



## Cytogenetic Observations in Infertile Men with Varying Clinical Findings: A Case-Only Study from Kashmir, North India

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### Abstract

**Objective:** Male infertility has been attributed to many conditions that include varicocele, cryptorchidism, spermatic duct obstruction, urogenital tract infections, antisperm antibodies, retrograde ejaculation, endocrine disturbances, systemic diseases, testicular malignancy and environmental factors. Besides, it has been associated with aneuploidies and structural chromosomal abnormalities, Y chromosome microdeletions and specific gene mutations. The present study aimed to determine the frequency and type of chromosomal abnormalities in infertile males having normal female partners in Kashmiri population.

**Materials and Methods:** A total of 40 men with a complaint of primary infertility were screened for chromosomal abnormalities through conventional analysis of GTG-banded metaphases from cultured lymphocytes.

**Results:** Cytogenetic analysis revealed abnormal karyotypes in 60% studied infertile men. Klinefelter's syndrome karyotype was found in 40% cases and was the most common numerical/sex chromosomal anomaly. Besides, 46,XY/47,XY,+19 mosaic karyotype was reported in one of the cases. Structural chromosomal anomalies were observed in 7.5% of the cases including two novel karyotypes 46,XY/46,XY,i(7q) and 46,XY/46,XY,t(5;20)(q14;q13.2) that have not been reported anywhere in male infertility.

**Conclusion:** The occurrence of chromosomal abnormalities in 60% of the studied infertile men strongly reiterates the inclusion of routine cytogenetic testing and counseling in the diagnosis and management of male infertility.

**Keywords:** Cytogenetics; Male infertility; Azoospermia; Kashmir population

### Introduction

Male infertility is defined as the inability of a sexually active, non contraceptive couple to achieve pregnancy within one year [1]. It is estimated that every year globally, 60–80 million couples suffer from infertility, of which probably 15–20 million are in India alone [2]. It is a worldwide problem affecting people of all communities, though the cause and magnitude may vary with geographical location [3]. At present, about 15% of couples are infertile because of several reasons, out of which the male factor is responsible for approximately 50% of the cases [4].

Male infertility may be caused by genetic abnormalities, varicocele, cryptorchidism, spermatic duct obstruction, urogenital tract infections, antisperm antibodies, retrograde ejaculation, endocrine disturbances, systemic diseases, testicular malignancy and environmental factors. Somatic and/or germ cell chromosomal abnormalities, deletions of the azoospermia factor regions in the proximal long arm of Y chromosome, DNA damage in sperm atozoa and single gene mutations constitute the genetic component. However, it is idiopathic in about 40% of the cases [5].

Chromosomal anomalies have been reported to contribute as one of the crucial genetic factors in male infertility. The prevalence of chromosomal abnormalities in infertile males has been estimated to fall within the range of 2.4% to 16.4% [6] compared to the frequency in general male population (0.3–0.4%) [7]. In azoospermic men, the incidence of chromosomal abnormalities is particularly high, varying from 13.1% to 23.6%; in males with oligospermia, the incidence is 2.1%

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to 6.6% and in men with severe oligospermia it is 10.6% [8,9]. The incidence of autosomal chromosome abnormalities in infertile men has been reported to fall in the range of 1.1% to 7.2% [10] and sex chromosome abnormalities in a range of 0.4% to 12.3% [8].

Kashmir population is an ethnic population as far as its culture and geography is considered. Worldwide, a number of studies have been carried out on male infertility due to chromosomal defects. However, there has been no study on cytogenetic profiling of male infertility from this part of the world. Therefore, the aim of this study was to determine the occurrence and the frequency of various chromosomal alterations of infertile men in Kashmir having primary infertility.

## Materials and Methods

The present study was carried out at Advanced Centre for Human Genetics, Sher-I-Kashmir Institute of Medical Sciences (SKIMS), Srinagar (J&K). Cytogenetic analysis of 40 infertile men, who were referred from Department of Endocrinology, SKIMS and Government Medical College Srinagar, was carried out after obtaining an informed consent from the subjects. The study was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments in humans. The history of the patients and all the related information was noted in a predesigned standard format.

The 3ml heparinised peripheral blood samples of each patient were cultured for 72h in RPMI-1640 medium supplemented with fetal bovine serum and phytohemagglutinin. Cytogenetic analysis was performed by using the GTG banding technique. Karyotyping was performed with the help of Cytovision software Version 3.9 (Applied Imaging, Michigan, USA) on well spread G-banded metaphase plates at 450 band resolution. At least 25 metaphases were examined for each subject to rule out any chromosomal anomaly and mosaicism. The karyotypes were designated according to ISCN (2009) nomenclature.

## Results

The present study reveals the frequency and types of chromosomal abnormalities encountered in 40 men with primary infertility and whose wives were healthy. All the female partners who were also subjected to chromosomal analysis revealed a normal karyotype. Semen analysis revealed 33 men to be azoospermic and one to be oligospermic. The FSH and LH levels were raised in infertile men with azoospermia and oligospermia and the testosterone levels were mostly reduced. However, normozoospermic men had normal testosterone, LH and FSH levels. Besides, hypogonadism was reported in 12(30%) of the investigated subjects (Table 1).

Cytogenetic analysis revealed abnormal karyotypes in 24(60%) infertile men. Numerical as well as structural chromosomal anomalies were found in these cases. Numerical abnormalities were observed in 21(52.5%) of the cases. 47,XXY (Klinefelter's syndrome) karyotype was found in 16(40%) cases and was the most common numerical anomaly (Figure 1). However, 4(10%) of the infertile men were found to have 46,XY/47,XXY mosaic Klinefelter's syndrome karyotype. 46,XY/47,XY,+19 mosaic karyotype was reported in one (2.5%) of the males (Figure 2). Structural chromosomal anomalies were observed in 3(7.5%) of the cases. These include translocation in one (Figure 3) manifested as 46,XY/46,XY,t(5;20)(q14;q13.2), inversion in one (Figure 4) manifested as 46,XY,inv(9) and isochromosome in one (Figure 5) manifested as 46,XY/46,XY,i(7q).

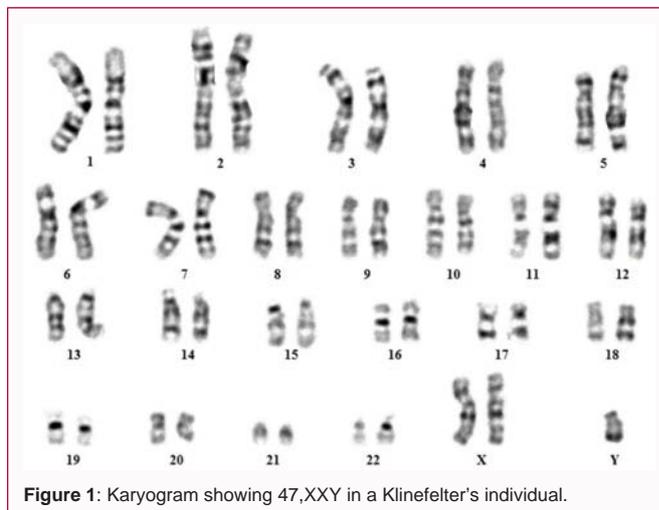


Figure 1: Karyogram showing 47,XXY in a Klinefelter's individual.

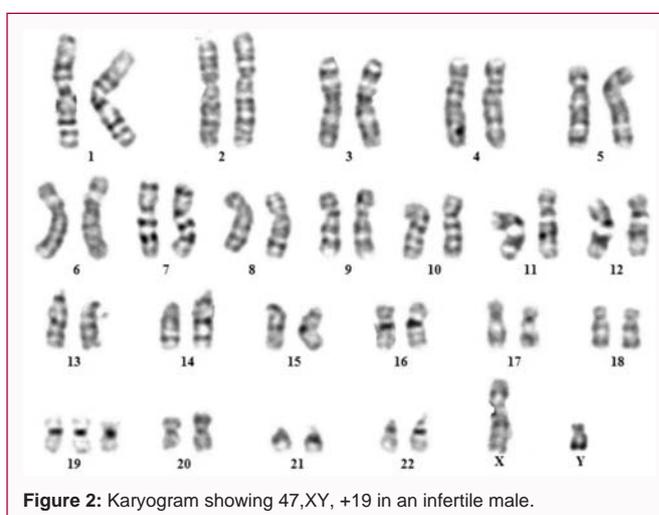


Figure 2: Karyogram showing 47,XY,+19 in an infertile male.

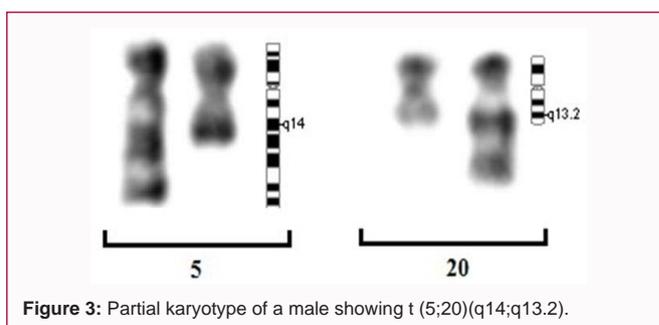


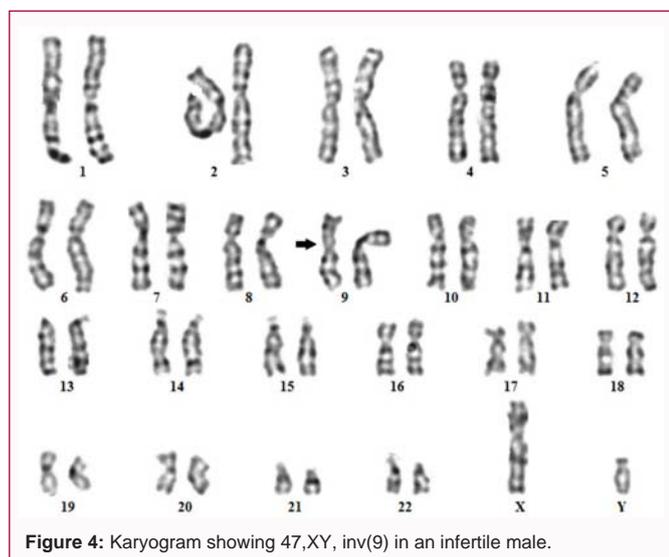
Figure 3: Partial karyotype of a male showing t(5;20)(q14;q13.2).

## Discussion

Cytogenetic abnormalities have been reported to be one of the major contributing factors towards male infertility and are the most common genetic abnormalities in infertile men [11]. In the present study 60% of the subjects with male infertility were found to have an abnormal chromosomal profile, which is very high as compared to other studies that have documented chromosomal anomalies in the range of 2.4% to 16.4% in infertile men [6]. In a recent study carried out in Southern India, 4.6% of 180 infertile men revealed an abnormal chromosomal pattern [5]. In another study in an Indian population, 10.2% of 78 infertile men were reported to have an abnormal karyotype [12]. The frequency of chromosomal abnormalities in infertile males depends on various factors and the most important of these is the

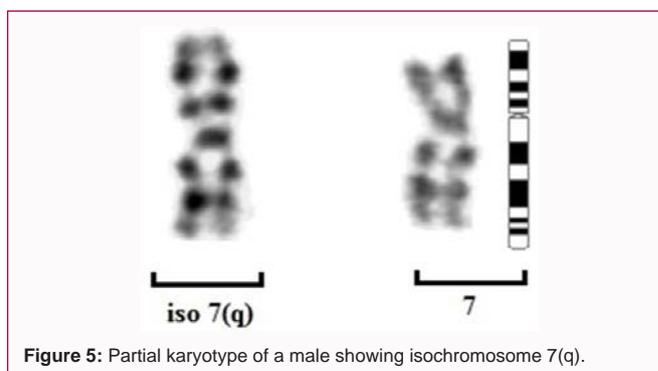
**Table 1:** Karyotype particulars, hormonal status and clinical findings of subjects with infertility. (No=40).

Cytogenetic grade	Karyotype	No. of cases	Frequency (%)	Hormonal Status (no)	Clinical Findings (no.)
Normal Karyotype	46,XY	16	40	Increased FSH (08), Increased LH(05), Decreased testos(06)	Azoospermia(13), hypogonadism(03)
<b>Numerical Abnormality</b>					
Klinefelter's Syndrome					
Non Mosaic	47,XXY	16	40	Increased FSH(16), Increased LH(13), Decreased Testos(10)	Azoospermia(16), hypogonadism(08),
Mosaic	46,XY/47,XXY	4	10	Increased FSH(04), Increased LH(02)	Azoospermia(02), Oligospermia(01), Hypogonadism(01)
Trisomy	46,XY/47,XY,+19	1	2.5	Normal FSH,LH, Testos	Primary Infertility
<b>Structural Abnormality</b>					
Isochromosome	46,XY/46,XY,i(7q)	1	2.5	Increased FSH	Azoospermia, Gynecomastia, Bilateral small testes
Inversion	46,XY,inv(9)	1	2.5	Increased FSH/LH	Azoospermia
Translocation	46,XY/46,XY,t(5;20)(q14;q13.2)	1	2.5	Increased FSH	Primary Infertility

**Figure 4:** Karyogram showing 47,XY, inv(9) in an infertile male.

selection of patients based on the sperm count. In the present study the incidence of chromosomal anomalies in males with azoospermia was 20/33(60.6%) which is very high as compared to 15.4% [12] and 4.6% [5]. The difference in the frequency of chromosomal alterations observed in the present study may be due to the differences in the ethnicity of the subjects, the selection criteria and the sample size.

Sex chromosome abnormalities are the most frequent chromosome-related cause of infertility. In our study, we found 47,XXY (Klinefelter's syndrome) as the most common numerical anomaly. Klinefelter syndrome is the major cytogenetic/sex chromosomal/ numerical anomaly detected in infertile men followed by translocations, deletions and inversions [13]. This abnormality is associated with severe spermatogenic failure causing a marked reduction in testicular size and azoospermia resulting in infertility [14]. Infertility in these subjects is also attributed to the direct harmful effect of an extra X chromosome causing lethal gene dosage effect in the cells in the testis resulting in azoospermia [7]. Besides, we had only one case with oligospermia who manifested a mosaic Klinefelter's syndrome karyotype. Mosaic cases have often been found to present with oligozoospermia [15].

**Figure 5:** Partial karyotype of a male showing isochromosome 7(q).

In the present study one male was found to have 46,XY/47,XY,+19 mosaic karyotype. Literature suggests that trisomy 19 is frequently encountered in chronic myeloid leukemia as a secondary abnormality and has also been reported as a sole abnormality in few cases of myeloid malignancies [16]. However, the trisomy 19 male of our study had no symptoms of any malignancy. Besides, there are no reports of trisomy 19 in male infertility.

Out of the 40 infertile men, one was found to have a mosaic karyotype (46,XY/46,XY,t(5;20)(q14;q13.2) with a reciprocal translocation. This is a unique autosomal translocation that has not been reported elsewhere in the literature. Autosomal translocations are the most commonly observed structural chromosomal anomaly in infertile men. Autosomal translocations impair spermatogenesis due to disrupted meiotic pairing and segregation [17-21]. Autosomal translocations are 4 to 10 times more common in infertile men than in the fertile population [22,23]. While most translocations do not influence other tissues, these can severely affect spermatogenesis. This is attributed to the disruption of genes responsible for spermatogenesis or to impaired synaptic complex pairing during meiosis. Literature suggests transcriptional silencing of unpaired regions during meiosis in reciprocal translocation carriers. Meiotic arrest is likely to occur if genes crucial for meiosis are present in the asynapsed regions. The breakpoint regions and the chromosomes involved in translocation are likely to be determining factors for the fidelity of synapsis, and therefore for the fertility status of the carrier [24]. These types of translocation carriers always have reduced number of gametes with

an unbalanced karyotype [13].

Pericentric inversion was observed on chromosome 9 in 2.5% of the studied subjects. Chromosome 9 pericentric inversions are the most common chromosomal inversions in humans that produce asymmetric bivalents in meiotic metaphase I spreads and structural chromosome aberrations in sperm studies [25,26]. In the general population, the frequency of this inversion is reported to be 1-1.65%. Even though it is known that chromosome 9 inversions do not have a phenotypic effect, it has been related to male infertility [27-29]. The inversions can perturb spermatogenesis and produce unbalanced gametes through the formation of an inversion loop [30]. Besides, it can delay meiosis and also lead to a breakdown of meiosis as the recombination is reduced within the pairing loop [31-33].

Isochromosome is the resultant of an abnormal split of the centromere followed by duplication of one of the arms. In our study, one of the subjects was found to have an isochromosome and manifested with a karyotype 46,XY/46,XY,i(7q). Although isochromosome 7q has been linked with myeloproliferative disorders [34], we for the first time report it in male infertility.

## Conclusion

The results of the present study conclude that chromosomal alterations do occur as an etiology in the infertile men of Kashmir with a very high frequency of chromosomal abnormalities as compared to the studies carried out in other Indian populations as well as by several other workers at isolated places. However, we report for the first time a unique case of reciprocal autosomal translocation associated with male infertility. However, precise molecular characterization of the breakpoint regions of the unique translocation is needed to identify new genes or genes involved in male infertility that can help in better understanding of underlying molecular mechanism. Besides, we report for the first time isochromosome 7q in an infertile male. Cytogenetic analysis, therefore, should be mandatory for all infertile men for their timely management and the post-test genetic counseling. The study being the first of its kind in this part of the world forms the basis for further studies on male infertility of this region.

## Author's Contributions

TMM involved in recording clinical and family history of the cases, performing cytogenetic analysis and writing the manuscript. MHZ involved in inception of the research work and performing cytogenetic analysis, NK, RA and FA, involved in carrying out the cell culture and processing of samples, ZAS involved in designing and directing all the experiments and evaluation of entire manuscript.

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