

## Cytogenetic Analysis of Male Infertility

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**Abstract:** *In order to assess the significance of chromosome abnormalities and polymorphic chromosomal variants in male infertility, the results of cytogenetic studies of 70 patients (16 azoospermic and 54 oligozoospermic men) were compared with those of 35 control fertile men. Total chromosome alterations were revealed in 31% of infertile men. Major chromosomal abnormalities had a 10-fold increase in infertile males compared to the control group. In azoospermics, the most prevalent were sex chromosomal abnormalities (47, XXY) (22.7%); mosaicism that is 47, XXY/48, XXYY was seen in one patient. One patient showed 46, XX karyotype and variations in Y chromosome was identified. Whereas in oligozoospermic men polymorphic variations were observed in chromosome 1, 9, Y and presence of satellite in chromosome 15 and 21 and robertsonian translocation (13/14) were also identified. The most frequently observed polymorphisms involved chromosome 9. In conclusion, chromosomal abnormalities found with a high frequency in infertile males are a major cause of male infertility and justify the requirement of cytogenetic analysis in every infertile man.*

**Keywords:** *azoospermia, chromosome aberrations, Infertility, karyotyping, oligozoospermia*

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### I. Introduction

“Infertility is the inability of a sexually active, non-contracepting couple to achieve pregnancy in one year” (WHO). About 25% of couples do not achieve pregnancy within 1 year, 15% of whom seek medical treatment for infertility and less than 5% remain unwillingly childless.

Infertility affects both men and women. Male causes for infertility are found in 50% of involuntarily childless couples. In case of a single factor, the fertile partner may compensate for the less fertile partner. In many couples, however, a male and a female factor coincide. Infertility usually becomes manifest if both partners are subfertile or have reduced fertility. Reduced male fertility can be the result of congenital and acquired urogenital abnormalities, infections of the genital tract, increased scrotal temperature (varicocele), endocrine disturbances, genetic abnormalities and immunological factors [1].

No causal factor is found in 60-75% of cases (idiopathic male infertility). These men present with no previous history associated with fertility problems and have normal findings on physical examination and endocrine laboratory testing.

Semen analysis reveals absence of spermatozoa in the semen (azoospermia), decreased number of spermatozoa (oligozoospermia), decreased motility (asthenozoospermia) and many abnormal forms on morphological examination (teratozoospermia). These abnormalities usually occur together and are described as the oligoastheno-teratozoospermia (OAT) syndrome. Unexplained forms of male infertility may be caused by several factors, such as chronic stress, endocrine disruption due to environmental pollution, reactive oxygen species and genetic abnormalities.

Andrological examination is indicated if semen analysis shows abnormalities. Because semen analysis still forms the basis of important decisions concerning appropriate treatment, standardization of the complete laboratory work up is highly desirable. Ejaculate analysis has been standardized by the WHO and propagated by continuing work and publications in the WHO Laboratory Manual for Human Semen and Sperm-Cervical Mucus Interaction (4th edition) [2]. The consensus is that modern spermatology has to follow these guidelines without exclusions.

Knowledge of genetic abnormalities in infertility is mandatory for every urologist working in andrology so that correct advice can be given to couples seeking fertility treatment because of male infertility. By means of invitro fertilization (IVF), ICSI (intracytoplasmic sperm injection) and TESE (testicular sperm extraction) men with very low sperm counts can be given a reasonable chance of paternity. However, this also increases the possibility of passing genetic abnormalities on to the next generation because the sperm of infertile men shows an increase in aneuploidy, other genetic abnormalities and DNA damage. Although there are prospects for screening of sperm [3], current routine clinical practice is based on the screening of peripheral blood samples.

Chromosome abnormalities can be numerical, such as trisomy, or structural, such as inversions or translocations. In a survey of pooled data from 11 publications including 9,766 infertile men, the incidence of chromosomal abnormalities was 5.8% [4]. Of these, sex chromosome abnormalities accounted for 4.2% and autosomal abnormalities for 1.5%. For comparison, the incidence of abnormalities in pooled data from three series totaling 94,465 newborn male infants was 0.38%, of which 131 (0.14%) were sex chromosome abnormalities and 232 (0.25%) autosomal abnormalities [5]. Standard karyotype analysis should be offered to all men with damaged spermatogenesis who are seeking fertility treatment by IVF/ICSI. In this study we have correlated the chromosome abnormality and male infertility.

### II. Materials And Methods

70 infertile men with non-obstructive azoospermia and severe oligozoospermia in the age group from 20 – 50 yrs attending the genetic clinic were enrolled in this study. Of these patients, 54 had severe oligozoospermia and 16 had non-obstructive azoospermia. A detailed medical history including pedigree analysis was procured from each patient. Routine

physical examination was carried out. 35 age matched males with normal reproductive function were included as a control group. 5 ml of peripheral blood was taken from each patient.

#### **Chromosomal Analysis:**

Chromosomal analysis of peripheral blood lymphocytes was performed based on the International System for Human Cytogenetic Nomenclature [6]. The peripheral blood lymphocytes were cultured with RPMI-1640 media along with all the supplements. After a period of 72 hrs incubation colchicines was added followed by hypotonic treatment and fixation. Metaphase chromosomes that were prepared by the standard protocols were banded using GT (Giemsa Trypsin) banding technique. At least 25 well spread metaphases were scored from each patient.

### **III. Results**

Different types of chromosomal aberrations and frequency were studied are shown in table 1, 2 and 3. Out of 70 males, 22 patients showed chromosomal aberrations which included variations in the heterochromatin region of 1, 9, Y chromosome, satellite region of chromosome no.21, 47, XXY, 48, XXYY and autosomal translocations. (Figure 1, 2, 3, 4, 5, 6, 7 and 8).

The type of chromosomal aberrations detected in the azoospermic and oligozoospermic men are given in Table 1. The abnormalities observed in the two groups included both sex chromosomal and autosomal numerical and structural abnormalities.

Table 2 indicated the frequency of chromosome aberrations. The most observed were 47, XXY (22.7%) and polymorphic variations in chromosome 9 and Y were also significant.

In azoospermic male the most prevalent were sex chromosomal abnormalities (47, XXY) (Fig.7) (22.7%); mosaicism that is 47, XXY/48, XXYY was seen in one patient (Fig.8). One patient showed 46, XX karyotype and heterochromatin variation in Y chromosome (46, XYqh+) (Fig.4), deletion in Y chromosome (46, XYp-) (Fig.5) was also identified. Whereas in oligozoospermic men, polymorphic variations were observed in chromosome 1, 9, Y (Fig. 1, 2 and 3) and presence of satellite in chromosome 15 and 21 (Fig. 6) and robertsonian translocation (13/14) (Table 3) was identified.

### **IV. Discussion**

Chromosomal abnormalities play a prime role in male infertility with abnormal semen parameters. It has been known for some 20 years that the prevalence of chromosomal abnormalities is higher in infertile men and is inversely related to the sperm count [7]. The results showed an inverse correlation between chromosomal anomalies and sperm count. Spermatozoa bearing abnormal chromosomes may cause abnormal embryonic development, which can in turn, cause early pregnancy loss [8].

The occurrence of chromosomal abnormalities among infertile men depends on a number of factors and the most important one is the sperm count. The present study shows 12.8 percent chromosomal abnormalities in males with azoospermia and 18.5 percent chromosomal abnormalities in men with oligozoospermia.

Sex chromosome abnormalities are the most frequent chromosome-related cause of infertility. In this study, we found 47, XXY karyotype in 5 men. This abnormality is associated with severe spermatogenic failure causing a marked reduction in testicular size and azoospermia resulting in infertility [8]. A mosaic 47, XXY/ 48, XXYY karyotype was found in one azoospermic male. Infertile men with gonosomal mosaicism have a range of spermatogenic profile ranging from severe impairment to apparent normality [9]. Gonosomal mosaicism may be a probable cause for the failure of assisted reproduction [10]. Deletion in the long arm of Y chromosome was seen in two patients. Studies have indicated that deletions on the long arm of the Y chromosome might lead to azoospermia [11] and sometimes to severe oligozoospermia [12].

A single case of azoospermic male with Robertsonian translocation t (13; 14) is reported in this study. An increased number of carriers of Robertsonian translocations have been reported among severely oligozoospermic and azoospermic men [13].

Several reports on male infertility mention the presence of chromosomal variants or polymorphisms. Y chromosome polymorphisms have been preferentially seen in azoospermia and severe oligozoospermia (13). 'Long Y chromosome' (Yqh+) and 'short Y chromosome' (Yqh-) is known to exist [14]. The variation in the length of Y chromosome is usually due to variation in the distal part of the long arm that is known to contain heterochromatin [14].

The present study showed 46, XX in one azoospermic men. The 46, XX maleness is characterized by testicular development despite the lack of normal Y chromosome. The frequency of XX males in the general population is very low (1 in 10,000) whereas they are found more frequently in azoospermic men [15].

Autosomal polymorphisms were observed in the present study which includes increase in the centeromeric heterochromatin in chromosomes 1 and 9. Some workers have considered variations in heterochromatin in chromosomes 1 and 9 to be associated with fetal wastage, recurrent abortions, and abnormal phenotypes. But studies indicated no significant difference in the heterochromatic regions between aborting and non-aborting couples [16], and also revealed that heterochromatic regions of chromosomes 1, 9, 16 and Y in children showed signs of embryonic development disorder [17]. Heterochromatin has a specific role and behaviour in the synapsis of human homologous chromosomes. Association of heterochromatic variation with bad obstetric history is also indicated in the studies [18].

Presence of satellites in acrocentric chromosomes 21 and 15 was observed in one patient. Positive correlation existed between the frequency of acrocentric variants and sterility, due to an increase of risk satellite association and of nondisjunction leading to aneuploidy in gametes [19]. The report was limited by only using cytogenetic detection methods, without confirmation by genetic testing. Analysis at the molecular level like polymerase chain reaction, restriction fragment length polymorphism, if required DNA sequencing may be needed to unveil any relation between heteromorphisms and reproductive failure taking into consideration, the heterochromatin have been regarded to have more crucial cellular roles than previously thought [20].

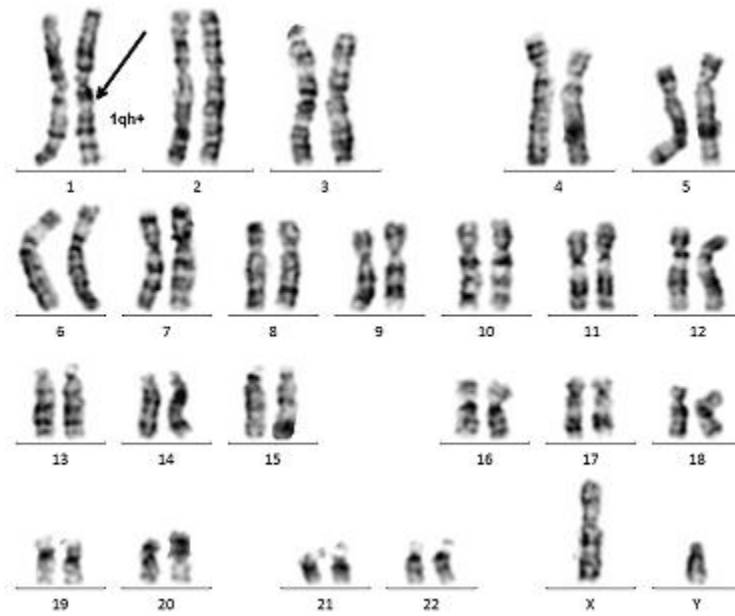


Fig1. 46, XY, 1qh+ (Male Karyotype with 46 chromosome involving increase in the secondary constriction region of long arm of chromosome 1)

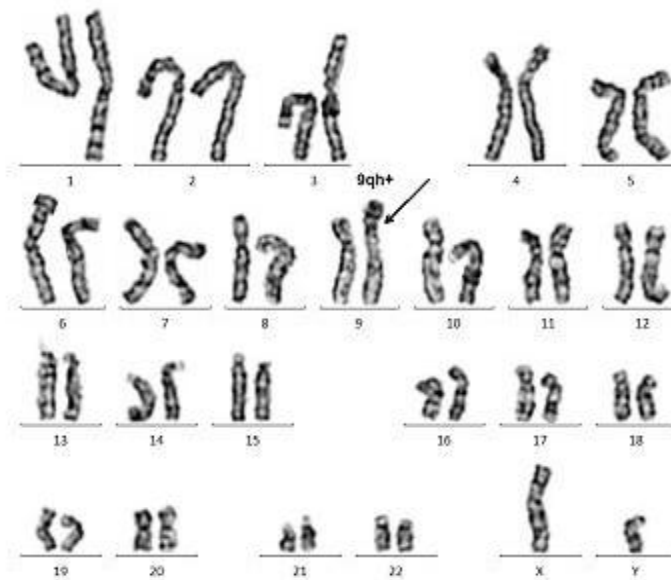


Fig2. 46, XY, 9qh+ (Male Karyotype with 46 chromosome involving increase in the secondary constriction region of long arm of chromosome 9)

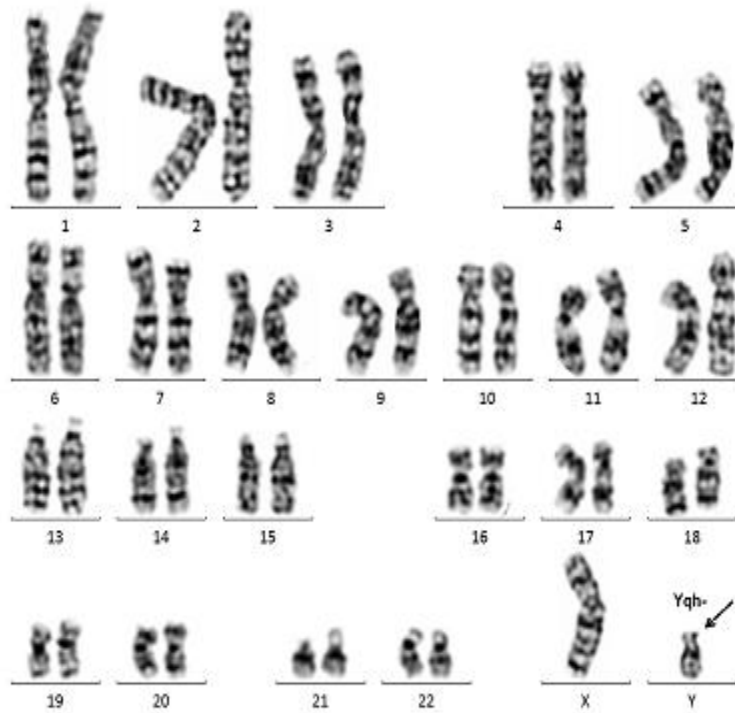


Fig3. 46, XYqh-(Male Karyotype with 46 chromosome involving decrease in the secondary constriction region of long arm of Y chromosome)

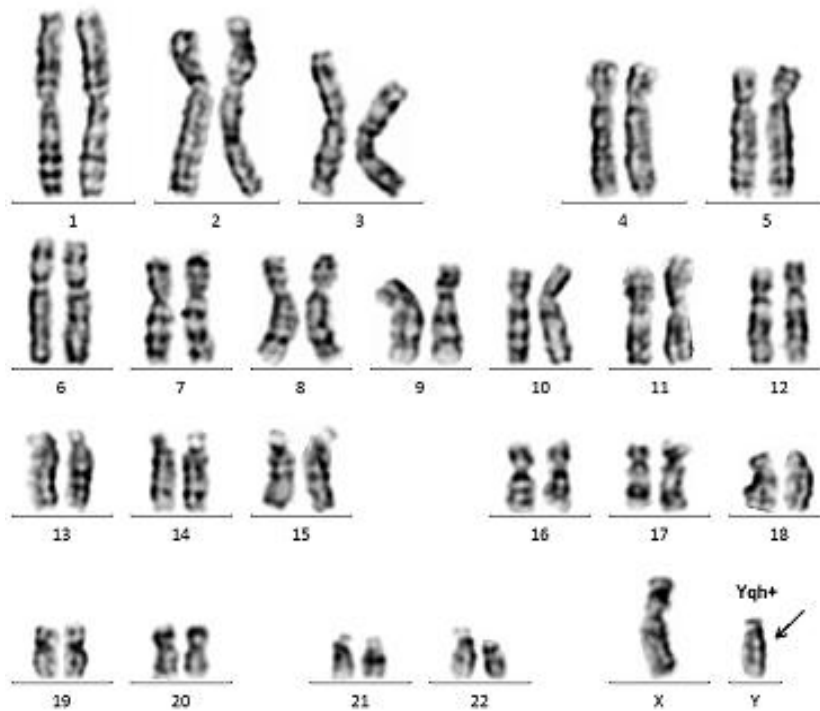


Fig4. 46, XYqh+ (Male Karyotype with 46 chromosome involving increase in the secondary constriction region of long arm of Y chromosome)

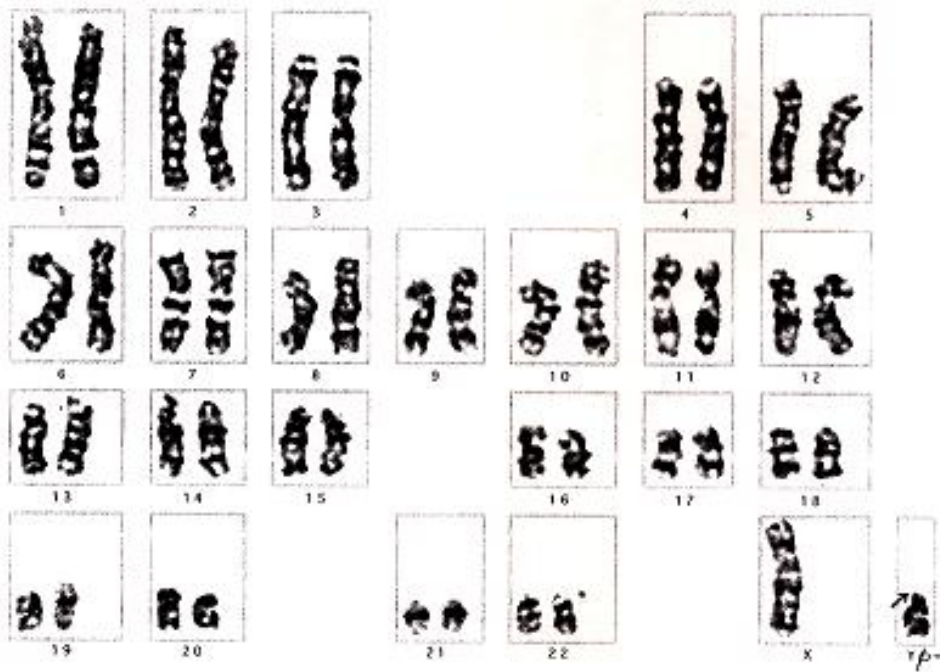


Fig5. 46, XYp- (Male Karyotype with 46 chromosome involving deletion in the short arm of Y chromosome)

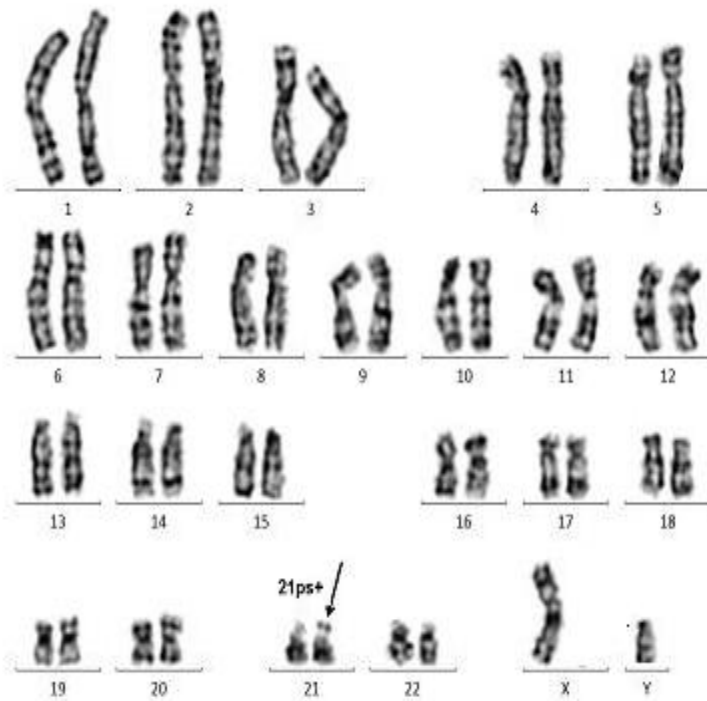


Fig6. 46, XY, 21ps+ (Male Karyotype with 46 chromosome involving presence of satellite in chromosome 21)

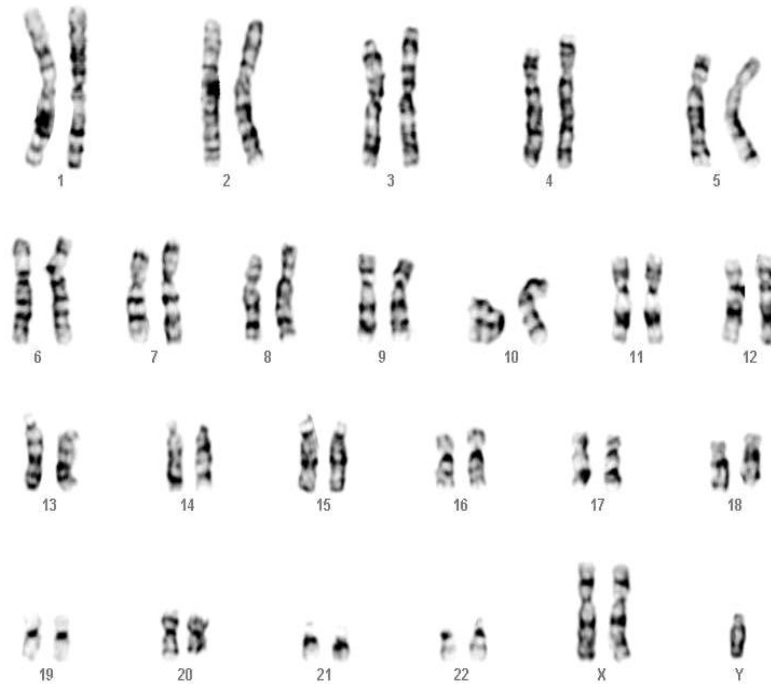


Fig7. 47, XXY (Klinefelter's Syndrome)

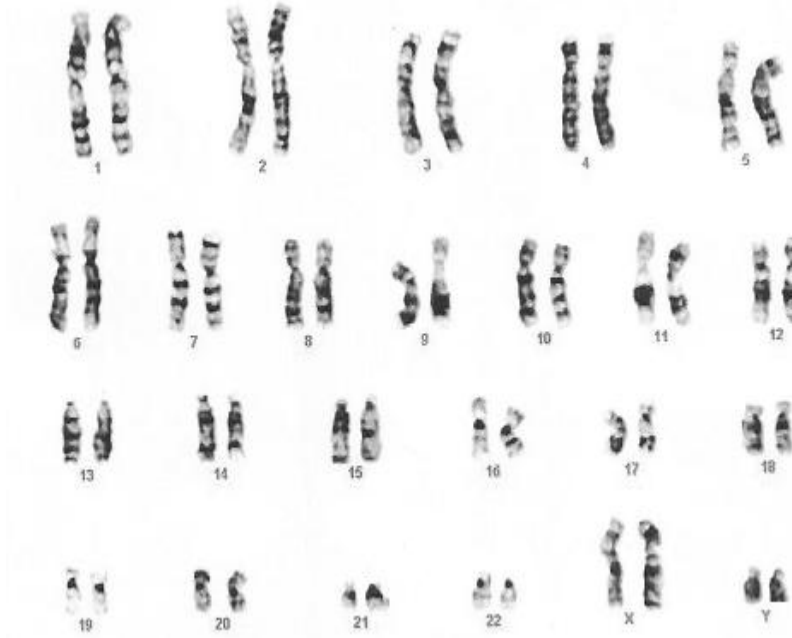


Fig8. 48, XXYY

Table 1: Different Type of Chromosomal Aberrations

S.No.	AGE	GENDER	PROVISIONAL DIAGNOSIS	KARYOTYPE
1.	31 yrs	M	Azoospermia	47,XXY
2.	30 yrs	M	Azoospermia	47,XXY
3.	30 yrs	M	Azoospermia	46,XYp-
4.	28 yrs	M	Azoospermia	47,XXY
5.	39 yrs	M	Azoospermia	46,XYqh-
6.	31 yrs	M	Oligozoospermia	46,XY,21ps+
7.	34 yrs	M	Oligozoospermia	46,XY,9qh+
8.	46 yrs	M	Oligozoospermia	45,XY,t(13;14)
9.	37 yrs	M	Oligozoospermia	46,XY,9qh-
10.	37 yrs	M	Oligozoospermia	46,XY,15ps+
11.	35 yrs	M	Oligozoospermia	46,XYp-
12.	30 yrs	M	Azoospermia	47,XXY
13.	32 yrs	M	Oligozoospermia	46,XY,1qh+
14.	31 yrs	M	Oligozoospermia	46,XY,9qh+

15.	20 yrs	M	Oligozoospermia	46,XY,9qh+
16	45 yrs	M	Azoospermia	47,XXY/48,XXYY
17	27 yrs	M	Oligozoospermia	46,XYqh+
18	28 yrs	M	Azoospermia	47,XXY
19	31 yrs	M	Oligozoospermia	46,XYqh+
20	36 yrs	M	Oligozoospermia	46,XY,9qh-
21	31 yrs	M	Oligozoospermia	46,XY,9qh+
22	21 yrs	M	Azoospermia	46,XX female

**Table 2: Frequency of Chromosome Aberrations**

KARYOTYPE	FREQUENCY	PERCENTAGE
47,XXY	5	22.7
46,XY,9qh+	4	18
46,XYqh+	2	9
46,XYp-	2	9
46,XYqh-	2	9
46,XY,21ps+	1	4.5
46,XY,1qh+	1	4.5
45,XY,t(13;14)	1	4.5
46,XY,9qh-	1	4.5
46,XY,15ps+	1	4.5
46,XX female	1	4.5
47,XXY/48,XXYY	1	4.5

**Table 3: Chromosomal Polymorphisms in Infertile Men**

CLASSIFICATION	KARYOTYPE	No. OF MEN WITH DEFECTS
Azoospermia	47,XXY	5
	47,XXY/48,XXYY	1
	46,XX female	1
	46,XYqh-	1
	46,XYp-	1
Oligozoospermia	46,XYqh+	2
	46,XYp-	1
	46,XY,21ps+	1
	46,XY,9qh+	4
	46,XY,9qh-	2
	46,XY,1qh+	1
	46,XY,15ps+	1
	45,XY,t(13;14)	1

### V. Conclusion

In conclusion, the occurrence of chromosomal anomalies among infertile men strongly suggests genetic testing like polymerase chain reaction and counselling prior to the ICSI treatment. Moreover, prenatal diagnosis in the case of abnormalities is of utmost importance. Such investigation is a pre-requisite to minimize the risk of propagation of chromosomal abnormalities into the next generation. Additionally, a thorough follow up of babies conceived through ICSI, in particular the male progeny is essential.

### References

- World Health Organization. WHO Manual for the Standardised Investigation and Diagnosis of the Infertile Couple. Cambridge, Cambridge University Press, 2000.
- World Health Organization. WHO Laboratory Manual for the Examination of Human Semen and Sperm-cervical Mucus Interaction.4th edn. Cambridge: Cambridge University Press, 1999.
- Johnson MD. Genetic risks of intracytoplasmic sperm injection in the treatment of male infertility: recommendations for genetic counseling and screening .Fertil Steril 1998; 70:397- 411
- Van Assche E., Bonduelle M, Tournaye H, Joris H, Verheyen G, Devroey P, et al. Cytogenetics of infertile men ,Hum Reprod 1996;11(Suppl4):1-24
- Chandley AC, Edmond P, Christie S, Gowans I, Fletcher J,Frackiewicz A, et al. Cytogenetics and infertility in man. I. Karyotype and seminal analysis: results of a five-year survey of men attending a subfertility clinic. Ann Hum Genet 1975; 39: 231-54.
- Mitelman F, editor. ISCN 1995: An International System for Human Cytogenetic Nomenclature., Switzerland. Basel; S Karger; 1995
- Foresta Carlo (2001). 'Guidelines for the genetic diagnosis of the infertile couple' Data collected from various sources.
- Pandiyam N, Jequier AM., Mitotic chromosomal anomalies among 1210 infertile men. Hum Reprod 1996; 11: 2604-8.
- Egozcue S, Blanco J, Vendrell JM, Garcia F, Veiga A, AranB, et al. Human male infertility: chromosome anomalies, meiotic disorders, abnormal spermatozoa and recurrent abortion. Hum Reprod Update 2000; 6: 93-105.
- Toncheva D, Ilieva P, Mavrudieva M. Detection of low-level sex-chromosomal mosaicism. Genet Couns 1994; 5:363-7.
- Reijo R, Lee TY, Salo P, Alagappan R, Brown LG,Rosenberg M, et al. Diverse spermatogenic defects in humans caused by Y chromosome deletions encompassing a novel RNA-binding protein gene. Nat Genet 1995; 10:383-93.
- Reijo R, Alagappan RK, Patrizio P, Page DC. Severe oligozoospermia resulting from deletions of azoospermia factor gene on Y chromosome. Lancet 1996; 347 :1290-93
- De Braekeleer M, Dao TN. Cytogenetic studies in male infertility: a review. Hum Reprod 1991; 6: 245-50.
- De Braekeleer M, Dao TN. Cytogenetic studies in couples experiencing repeated pregnancy losses. Hum Reprod 1990; 5: 519-28.
- Rao A. Kamini.'The Infertility Manual' 2<sup>nd</sup> edition: 42-53,126-131 and 528-535
- Hemming L, Burns C., Heterochromatic polymorphism in spontaneous abortions. J Med Genet, 1979; 16(5): 358-362.
- Podugol'nikova OA, Bliumina MG, Heterochromatic regions of chromosomes 1, 9, 16 and Y in children with signs of embryonic development disorder. G.enetica, 1982; 15(10): 1870-1879.
- Minocherhomji S, Anthalye AS, Madon PF, Kulkarni D, Uttamchandani SA, Parikh FR. A case-control study identifying chromosomal polymorphic variations as forms of epigenetic alterations associated with the infertility phenotype. Fertil Steril, 2008; 92: 88-95
- Moreau N., Teyssier M., Variant D and G-Chromosomes in Male-Related Infertility. Archives of Andrology: Journal of Reproductive Systems, 1982, 9 (4), issued on line, 2009:307-310.
- Lissitsina J, Mikelsaar R, Punab M. Cytogenetic analyses in infertile men. Arch Androl.2006; 52(2):91-95.