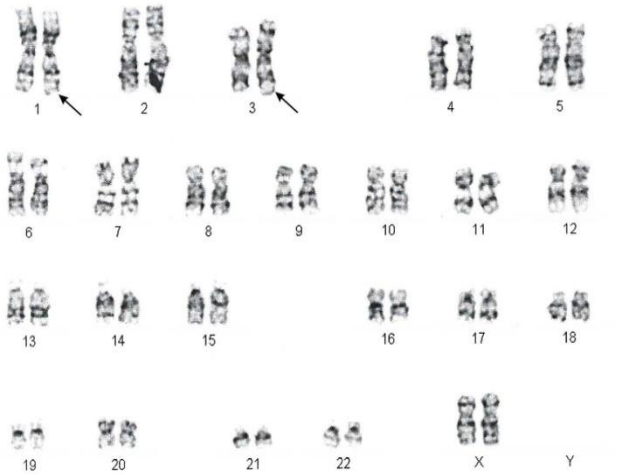

FIG.8

Fig.9: Karyotype showing terminal deletion of chromosome 10

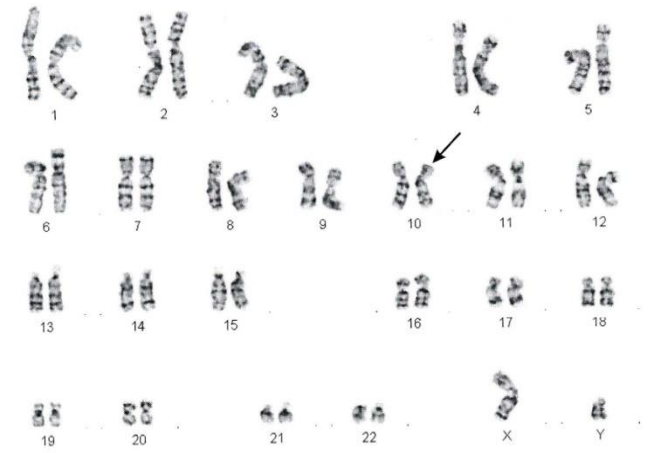

FIG.9

Discussion: In the present study, we found significant differences for chromosomal aberrations while comparing couples with recurrent spontaneous abortion(6.7%) to control(0%). The frequency of chromosomal aberrations was similar to that observed in other reports[15,16,17,18]. However lower frequencies were also observed by other authors[19,20,21,22]. Many studies attribute this variability to differences in the sample size and variations in the criteria for selection of the cases, such as the number of abortions and the exclusion of those with different aetiology.

In our study, Chromosomal aberrations were found in 10 cases with recurrent spontaneous abortion including mosaicism in the X chromosome in 4 cases(40%). Similar observations were made by Kiss et al[23] and Pal et al[24] who reported X-chromosome mosaicism in 50% and 40% cases respectively. Out of the 4 cases of X chromosome mosaicism, one case was male (10%) and 3 cases(30%) were female. It has been related that approximately 3% of infertile males and 5-10% of those with oligospermia or azoospermia had mosaic Klinefelter syndrome[25].

Balanced chromosomal rearrangements in either parent are an important cause of RSA, particularly in the first trimester[26,27,28].Couples with balanced reciprocal translocation have a chance of suffering from recurrent miscarriage and a20% risk of bearing children with abnormal genetic makeup[29].

In the present study, we found 3 cases of reciprocal translocation in women with recurrent miscarriages and one case of robertsonian translocation in a male partner of a couple with recurrent spontaneous abortion. Translocations were found to be more common in women (30%) compared to men (10%). Most studies have reported that in couples with recurrent pregnancy loss the number of female carriers with balanced chromosomal aberrations significantly exceeds the male carriers[30].As male carriers of transloations have reduced fertility, chromosomal abnormalities in the female partner are a more common finding in couples with recurrent miscarriages[24].The significant increase in reciprocal translocation in couples with reproductive failure is an expected finding because the carrier of reciprocal translocation have a significantly increased risk of chromosomal imbalance during gametogenesis due to unequal meiotic segregation of the balanced translocation[31,32,33].

In this study, inversion of chromosome 16 was observed in one woman with recurrent miscarriage. Similar observation was also made by Fuente-Cortes et al[34].

In our study terminal deletion of chromosome 10 was observed in one male partner of a couple with recurrent spontaneous abortion. Dubey et al[20] in their study on 742 couples with recurrent spontaneous abortions found deletion of Chromosome 10 in one male partner.

Conclusion: Recurrent spontaneous abortion continues to be a challenging reproductive problem for the patient and the clinician. Therefore, identifying a cytogenetic cause for a miscarriage may be of great significance for the management of such patients. In case of detected chromosomal aberration, the patient should be counseled individually according to the type of anomaly. Cytogenetic analysis should be recommended in all couples with recurrent spontaneous abortion because the results could provide vital information for their genetic counseling and future genetic intervention.

In today's scenario, further studies focusing on the molecular mechanisms using more sophisticated technologies such as assay comparative genomic hybridization(aCGH) and Next Generation Sequencing (NGS) are highly recommended.

Acknowledgement: I want to acknowledge Indian Council of Medical Research (I.C.M.R), New Delhi for financial support to carry out this study. I would also like to acknowledge Mr. Saurav Sarma, Research scholar, Department of Statistics, Gauhati University for helping me in statistical analysis .

References:

1. Hamman AK, Wang NE, Chona S. The pregnant adolescent with vaginal bleeding: Etiology, diagnosis and management. Paediatric emergency care. 2006;22(10):76-77
2. Meka A. and Reddy M. Recurrent Spontaneous Abortions: An Overview of Genetic and Non-Genetic Backgrounds. Int J Hum Genet. 2006; 6(2):109-117
3. Edmonds DK. Recurrent Miscarriage. Dew Hurst's Text Book OfObstetrics and Gynaecology. 7th ed. Blackwell Publishing. Oxford, Massachusettes,2007 :100-105
4. ImpeyL. Disorders of early pregnancy. Obstetrics and Gynaecology. 2nd ed. John Wiley & Sons Ltd. Oxford,2004:94-95
5. Schoenwo lf GC, Bleyl SB, Brauer PR, Francis-West PH. Gametogenesis, Fertilization and the First week. Larsen's Human Embryology. 5thed.ChurcillLivingstone. Philadelphia, 2015:14-42
6. Hassold T, Jacobs PA, Pettay D. Cytogenetic studies of couples with repeated spontaneous abortions of known karyotype. Genet Epidemiol.1988; 5(2):65-74
7. Exalto N. Recurrent miscarriage. IntCongr Ser.2005;1279: 247-50
8. Yuce H, Tekederell L, Elyas H. Cytogenetic results of recurrent spontaneous abortions in Turkey. Medical Science Monitor.2007;13(6):286-289
9. Khedekar DN, Kesari GV, Kotwaliwale SS, Hattangdi SS. Cytogenetic study of leucocyte culture in multiple pregnancy loss in Mumbai. International J of Genetics.2012;4(2):80-84
10. PurandareH, Chakravarty A. Human Cytogenetic techniques and clinical applications. Bhilani Publishing House, Mumbai.2000;182-203
11. Cheng L, Zhang DY. Molecular Genetic Pathology in Principles of Clinical Cytogenetics. Human Press, Indiapolis, New York,2008;41-61
12. Carp H, Guetta E, Dore H, Soriano D, Barkai G, Schiff E. Embryonic Karyotype in recurrent miscarriage with parental aberrations. Fertil Steril.2006;85:446-50
13. 13. Lee RM, Silver RM. Recurrent pregnancy loss; summary and clinical recommendations. Semin Reprod Med. 2000;18:433-40
14. 14. McGowan-Jordan J, Simons A, Schmid, editors. ISCN 2016: An International System of Human Cytogenomic Nomenclature. Basil: S karger AG.2016
15. 15.Cortes B, Cerda R, Davila M, Garcia C, De La Rosa R, Cortes E. Chromosomal abnormalities and polymorphic variants in couples with repeated miscarriages in Mexico. Reprod Biomed online.2009;18(4):543-548
16. 16.El-Dahtory FA. Chromosomal abnormalities as a cause of recurrent abortions in Egypt. Ind J Hum Genet.2011;17(2):82-84
17. Al-Hassanee A, Abdulameer S, Kashmoola M. Chromosomal abnormality in couple with repeated abortion. Tikrit Medical Journal.2012;8(1):48-53
18. Gaboon N, Mohamed A, Elsayed S, Zaki O, Elsayed M. Structural chromosomal abnormalities in

couples with recurrent abortion in Egypt. Turk J Med Sci.2015;45:208-213
19. Azim M, Khan AH, Khilji ZL, Pal JA, Khurshid M. Chromosomal abnormalities as a cause of recurrent abortions: a hospital experience. J Pak Med Assoc.2003; 53:117-119.
20. Dubey S, Chowdhury M, Prahlad B, Kumar V, Mathur R, Hamilton S, Kabra M, Menon PSN, Verma IC. Cytogenetic causes for recurrent spontaneous abortions-An experience of 742 couples(1484 cases). Indian J Hum Genet.2005;11:94-98.
21. Dutta U, Rajitha P, Pidugu V, Dalal A. Cytogenetic abnormalities in 1162 couples with recurrent miscarriages in southern region of India: Report and Review. J Assist Reprod Genet.2010;28:145-149
22. Meshkat Z, Khadem Ghaebi N, Khajedaluee M, Aghili Z, Rostami S. Prevalance of chromosomal aberrations in couples with recurrent miscarriages in the city of Mashhad, Iran: A cross sectional study. Arch Med Lab Sci.2016;2(1):19-23
23. Kiss A, Rosa RFM, Dibi RP, Zen PRG, Pfeil JN, Graziadio C, etal. Chromosomal abnormalities in couples with history of recurrent abortion. Rev Bras Ginecol Obstet.2009;31(2):68-74.
24. Pal S, Ma S, Norhasimah M, Suhaida M, Siti Mariam I, Ankathil R, Zilfalil B. Chromosomal abnormalities and reproductive outcome in Malaysian couples with miscarriage. Singapore Med J.2009;50(10):1008-1012
25. Abdel-Razia MM, Abdel-Hamid IA, Elsobky E, EL-Dahtory F. Further evidence of the clinical, hormonal and genetic heterogenicity of Klinefelter syndrome: a study of 216 infertile Egyptian patients. J Androl.2012;33(3):441-448
26. Makino T, Tabuchi T, Nakada K, Iwasaki K et al. Chromosomal analysis in Japanese couples with repeated spontaneous abortion. Int J Fertil.1990;35:266-270
27. Sheth FJ, Liehr T, Kumari P, Akinde R et al. Chromosomal abnormalities in couples with repeated fetal loss: An Indian retrospective study. Indian J Hum Genet.2013;19:415-422
28. Alaraji SMH. Chromosomal abnormalities associated with recurrent spontaneous abortions in Iraqi women. Med J Babylon.2014;7:2
29. De P, Chakravarty S, Chakravarty A. Novel balance chromosomal translocations in females with recurrent spontaneous abortions: Two case studies. J Hum Reprod Sci.2015;8:114-117
30. Kochhar PK and Ghosh P. Reproductive outcome of couples with recurrent miscarriage and balanced chromosomal abnormalities. J Obstet Gynaecol Res.2013;39:113-120
31. Celep F, Karaguzel A, Ozeren M, Bozkaya H. The frequency of chromosomal abnormalities in patients with reproductive failure. Eur J Obstet Gynecol Reprod Biol.2006;127(1):106-109
32. Fryus JP, Van Buggenhout G. Structural chromosome rearrangements in couples with recurrent fetal wastage. Eur J Obstet Gynecol Reprod Biol.1998;8(2):171-6
33. Panasink B, Danik J, Lurie IW, Stasiemiz-Jaracka B, Leuniewicz R, Sawicka A et al. Reciprocal chromosome translocations involving short arm of chromosome 9 as a risk factor of unfavourable pregnancy outcomes after meiotic malsegregation 2:2.Adv Med Sci.2009;54(2):203-10
34. De la Fuente-Cortes BE, Cerda-Flores RM, Davila-Rodrigauez MI, Garcia-Vielma C, De la Rosa Alvardo RM, Cortes-Gutierrez EL. Chromosomal abnormalities and polymorphic variants in couples with repeated miscarriage in Mexico. Reproductive Biomedicine online;www.rbmonline.com/Article/3534 on web 29 Jan 2009.vol18 (4):543-548.

Conflict of interest: None

Funding: ICMR, New Delhi
(Seed Money Grant Project)

Cite this Article as: M Dutta, J Mahanta, G Das, ARai. Chromosomal Analysis In Couples With Recurrent Spontaneous Abortion: A Case Control Study. Natl J Integr Res Med 2018; 9(4):25-31