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Der Pharmacia Lettre, 2017, 9 [4]:44-56
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Scholars Research Library
ISSN 0975-5071
USA CODEN: DPLEB4

A Retrospective Cytogenetic Study of Chromosomal Abnormalities in Infertile Couples of Indian Origin

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ABSTRACT

Declining human fertility rates are becoming a cause of major global concern. Though this decline is more pronounced in industrially developed countries, recent figures have projected a decline in fertility rates in developing countries such as India also. It has been established that structural and numerical chromosome abnormalities are the common causes of infertility, loss of pregnancy, and the birth of abnormal offspring. In the present study, we have carried out a cytogenetic analysis of patient with infertility. A higher incidence of chromosomal and structural abnormalities was observed in the infertile patients. It was observed that, 92.5% belonged to cytogenetically normal group whereas 4.4% revealed abnormal karyotype and 3.1% showed variant type of chromosome. It was observed that amongst the study group chromosomal abnormalities were more prevalent amongst females (63.63%) as compared to male (36.36%) subjects. In the cytogenetically abnormal group the most

frequent chromosomal abnormalities were balanced translations observed in approximately about 43% of the subjects. These balanced translations also included four Robertsonian translocations, Inversion was recorded in 18% of the subjects.

Keywords: *Infertility, cytogenetics, karyotyping reproductive failure, chromosomal abnormality, variant chromosome*

INTRODUCTION

Most of the recurrent spontaneous abortions that occur during first trimester of pregnancy are caused by chromosomal abnormalities in the fetus [1]. These abnormalities may arise de novo or may be inherited from one of the parent. It has been reported that in 4-8% of couples with history of recurrent spontaneous abortion, at least one of the partners has chromosomal abnormality [2].

Conventional cytogenetic analysis (karyotyping) provides an overview of all chromosomes giving vital information on aneuploidies such as Klinefelter and Turner syndrome as well as major structural alterations, such as balanced translocations, unbalanced translocations, deletions and inversions. Cytogenetic studies are essential for evaluating the role of chromosomal aberrations and heterochromatin variations in reproductive failure. Karyotyping of the subjects with reproductive failure is important not only for diagnosis but also to predict the success rate of the assisted reproduction treatment strategies [3]. The present study comprises of 1000 patients that is 500 couples of Indian origin with no known cause of infertility (idiopathic). Cytogenetic studies were performed on a total of 500 infertile couples that were referred to our laboratory during 2009-2011 in the Dr. Lal Path labs, India.

MATERIAL AND METHODS

An informed consent was taken from all the subjects before taking the blood sample. The karyotyping investigations were carried out on the cultures of the peripheral blood lymphocytes by following internationally accepted standard Cytogenetic techniques as detailed in chapter 3 Material and Methods. Short-term cultures of peripheral blood lymphocytes were established in RPMI supplemented with 10% FBS and antibiotics. After 72 h of culture, the culture was arrested in the metaphase stage using colchicine reagent. The metaphase spreads were analyzed by the standard GTG-banding technique. The chromosomal abnormalities which were observed in the data set were reported in accordance with the ISCN guidelines (International System for Human Cytogenetic Nomenclature) [4].

RESULTS

Amongst the selected 1000 subjects (500 couples); 925 patients (92.5%) were found to possess normal karyotype whereas 44 patients (8.8%) revealed abnormal karyotype. The distribution of chromosomal abnormality found in the patients with Infertility has been tabulated in Table (1). It was observed that amongst the cytogenetically abnormal group chromosomal abnormalities were more prevalent amongst females (63.63%) as compared to male (36.36%) subjects (Figure 1). A significantly higher incidence of numerical as well as structural chromosomal abnormalities was observed in female patients (Table.2). Frequency and percentage were presented for categorical data and Pearson chi square test were used for calculating the p-value.

Table-1: Distribution of chromosomal abnormalities found in the patients with Infertility

S. No	Age	Gender	Numerical abnormalities	Structural abnormalities
1.	23	F		46, X, PSU IDIC(X)(Q22)
2.	27	F		46, XX, t (2;14) (q21; q11.2)
3.	21	F		45, XX, rob (13;15) (q10; q10)
4.	27	F		46, XX, t (1;3) (p36.1;q26)
5.	30	M		46, XY, t (4;7) (q25; q22)
6.	33	M		46, XY, inv (17) (p12q23)
7.	36	M		46, XY, add (15) (q10)
8.	35	M		45, XY, rob (13;14) (q10; q10)
9.	32	F	mos 45, X [15]/46, X, +mar [15]	
10.	25	F		45, XX, rob (13;14) (q10; q10)
11.	26	F		46, XX, t (2;13) (p23; q34)
12.	32	F		46, XY Androgen Insensitivity syndrome
13.	36	M		46, XY, inv (2) (p24q35)
14.	26	F		46, XX, inv(8)(p21q22)
15.	32	F	45, X	
16.	31	F		46, XX, t(16;19)(q10;p13.3)
17.	26	F		46, XX,t(4;5)(q28;p15)
18.	27	M		46,XY,t(4;5)(q28;p15)
19.	22	F	46,X,i(X)(q10)[25]/45,X[5]	
20.	59	M		46,XY,inv(2)(q11.2q31)
21.	24	M	47,XXY	
22.	26	F		46,X,der(X)del(X)(q22)add(X)(q22)
23.	24	M	47,XXY	
24.	27	F		46,XX,t(2;12)(q33;q22)
25.	24	M		46,XY,15pstk+
26.	26	F		46,X,t(X;15)(p22.1;q22)
27.	34	F		46,XX,15pstk+
28.	28	M		46,X,inv(Y)(p11q11)

29.	25	F		46,XX,t(4;8)(p15;p21)
30.	45	F	mos 47,XXX[4]/46,XX[26]	
31.	28	F	45,X	
32.	30	F		46,XX,t(11;22)(q23;q11.2)
33.	26	F	mos 45,X[4]/46,XX[26]	
34.	35	M		46,XY,t(2;20)(q31;q13.3)
35.	26	F		46,XX,inv(5)(p15q35)
36.	25	F		46,XX,15pstk+
37.	36	M		46,X,inv(Y)(p11q11)
38.	33	F		46,XX,t(8;11)(q24;q23)
39.	28	M		46,X,inv(Y)(p11;q11)
40.	32	M	mos 47,XY,+mar[13]/46,XY[17]	
41.	38	F		46,XX,t(3;17)(p21;p11.2)
42.	23	F		45,XX,rob(13;14)(q10;q10)
43.	36	M	mos 47,XYY[10]/46,XY[20]	
44.	29	F		46,XX,t(5;7)(q31;q11.2)

Figure-1: Distribution of chromosomal abnormalities in male & female patients.

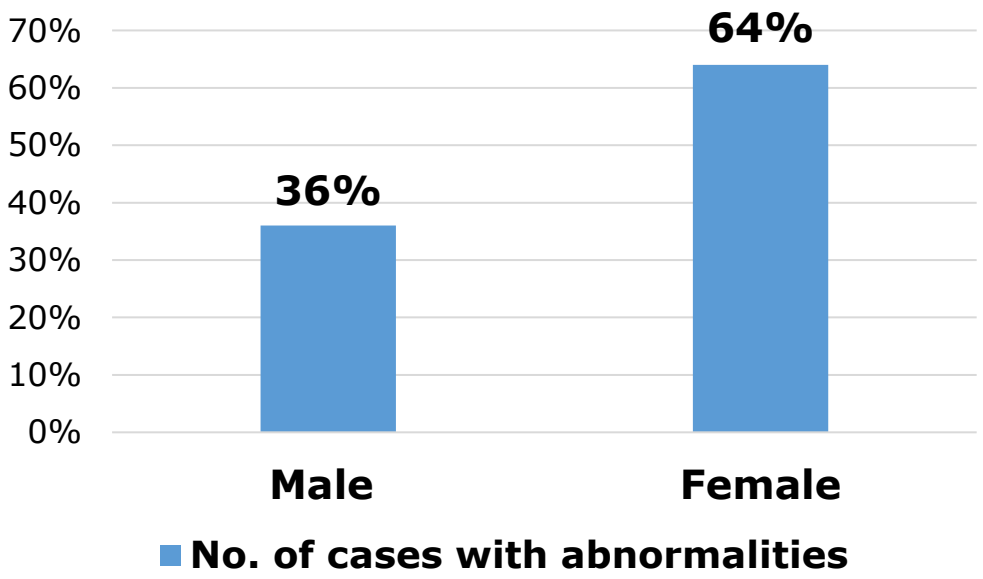
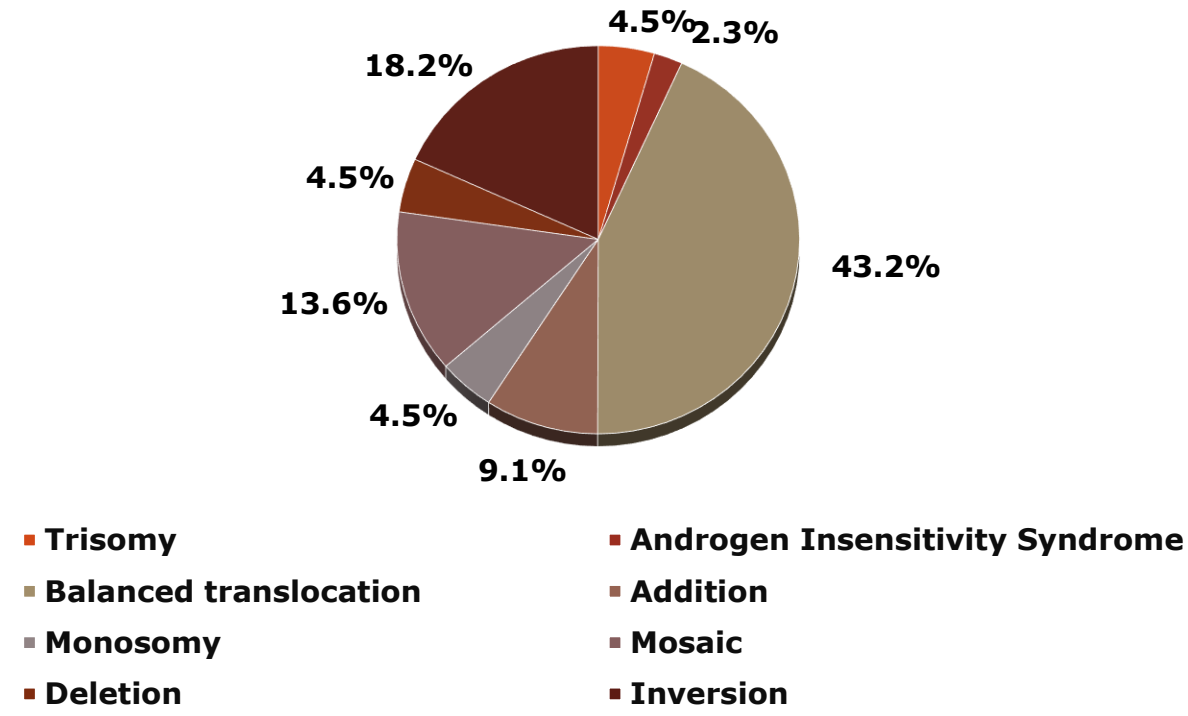


Table-2: Gender wise distribution of numerical and structural chromosomal aberrations.

	Numerical Abnormalities		Structural Abnormalities	
	TOTAL NO: 10		TOTAL NO: 34	
	Male	Female	Male	Female
Number of samples	4	6	13	21
Percentage (%)	40	60	38.2	61.8

The constitutional chromosomal abnormalities can be classified as numerical and structural. Klinefelters syndrome (XXY) was the most frequent numerical anomaly affecting 2 patients 50% male partners in the study group whereas monosomy of chromosome X (Turner syndrome) was the most frequent anomaly affecting 5 patients 83% of female partner in the subject couples. The most frequent structural chromosomal abnormalities were balanced translocations that were observed in 43.18% of the subjects. These balanced translocations also included four Robertsonian translocations, Inversion was observed in 18.18% of the subjects. Figure .4 shows the proportion of different types of anomalies amongst the patients.

Figure-2: Different type of chromosomal abnormalities found in the study group



The representative karyograms depicting major type of chromosomal of typical abnormalities has been given (Figure 3 and Figure 4).

Figure-3: Karyotype Shows 45, XX, t .0(13;15) (q10;q10)

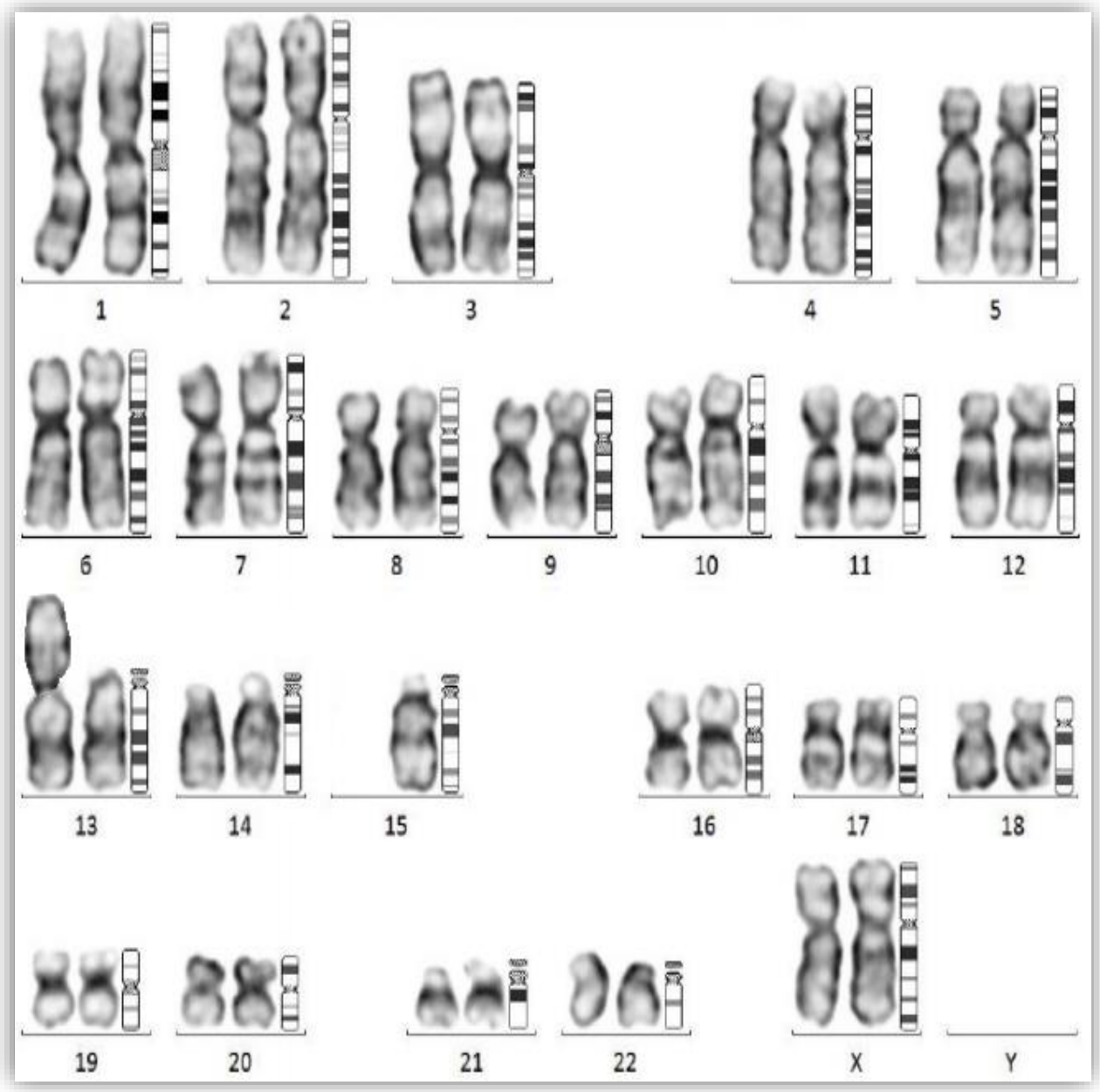
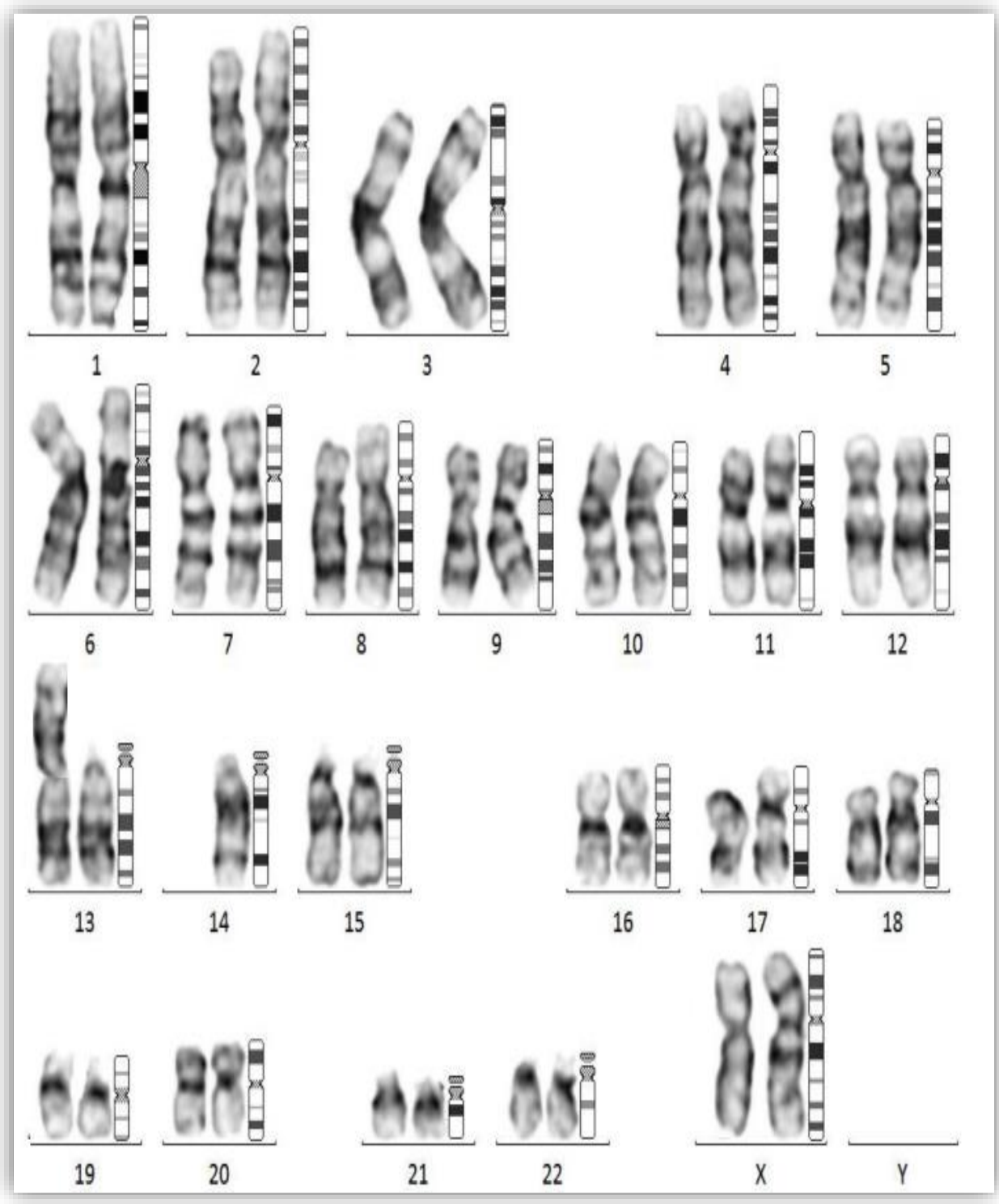


Figure-4: Karyotype Shows 45, XX, t(13;14) (q10;q10)



Study of chromosomal variants has an important role in the evaluation of Primary and secondary infertility. Polymorphism or heteromorphism are variations observed in heterochromatin regions. Heterochromatin has been defined as formed by randomly

organized and highly repeated sequences of DNA that do not encode proteins. The chromosomal variations in heterochromatin regions are considered as normal karyotypes [5]. Polymorphic variants on non-acrocentric chromosomes usually occur in the paracentric heterochromatin on the long arms of chromosomes and include varying sizes of heterochromatin blocks, satellites, repeat sequence regions and inversions. These include heterochromatin regions of chromosomes 1, 9, 16 and Y and also prominent acrocentric short arms, satellites and stalks [6].

Some studies have reported that chromosomal variations in heterochromatic regions might have deleterious effects [,7,8,9,10]. Studies by Minocherhomji et al. 2009 [11] showed that heteromorphism shown by paracentric long-arm regions of chromosomes 1, 9, 16, and inv (9) were associated with secondary infertility. In recent years, increasing evidence has been accumulated establishing association of chromosomal polymorphism in infertility [11, 12].

A large proportion of chromosomal polymorphisms were observed in the present study. In the present study, the inversion of chromosome # 9 was the most frequent anomaly, 4.2% present in the subjects (67.7 % from the total variants) (Table-3). Inv 9 (p12q13) has been reported as the most common inversion variant by many other researchers. A study was performed on 334 carriers of heterochromatin variants of chromosome 9, including 192 patients from Western Europe and 142 patients from Easter-European origin. Out of these, 21 patients had inv (9) and 10 patients found with other heterochromatin variants [13].

Table-3: The distribution of chromosomal Variant found in the patients with repeated spontaneous abortion.

S. No	Age	Gender	Chromosomal aberrations
1.	28	M	Inv(9)(p11q13)
2.	23	F	16qh+
3.	41	F	Inv(9)(p11q13)
4.	29	M	Inv(9)(p11q13)
5.	23	F	21ps+
6.	25	M	Inv(9)(p11q13)
7.	32	F	1qh+
8.	31	F	Inv(9)(p11q13)
9.	28	M	Inv(9)(p11q13)
10.	22	F	Inv(9)(p11q13)
11.	24	F	21ps+
12.	31	M	Inv(9)(p11q13)
13.	19	M	22ps+
14.	29	M	Inv(9)(p11q13)
15.	35	F	1qh+
16.	31	F	16qh+

17.	27	M	Inv(9)(p11q13)
18.	24	F	Inv(9)(p11q13),x2
19.	28	M	Inv(9)(p11q13)
20.	24	M	Inv(9)(p11q13)
21.	21	F	16qh+
22.	28	F	1qh+
23.	27	M	Inv(9)(p11q13)
24.	23	M	Inv(9)(p11q13)
25.	21	F	Inv(9)(p11q13)
26.	28	F	16qh+
27.	25	M	Inv(9)(p11q13)
28.	24	M	Inv(9)(p11q13)
29.	36	M	Inv(9)(p11q13)
30.	42	F	Inv(9)(p11q13)
31.	38	M	Inv(9)(p11q13)

In both male and female groups, te inversion of chromosome 9 was the most frequent chromosomal anomaly associated with infertility. Therefore, in another retrospective study on subjects with reported primary or secondary infertility to investigate the role of inv (9) in infertility (500 couples or 1000 individual). [14]

Figure-5: The distribution of chromosomal variant found in percentage

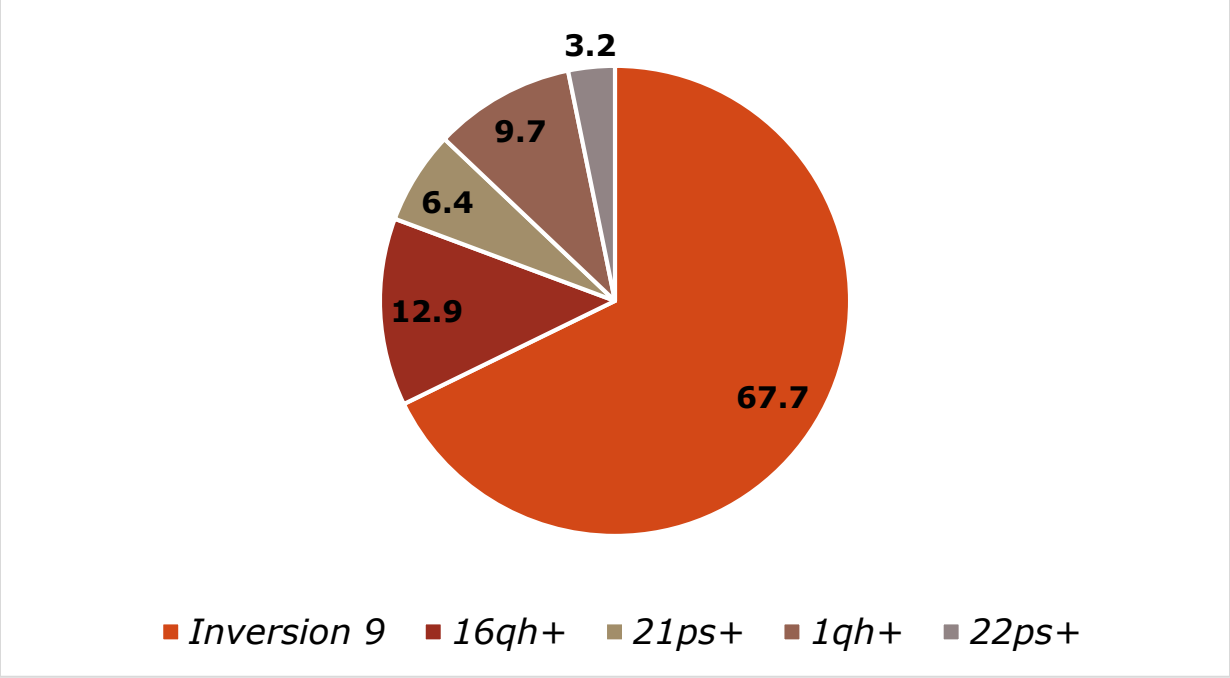


Table-4: Distribution of chromosomal variant in male & female patients.

Chromosomal aberrations	Female	Male	Total cases	p-value*
Inv (9) (p11q13)	6	15	21	0.007
1qh+	3	0	3	
16qh+	4	0	4	
21ps+	2	0	2	
22ps+	0	1	1	
p-value was calculated using chi square test, significant p-value was <0.05.				

DISCUSSION

It has been reported that the most prominent cause of first trimester abortion is fetal chromosomal abnormality. Different investigators have observed a high incidence of cytogenetic abnormalities in parents, who have history of more than two recurrent spontaneous abortions. The genetic etiology for repeated spontaneous pregnancy loss includes an unbalanced chromosome rearrangement in fetus, which may be due to presence of balanced translocation or any other chromosomal rearrangement in any of the parent. In 4-8% of couples with repeated spontaneous abortion, at least one of the parents has chromosomal abnormality that probably contains balanced chromosomal abnormalities [15].

In our study that comprised of 500 couples with infertility, large proportion 7.5% of the infertile couples carry chromosomal abnormalities in at least one of the partner. Out of these 7.5%, 4.4% couples showed chromosomal abnormalities whereas the rest 3.1% i.e. 31 individuals had chromosomal changes that have been considered as variant chromosomes. There were 15 male partners and 16 female partners in this group. The most frequent variant observed was inv (9) (67.7%). The prevalence of chromosomal abnormalities amongst couples with repeated spontaneous abortions varied in different studies, from none to as high as 21.4% [2, 16, 17].

Dubey et al. in 2005 [18], studied the chromosomal abnormalities in total number of patients, who had at least two spontaneous abortions. They reported chromosomal abnormalities in 31 (4% for couples and 2% of individual) patients comprising of 24 females and 7 male partners. Among 31 subjects, 22 (71%) showed structural aberrations, and 9 (29%) carried numerical abnormalities. In present study, also inclusion criteria was same at least two abortion 44 patients (4.4% for couples and 8.8 for

individual) had chromosomal abnormalities. In the present study, 27(63.63%) cases with abnormalities were found in female group and 17 cases were found in male partner. Out of 44 cases, only 10 (22.5%) cases were found numerical chromosomal abnormalities and other 34 (77.5%) cases revealed structural chromosomal abnormalities.

Mierla D et al. (2015) studied the correlation of chromosomal aberration associated with infertility in European population. This retrospective study was performed on 2195 (couples) using conventional cytogenetic techniques. In the study group, numerical chromosomal abnormalities were detected in 1.76 % of infertile couples (1.14% of infertile men and 0.62% of infertile women) [19]. In the present study, numerical abnormalities were detected in 2 % of infertile couple (0.8% in infertile male and 1.2% of infertile female. It is interesting that a greater proportion of men carried the numerical chromosomal abnormality as compared to the women. Give a comparison of the abnormalities found in European population graphically.

Table-5: Structural chromosomal rearrangements found in couples with recurrent abortions compared to similar studies.

Country/ Author	**No. of couples studied**	**Robertsonian**	**Reciprocal**	**Inversion**	**Others**	**Number**	**%**
Iraq (Musal) 2010	50	-	1	-	2	3	6
Netherlands (Leiden) 2005	67	3	5	1	-	9	13.4
Iran (Tehran) 2008	142	4	4	4	7	19	13.9
Netherlands (Rotterdam) 2005	148	3	6	3	2	14	9.6
Saudi Arabia (Riyadh) 2000	193	1	10	2	-	13	7.7
*Japan 2004	639	9	19	1	-	29	4.2
France (Strasbourg) 2004	217	4	-	2	-	6	2.8
China(Ruijin)2001	61	5	1	1	-	7	11.5
***Present study**	**500**	**4**	**15**	**8**	**17**	**44**	**8.8**
*p-value = <0.0001 (Japan 2004 and present study); p-value was calculated using chi square test, significant p-value was <0.05.							

We have compared this study with Japanese population using chi square test and significant p value was found. We have observed 8 cases of deferent type of Inversion, which was very rarely found in Japanese population. In the other studies, Inversion was not found frequently as compare to Indian population. Total percentage of abnormal cases was also significantly higher than Japanese population.

Previous studies have reported that in couples with secondary infertility, the number of female patients with balanced chromosomal aberrations significantly exceeds the male patients [20]. The frequency of Numerical chromosomal abnormalities, balanced chromosomal abnormalities and inversion were found to be higher in women with secondary infertility (7.3%) than in men (2.1%) [20]. A proposed mechanism contributing to the higher incidence of female translocation carriers is that only one ovum matures each month, whereas male carriers release millions of sperm in every ejaculation, resulting in possible pre-zygotic selection against unbalanced gametes [21].

The present study shows that the ratio of prevalence of chromosomal abnormalities amongst male and female subjects was about 1:1.75. A similar male to female ratio has been found in previous studies involving different ethnic groups [22,23,24,25,26]. In the present study, 8 balanced reciprocal chromosomal translocations with unique and novel break points were found and recorded in female patients with secondary infertility as t(2;14)(q21;q11.2), t(1;3)(p36.1;q26), t(16;19)(q10;p13.3), t(4;5)(q28;p15), t(2;12)(q33;q22), t(4;8)(p15;p21), t(8;11)(q24;q23), t(3;17)(p21;p11.2) and t(5;7)(q31;q11.2). In the male patients with secondary infertility three novel balanced reciprocal translocation are observed t(4;7) (q25;q22), t(4;5)(q28;p15) and t(2;20)(q31;q13.3).

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