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Original Article

Genetics Section

Chromosomal Abnormalities in Infertile Men from Southern India

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ABSTRACT

Background and Objective: Male infertility has been associated with aneuploidies and structural chromosomal abnormalities, Yq microdeletions and specific gene mutations and/or polymorphisms. Besides genetic factors, any block in sperm delivery, endocrine disorders, testicular tumours, infectious diseases, medications, lifestyle factors and environmental toxins can also play a causative role. This study aimed to determine the constitutional karyotype in infertile males having normal female partners in a south Indian population.

Materials and Methods: A total of 180 men with a complaint of primary infertility ranging from 1 to 25 years were screened for chromosomal abnormalities through conventional analysis of GTG-banded metaphases from cultured lymphocytes.

Results: Four individuals were diagnosed to have Klinefelter syndrome. Two cases exhibited reciprocal translocations and one showed a maternally inherited insertion. Polymorphisms were seen in sixty-seven patients (37.2%).

Conclusion: The occurrence of chromosomal abnormalities in 4.6% and variants involving the heterochromatic regions of Y, chromosome 9 and the acrocentric chromosomes in 38.2% of the infertile men with an abnormal seminogram strongly reiterates the inclusion of routine cytogenetic testing and counselling in the diagnostic work-up prior to the use of assisted reproduction technologies.

Keywords: Azoospermia, Chromosomal polymorphisms, Insertion, Klinefelter syndrome, Male infertility, Oligozoospermia, Reciprocal translocation

INTRODUCTION

Infertility is a major social and health problem worldwide affecting 10% to 15% of sexually active couples but unable to conceive after one year without contraception [1]. While on one hand there is an increasing concern about human overpopulation, on the other hand there is also anxiety over increasing rates of infertility. It is estimated that about 60–80 million couples suffer from infertility every year across the globe and that nearly 15–20 million reside in India [2]. The male factor accounts for about one-half of the cases [3]. 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 spermatozoa and single gene mutations constitute the genetic component [4]. However, it is idiopathic in about 40% of the cases [5,6].

Chromosomal abnormalities have been recognized to occur in infertile men with azoospermia/oligozoospermia and/or abnormalities involving sperm morphology/ motility. These anomalies may be numerical or structural and involve the sex chromosomes or autosomes. The frequency of an abnormal karyotype ranged from 2-8% in unselected infertile men [7] as compared to only less than one per cent in the newborn males [8]. These figures were increased to 5-7% in oligozoospermic males and 10-15% in azoospermic males [9]. However, the exact mechanism of induction of infertility by these aberrations is not clear. The most frequent chromosomal abnormality in azoospermic individuals is 47,XXY or Klinefelter syndrome. It is observed in about 10-15% of cases with azoospermia and up to 5% in severely oligozoospermic men while it occurs in approximately 0.1-0.2% of newborn males [7,10]. A small proportion of the males may be 47,XXY/46,XY mosaics or variants having higher-grade aneuploidies or structurally abnormal X chromosomes [10].

Balanced autosomal translocations have been reported seven times more frequently in infertile men compared with newborns [3]. These translocations are found more frequently in oligozoospermic patients (1.7%) than in azoospermic patients (0.6%) [11]. Reciprocal translocations involving the X chromosome was found to commonly result in infertility while those involving the Y chromosome resulted in infertility when the breakpoint was confined to the euchromatic region [12]. On the other hand, Robertsonian translocations, involving more frequently chromosomes 13 and 14 or chromosomes 14 and 21, are reported in 0.9% of the oligozoospermic and 0.3% of the azoospermic patients [11]. Studies on infertile men with translocations and those with normal karyotype have shown aneuploidy in their sperm, particularly sex chromosome aneuploidy [13].

The contribution of polymorphic chromosomal variants to infertility is still questionable and future investigations including those using microarray and gene expression profiling technologies on larger study populations are required to delineate the role of the apparently "harmless" chromosomal polymorphisms. The present study was undertaken to determine the frequency of somatic chromosomal anomalies in referred cases of infertile men opting for assisted reproduction in order to rule out a chromosomal etiology and to contribute to the existing database.

MATERIALS AND METHODS

Cytogenetic evaluation of 180 infertile men opting for assisted reproduction was carried out at the Department of Genetics, University of Madras, Chennai, India. This group comprised of 124 cases investigated during June 2009 to May 2012 and 56 men during July 2013 to August 2014. The Institutional Human Ethics Committee granted ethical clearance for this study. After having obtained written informed consent, heparinised blood samples were collected from infertile men whose wives were found to be normal upon clinical examination. They were recruited from three Fertility Centres in Chennai – i) G.G Hospital (n = 72), ii) Kanmani Fertility

Centre (n = 13) and iii) Prashanth Fertility Research Centre (n = 56). Cases referred to the department of Genetics (n = 39) by private practitioners were also included. Information pertaining to personal identity, parental age and consanguinity, duration of infertility, family history, habits relating to smoking, alcohol consumption and drug intake, clinical features, past medical history, spermiogram and hormonal tests were documented using predesigned questionnaire. The patients were classified into different categories based on semen analysis according to WHO guidelines [1].

Chromosomal preparations were obtained from phytohemagglutinin-stimulated lymphocyte cultures and subsequently GTG-banded using standard procedures. CBG- and AgNOR- banding methods were employed to confirm the variants. Twenty-five metaphases were analyzed for each individual and karyograms were prepared from five well-banded metaphases at a resolution of 550 BPHS using Applied Spectral Imaging Systems karyotyping software (BandView version 6.0). Chromosomal abnormalities were designated using standard ISCN guidelines. Fluorescence *in situ* hybridization (FISH) using whole chromosome painting probe for chromosome 17 (WCP17-Green signal, Kreatech Diagnostics, The Netherlands) was performed on metaphases on unstained slides to confirm the structurally abnormal chromosome 17 detected in a patient according to manufacturer's instructions.

RESULTS

This study details the frequency and types of chromosomal abnormalities encountered in 180 men with primary infertility but whose wives were healthy. Eighty-eight female partners who were also subjected to chromosomal analysis revealed a normal karyotype. Male infertility is usually evidenced by the production of low number, abnormal and/ or immotile sperm. Semen analysis revealed 28 men to be normozoospermic and all of them showed a normal kayotype [Table/Fig-1]. Fifty men were classified to have azoospermia, 24 with severe oligoasthenoteratozoospermia, 72 oligoasthenoteratozoospermia and 6 cryptozoospermia. While the FSH and LH levels were within normal limits in the different categories of infertile men, total testosterone levels were mostly reduced [Table/Fig-2]. However, normozoospermic men had normal testosterone, LH and FSH levels [Table/Fig-1]. Infertile men with abnormal semen parameters were twice as frequently born to consanguineous parents as normozoospermic infertile men. Further instances of infertility were observed among relatives in 73 (48.03%) individuals with an abnormal spermiogram. First-, second- and third- degree relatives exhibited infertility in 30, 29 and 14 cases respectively and the corresponding figures were 3, 5 and 1 amongst the normozoospermic men. Twenty-two men were treated for varicocele.

Chromosomal analysis revealed an abnormal kayotype in seven out of 152 men (4.6%) all of whom were found to be azoospermic. Four men showed Klinefelter syndrome (47,XXY) [Table/Fig-3] and they typically exhibited tall stature, small testes, increased FSH and LH levels, low or low-normal testosterone, reduced seminal fluid volume and azoospermia. Reciprocal translocations involving autosomes t(1;11)(p22.3;p13) [Table/Fig-4] and t(1;10)(q25;q24) [Table/Fig-5] were seen in two cases and one individual exhibited a structurally abnormal chromosome 17, der(17)t(17;?)(p12;?) [Table/Fig-6]. However, FISH using whole chromosome paint probe WCP-17 revealed an insertion ins(17;?)(p12;?) [Table/Fig-7]. It was of interest to observe the same abnormality in his mother [Table/Fig-8,9]. The donor chromosome could not be characterized further due to non-cooperation of the patient. Further, his maternal cousin, another maternal cousin's daughter and a paternal cousin's daughter who were also found to be infertile were not available for investigation.

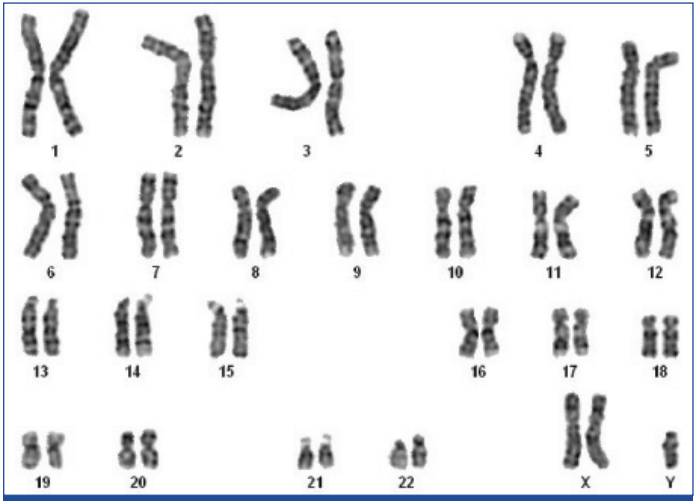
Chromosomal polymorphisms were observed in 58 infertile men with an abnormal spermiogram and in 9 normozoospermic men [Table/Fig-10,11]. Fifteen men exhibited two or more variant

	Infertile men with abnormal semen parameters (mean ± s.d.)(range)	Normozoospermic infertile men (mean ± s.d.)(range)
No. of patients (n)	152	28
Age (years)	35 ± 6 (20-53)	35 ± 6 (21-48)
BMI	25 ± 4.4 (14-41)	25.9 ± 4.2 (16.8-42.7)
Duration of infertility (years)	7.1 ± 4.5 (1-25)	7 ± 3.7 (2-18)
Consanguineous parents	43	4
Patients with history of infertility	73	9
Alcohol consumption	53	5
Smoking	26	4
Varicocele	22	1
FSH (mIU/ml)	5.77 (3.44-12.0)	7.02 (4-14.3)
LH (mIU/ml)	5.98 (4.23-8.39)	8.98 (7.07-15.05)
Total Testosterone (ng/dL)	235.5 (108-372.8)	433.5 (360.8-540)
Normal karyotype	95.4%	100%

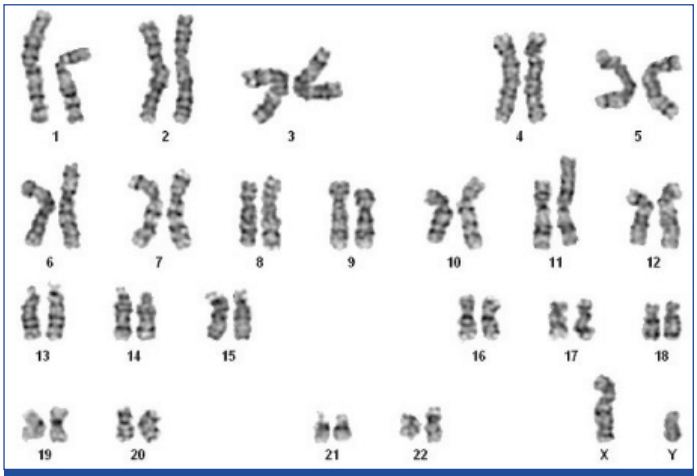
[Table/Fig-1]: Clinical, hormonal [as median with interquartile range] and karyotypic features in infertile men

Category	Azoospermia (n = 50)	OAT (n = 72)	SOAT (n = 24)	Cryptozo-ospermia (n = 6)
FSH (mIU/ml)	4.84 (3.2–16.1)	5.45 (3.31–10.7)	6.73 (4.9–9.4)	12.6 (4.4 - 14.7)
LH (mIU/ml)	5.55 (3.9–12.4)	5.98 (4.3–7.6)	6.22 (4.31–8.3)	6.75 (4.63 - 8.2)
Testosterone (ng/dL)	170 (12.2–278.3)	275.9 (210.2 - 439.0)	277.85 (188.2–378.1)	232.6 (55.5 - 269.2)

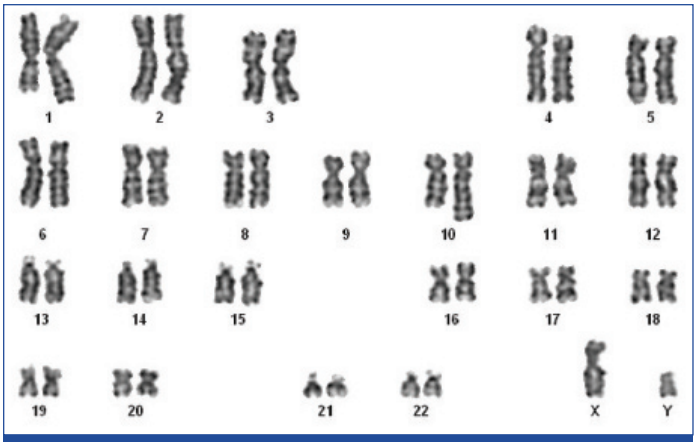
[Table/Fig-2]: Summary of the hormonal values (as median with interquartile range) recorded in the different categories of infertile men based on their spermiogram.



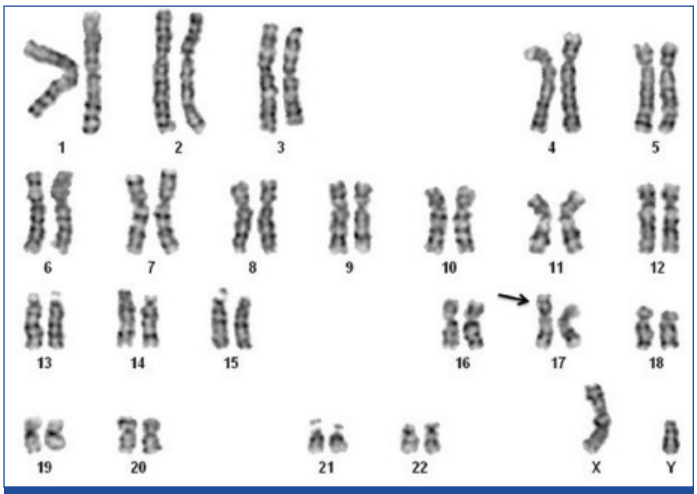
[Table/Fig-3]: Karyogram showing 47,XXY in a Klinefelter individual (IF84)



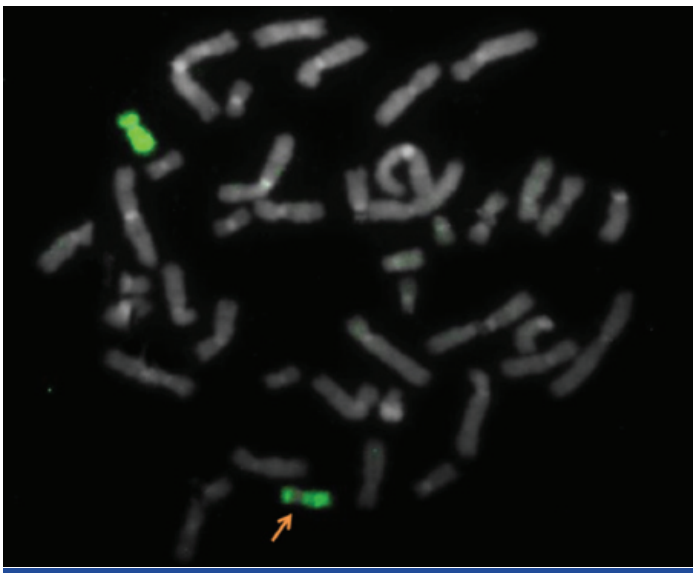
[Table/Fig-4]: Karyogram showing t(1;11)(p22.3;p13) (IF125)



[Table/Fig-5]: Karyogram showing t(1;10)(q25;q24) in IF129

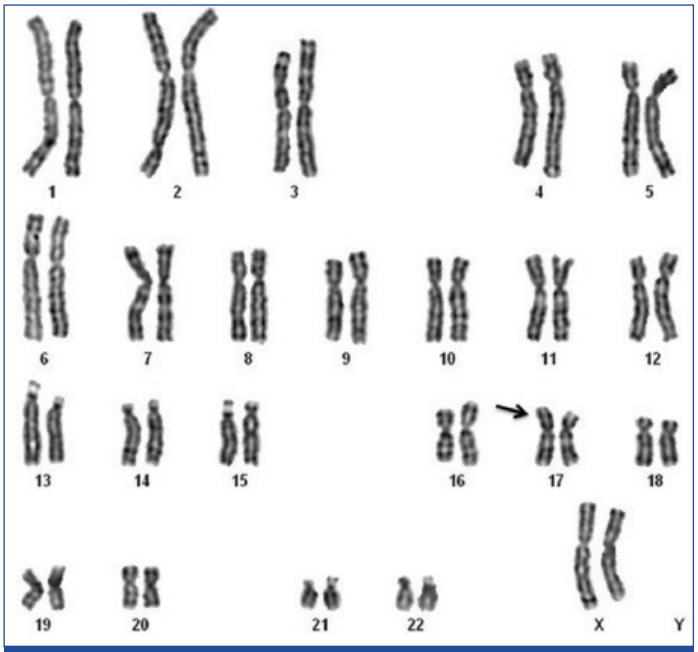


[Table/Fig-6]: Karyogram showing 46,XY,ins(17;?)(p12;?) (IF57P)

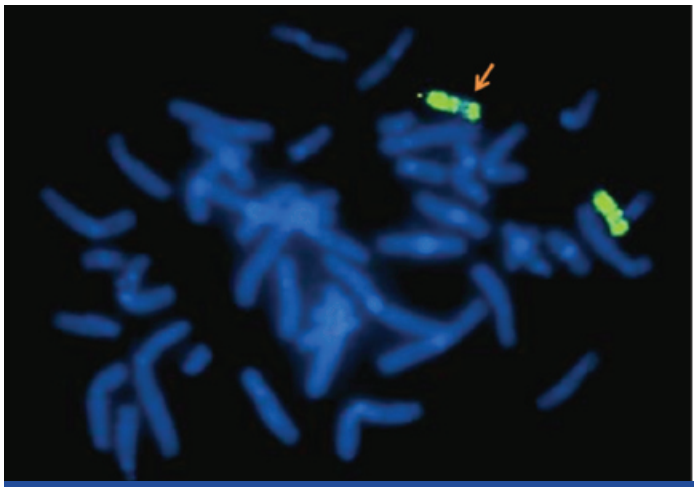


[Table/Fig-7]: FISH analysis with WCP-17 probe showing insertion of material of unknown origin into 17p (IF57P)

chromosomes. A variation in the heterochromatic region of the Y chromosome resulting in long Y (Yqh+; 9 cases) or short Y (Yqh-; 12 men) was commonly noticed. A pericentric inversion inv(Yqh) was noted in four cases including two cases of short Y. Other variants included an increase in the length of heterochromatic region on chromosome 9, 9qh+ (n=9) and those involving the heterochromatic regions of the acrocentric chromosomes - large satellites, increased or decreased length of the stalk and double satellites (n=45). One individual with cryptozoospermia showed absence of short arm of chromosome 21 [Table/Fig-10].



[Table/Fig-8]: Karyogram of mother of IF57P patient showing the same abnormal chromosome 17



[Table/Fig-9]: FISH analysis with WCP-17 probe in the patient's (IF57P) mother showing the same insertion

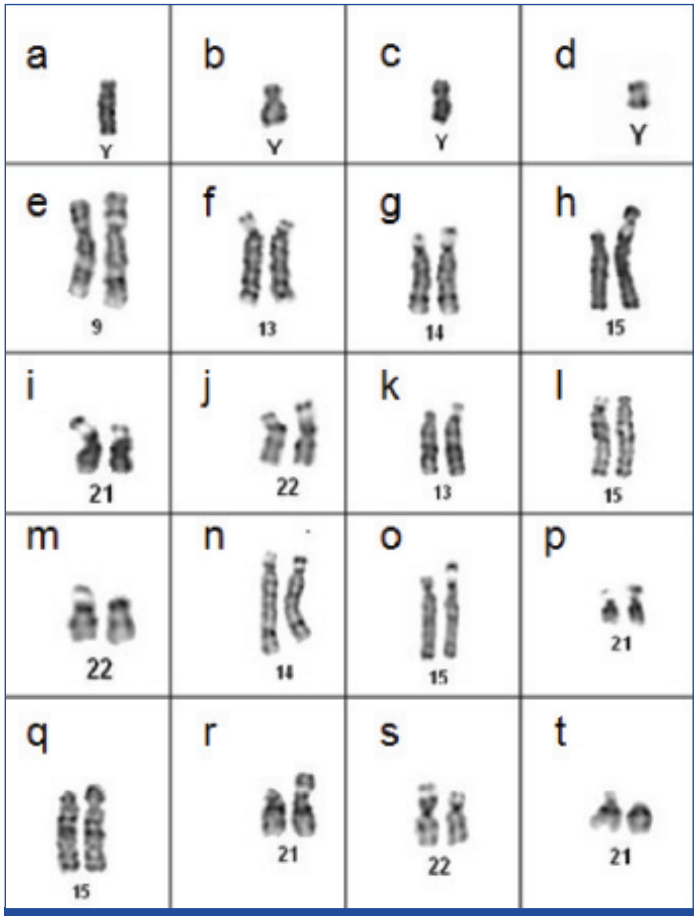
DISCUSSION

Most infertile men in the present study exhibited reduced levels of testosterone. Patients with Robertsonian and sex chromosome–autosome reciprocal translocations were reported to have significantly lowered testosterone levels when compared with those who had an autosome-autosome translocation and the fertile control group [14]. The authors suggested that karyotype analyses should be performed in infertile men having low or even normal hormone levels. A significant correlation between varicocele and poor sperm quality could be due to the effect of increased intra-testicular temperature with subsequent effect on testicular function or due to the statistically significant high levels of DNA damage [15].

Infertility in men can be diagnosed mostly by absence (azoospermia) or decreased number of sperm (oligozoospermia), decrease in progressive sperm motility (asthenozoospermia), presence of morphologically abnormal sperm (teratozoospermia) or any combination of these [1]. This study consisted of 28 infertile men (15.6%) who were normozoospermic upon repeated semen analysis. All of them exhibited a normal chromosomal pattern. However, they could harbour DNA damage in their sperm. Saleh et al., found a significant difference between the levels of DNA damage in sperm from infertile men with normal semen parameters as compared with sperm from fertile donors [16].

Azoospermia (n = 50)	OAT (n = 72)	SOAT (n = 24)	Cryptozo-ospermia (n = 6)	Normozo-ospermia (n = 28)
Yqh- 2	Yqh- 2	Yqh-	15pstk+	Yqh-
Yqh+ 3	Yqh+ 2	9qh+	21ph-	Yqh+ 2
inv(Yqh)	9qh+	14pstk+		inv(Yqh-)
inv(Yqh-)	14ps+ 2	22pstk+		21ps+
9qh+	21ps+ 4			14pstk+
15pstk+	13pstk+			15pstk+
21pstk+	14pstk+			
22pss	15pstk+ 3			
	21pstk+ 6			
	22pstk+ 3			
	13pstk-			
	15pstk-			
	15pss			
Cases with two or more variant chromosomes				
Yqh-, 9qh+ 2	Yqh+,15ps+		9qh+,14pstk+	Yqh+,21pstk+
Yqh-, 15pstk+	inv (Yqh), 21pstk, 21pss			9qh+,21pstk+
Yqh-, 21pstk+, 22ps+	9qh+, 21pstk+			
9qh+, 22pstk+	13pstk+, 21pstk+			
15pstk+, 22pstk+	15pstk+, 21pstk+			
	15ps+, 22pstk+			
Total 17	34	4	3	9

[Table/Fig-10]: Chromosomal polymorphisms encountered in the different categories of infertile men based on their spermiogram



[Table/Fig-11]: Chromosomal polymorphisms observed – a) Yqh+ b) Yqh- c) inv(Yqh) d) inv(Yqh-) e) 9qh+ f) 13pstk+ g)14pstk+ h) 15pstk+ i) 21pstk+ j) 22pstk+ k) 13pstk- l) 15pstk- m) 22pstk- n) 14ps+ o) 15ps+ p) 21ps+ q) 15pss r) 21pss s) 22pss t) 21ph-

An association between human male infertility and chromosomal anomalies (both somatic and meiotic) has been known for a very long time [9,17] and since then numerous studies have been carried out on chromosome abnormalities in infertile men. An abnormal chromosomal pattern observed in 4.6% of infertile men with abnormal spermiogram in the present study was in accordance with other investigations on more than 150 infertile men (3.0–11.7%) [Table/Fig-12]. These values were significantly higher than those reported (<1%) in newborns [8]. Further numerical sex chromosomal abnormalities were more frequent in the azoospermic group while oligozoospermic men showed mainly structural autosomal anomalies [5]. The occurrence of chromosomal abnormalities was confined to the azoospermic group only in this study which could be due to small sample size. The rest of the infertile men were noticed to have a normal karyotype although their seminogram were abnormal. It is probable that many of these individuals may have an increased incidence of aneuploid and/or diploid sperm [11,13].

Author	Cases	Chromosome abnormalities % (n)	Variants % (n)	Azoospermia % (n) [a + b]	Oligozo-ospermia % (n) [a + b]
Foresta et al., [18]	750	5.2 (39)	0.27 (2)	-	5.2 (39/750) [29 + 10]
Pina-Neto et al., [19]	165	9.6 (16)	1.8 (3)	20.0 (12/60) [12 + 0]	4.0 (4/100) [0 + 4]
Zhou-Cun et al., [20]	358	8.9 (32/358)	2.2 (8/358)	12.1 (31/256) [27 + 4]	0.98 (1/102) [1 + 0]
Etem et al., [6]	214	10.7 (23)	12.1 (26)	13.8 (19/138)	6.6 (4/76)
Vijayalakshmi et al., [21]	150	3.33 (5)	7.33 (11)	3.2(4/125) [4 + 0]	4.5 (1/22) [0 + 1]
Fu et al., [22]	1333	7.4 (99)	4.12 (55)	9.41 (89/945) [70 + 19]	2.57 (10/388) [3 + 7]
Al-Achkar et al., [23]	162	11.7 (19)	0.62 (1)	16.5 (16/97) [16 + 0]	4.6 (3/65) [1 + 2]
Han et al., [24]	189	8.5 (16)	0.53 (1)	11.2 (14/125) [14 + 0]	3.1 (2/64) [2† + 1]
Sreenivasa et al .,[25]	200	3.0(6)	4.5 (9)	8.3 (4/48) [4 + 0]	1.4 (1/73) [0 + 1]

[Table/Fig-12]: Frequency of chromosomal abnormalities in infertile men from published reports (on more than 150 men).
a & b – number of cases showing sex chromosomal & autosomal abnormalities respectively
† case with t(X-A)

Infertile men with normal 46,XY karyotype and oligozoospermia or teratozoospermia were found to exhibit a significantly increased risk of sperm aneuploidy, particularly for the sex chromosomes as compared with those with normal semen parameters or normal donors [26].

The most prevalent cytogenetic cause of hypogonadism and infertility in males is 47,XXY characteristic of Klinefelter syndrome. It is found in approximately 1 in 575-1000 newborn males [10,19,27]. Four patients (2.2%) out of 180 infertile males showed a 47,XXY karyotype in this study which is comparatively lower than reported in the literature [5]. FISH analysis using probes for sex chromosomes has demonstrated that the frequency of aneuploidy varied from 1.5 - 7% in sperm from Klinefelter mosaics and 2 - 25% in the sperm of men with nonmosaic 47,XXY karyotype [26].

Reciprocal translocations have been reported to be the most common chromosomal abnormality occurring in 1 in 500 newborns [8]. Carriers of these translocations usually do not exhibit any abnormal phenotype but may complain of recurrent miscarriage, chromosomally abnormal offspring and in some cases sterility [28]. The men may have azoospermia often due to an arrest at prophase I following an association between XY bivalents and quadrivalent formations [29]. Sperm karyotyping, using the hamster system, in 37 reciprocal translocation heterozygotes has shown that 19–77% of spermatozoa are unbalanced and these result from abnormal

segregation and/or interchromosomal effect leading to aneuploidy of chromosomes including those not involved in the translocation [7,30]. Rearrangements of human chromosome 1 were found to be linked to male factor infertility and especially to azoospermia [31]. In this study two azoospermic men exhibited reciprocal translocation involving chromosome 1. A *de novo* translocation t(Y;1)(q12;q12) in an azoospermic male was suggested to cause loss of Xp/Yp pairing following translocation of Y-PAR2 to der(1) chromosome and subsequently lead to the arrest of spermatogenesis at zygotene/pachytene and apoptotic degeneration of germ cells [32].

A balanced interchromosomal insertional translocation (IT) would involve an interstitial deletion of one chromosome and an interstitial insertion in another chromosome. The carriers of balanced ITs are thus phenotypically normal but carry an enhanced risk for repeated miscarriages or malformed offspring. The prevalence of ITs is expected to be lower than for reciprocal translocations. Van Hemel and Eussen identified five cases of unbalanced rearrangements among 40,000 patients that resulted from an unbalanced transmission of a derivative chromosome from a carrier parent for a balanced IT [33]. In the present study an azoospermic man was inferred to carry an IT which he had inherited from his mother. Studies on families with balanced structural chromosomal rearrangements have shown that sperm count is inversely correlated with the frequency of chromosome abnormalities while oogenesis is relatively preserved as exemplified through association of fertility in female carriers of these rearrangements [34].

The overall occurrence of the common C-band variants involving the heterochromatic region of chromosomes 1, 9, 16 and Y, and the acrocentric short arm variants in the present study was 37.2% (38.2 % in men with an abnormal seminogram). Comparable frequencies were reported by Madon et al., who observed these variants in 28.82% of male partners attending an IVF clinic for primary infertility or repeated miscarriages [35], Nagvenkar et al., in 37.5% of men prior to ICSI treatment [36], and Lissitsina et al., in 37.8% of the infertile men examined [27]. While Minocherhomji et al., observed a significantly higher frequency of chromosomal variants in infertile men (58.68%) relative to the control group [37]; lower frequencies were recorded by others (16.7% in Kate et al.,) [38]. Lissitsina et al., reported a similar frequency of polymorphic chromosomal variants in the control group also [27].

Hong et al., in a study on 1671 infertile couples did not find any significant difference in the implantation rate and clinical pregnancy rate between the group with variants seen in male partners when compared with the group with variants in females only or the control group showing no polymorphic variant chromosomes [39]. Quantitative and positional alterations of the constitutive heterochromatin were postulated to disturb normal pairing of homologous chromosomes resulting in meiotic arrest or inhibit gene transcription through a silencing effect on normally expressed genes in close proximity [35,37]. Among patients with abnormal spermiogram 62% of those carrying polymorphic variants revealed decreased ability of the spermatozoa to penetrate hamster oocytes when compared with 51% of the patients with normal karyotype [40]. It has been thus suggested to screen prospective gamete donors for variant chromosomes to enhance the success of IVF [35,37,41].

Nagvenkar et al., reported the occurrence of long Y (Yqh+) and short Y (Yqh-) in 3.4 and 27.3 per cent of the infertile men respectively [36]. Further, PCR analysis revealed AZFc microdeletion along with deletion of the heterochromatin in a single male who presented with a karyotype of 46,X,del(Y)(q11.2). The short Y chromosome was suggested to represent deletion of genes on Yq11.23 playing a role in spermatogenesis [36]. Four men (2.2%) possessed an inverted Y in this study. In most cases, inverted Y chromosomes are not associated with any phenotypic abnormalities or infertility [42]. Conversely a small deletion in Yq11.1-q11.2 or interruption of the *DAZ* genes due to the pericentric inversion has been reported in infertile men [42,43].

Chromosome 9 exhibits the highest degree of morphological variations and the incidence of an elongation of the heterochromatin 9qh+ is estimated to be 6-8% in the general population [44,45]. This variant chromosome was found in 5% of the infertile men and in 13.4% of those possessing heteromorphisms in the present study. Heterochromatic variants such as 9qh+ were suggested to influence the formation of synaptonemal complex through morphological differences and consequently to cause meiotic arrest [44]. Purandare et al., observed heterochromatic variations of chromosomes 1 and 9 (qh+ and inversions), and of short arm regions of D- and G- group chromosomes especially 15ps+ and 22ps+ to be significantly higher in couples with bad obstetric history [41]. In the present study, 45 infertile men (2.2%) exhibited short arm variants of acrocentric chromosomes.

CONCLUSION

The present study emphasizes the importance of routine cytogenetic screening of all infertile men prior to the use of assisted reproduction technologies not only in the identification of the underlying cause of infertility but also in minimizing the potential risk of transmission of chromosomal abnormalities as they tend to override the protective mechanism of natural selection. The couples must also be offered pre-implantation genetic diagnosis and chorionic villus sampling/amniocentesis for prenatal diagnosis or if necessary sperm donation.

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