Chapter III

Chromosomal analysis in infertile men
3.1 Introduction

Infertility is defined as failure to conceive after at least one year of unprotected intercourse [1]. It is a major health problem affecting 10-20% of couples, approximately 50% of the cases represents male factor infertility [2]. The etiology of male infertility is complex and may include anatomical problems: congenital bilateral absence of vas deferens; infections: mumps and herpes; life style and environmental factors: smoking, alcohol consumptions and air pollution; hormone imbalance: increase/decrease in levels of gonadal steroids and gonadotropic hormones; immunologic problems: antisperm antibodies; and genetic causes [3, 4]

Genetic factors (30%) are considered to be significant specifically CA and gene mutations associated with male infertility [5], as they have the risk of producing, spontaneous abortions or aneuploid offspring [6]. Most infertile men have normal karyotypes, but their spermiogram are abnormal, with an increased incidence of aneuploid and diploid sperm. The incidence of chromosomal abnormality in infertile men is reported to be 2.1 to 19.6% [7, 8] which is higher than in the general population.

About 7.1% of major CA were reported in 496 infertile men, of which 21% were azoospermic [9]. Bourrouillou et al., (1985) found a higher incidence of (10.3%) in 952 infertile men; 40% of infertile men were azoospermic suggesting that the frequency of chromosomal abnormalities were high in patients with abnormal spermatozoa [10]. Further, in azoospermic patients more than 90% of abnormalities affect sex chromosomes while autosomal chromosomal abnormalities are more frequent in patients with oligozoospermia [11, 12, 13]. Thus, the impact of chromosomal abnormalities in male infertility is very high and inversely related to sperm count. In this prospective study, it was proposed to analyze the type and frequency of various chromosomal abnormalities in fertile controls and infertile men with abnormal semen parameters.

3.2. Materials and methods

The study subjects include control (n=110) and infertile men (n=175). The age group and spermiogram are detailed in Section 2.1. The preparation of chromosomes were detailed in
the methodology Section 2.3. The chromosomes were analyzed after the banding techniques: GTG, AgNOR and Q as mentioned in Section 2.3.4, 2.3.6 and 2.3.7. Finally, the results of the chromosomal analysis were interpreted as per ISCN [14].

3.3. Results

The present study included fertile controls \((n=110)\) and infertile cases \((n=175)\). The spermiogram of cases showed absence of sperm, decreased sperm count (range 0-4 million/ml), reduced motility (range 6-12%) and abnormal morphology (normal ranges from 2-10%); based on those parameters the cases were classified into azoospermia, oligoasthenoteratozoospermia, severe oligoasthenoteratozoospermia and globozoospermia. The proportion of each category is shown in Table 3.1.

<table>
<thead>
<tr>
<th>Abnormal spermiogram</th>
<th>Sample size (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azoospermia (AZF)</td>
<td>84.60</td>
</tr>
<tr>
<td>Oligoasthenoteratozoospermia (OAT)</td>
<td>13.70</td>
</tr>
<tr>
<td>Severe Oligoasthenoteratozoospermia (SOAT)</td>
<td>1.10</td>
</tr>
<tr>
<td>Globozoospermia</td>
<td>0.60</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>100.00</strong></td>
</tr>
</tbody>
</table>

Chromosomal analysis of control subjects showed a normal karyotype of 46,XY without any structural or numerical abnormalities. However, in infertile cases 86.32% showed a normal karyotype of 46,XY and remaining 13.68% displayed different types of chromosomal abnormalities (Figure. 3.1).
Among azoospermia subjects, 2.85% showed an abnormal karyotype: 47,XXY (non-mosaic) Klinefelter’s and 46,X,del(Y)(q11.2), deletion on Y chromosome. A 0.57% of OAT cases showed Robertsonian translocation [45,XY,t(13;14)(q10;10)]. Thus, an overall 3.42% infertile subjects (Table 3.2) displayed constitutional chromosomal abnormalities (associated with both autosomes and sex chromosomes); 6.84% of variants (Table 3.3) and 3.42% (Table 3.4) with low-level mosaicism.

Table 3.2. Constitutional chromosomal abnormality (CCA) observed in infertile men (n=6)

<table>
<thead>
<tr>
<th>Classification</th>
<th>Karyotype</th>
<th>Percentage (%)</th>
<th>Figure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azoospermia</td>
<td>47,XXY</td>
<td>1.14</td>
<td>3.2</td>
</tr>
<tr>
<td></td>
<td>46,X,del(Y)(q11.2)</td>
<td>1.71</td>
<td>3.3</td>
</tr>
<tr>
<td>Oligoasthenoteratozoospermia</td>
<td>[45,XY,t(13;14)(q10;10)]</td>
<td>0.57</td>
<td>3.4</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>3.42</strong></td>
<td></td>
</tr>
</tbody>
</table>
### Table 3.3. Chromosomal variants observed in infertile men \((n=12)\)

<table>
<thead>
<tr>
<th>Classification</th>
<th>Karyotype</th>
<th>Percentage (%)</th>
<th>Figure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azoospermia</td>
<td>46,XY,Yqh+</td>
<td>3.42</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>46,XY,1qh+</td>
<td>0.57</td>
<td>3.6</td>
</tr>
<tr>
<td></td>
<td>46,XY,9qh+</td>
<td>0.57</td>
<td>3.7</td>
</tr>
<tr>
<td></td>
<td>46,XY,inv(9)(p11q13)</td>
<td>0.57</td>
<td>3.8</td>
</tr>
<tr>
<td></td>
<td>46,XY,15p+</td>
<td>0.57</td>
<td>3.9</td>
</tr>
<tr>
<td></td>
<td>46,XY,22p+</td>
<td>0.57</td>
<td>3.10</td>
</tr>
<tr>
<td>OAT*</td>
<td>46,XY,21p+</td>
<td>0.57</td>
<td>3.11</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>6.84</td>
<td></td>
</tr>
</tbody>
</table>

OAT*: Oligoasthenoteratozoospermia

### Table 3.4. Single cell abnormality observed in infertile men \((n=6)\)

<table>
<thead>
<tr>
<th>Classification</th>
<th>Karyotype</th>
<th>Percentage (%)</th>
<th>Figure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azoospermia</td>
<td>46,XY(99%)/46,XY,t(3;6)(1%)</td>
<td>0.57</td>
<td>3.12</td>
</tr>
<tr>
<td></td>
<td>46,XY(99%)/46,XY,del(9q)(1%)</td>
<td>0.57</td>
<td>3.13</td>
</tr>
<tr>
<td></td>
<td>46,XY(99%)/45,XY,t(7;14)(1%)</td>
<td>0.57</td>
<td>3.14</td>
</tr>
<tr>
<td></td>
<td>46,XY(99%)/45,XY,t(19;22)(1%)</td>
<td>0.57</td>
<td>3.15</td>
</tr>
<tr>
<td></td>
<td>46,XY(99%)/47,XXY(1%)</td>
<td>0.57</td>
<td>3.16</td>
</tr>
<tr>
<td></td>
<td>46,XY(99%)/45,X(1%)</td>
<td>0.57</td>
<td>3.17</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>3.42</td>
<td></td>
</tr>
</tbody>
</table>
Figure 3.2. Karyotype 47,XXY

Arrow indicates an extra X chromosome
Chromosomal analysis in infertile men

Figure 3.3. Karyotype 46, X, del(Y)(q11.2)

(a) Arrow displays del(Y)(q11.2)

(b) Arrow indicates the absence of heterochromatin region (Q-banding).
Figure 3.4. Karyotype [45,XY,t(13;14)(q10;10)]

(a) Arrow displays t(13;14)(q10;10)
(b) Partial metaphase showing the presence of t(13;14)
(c) Arrow represents the presence of D/D by AgNOR-banding.
Figure 3.5. Karyotype 46,XY,Yqh+

Arrow displays Yqh+
Chromosomal analysis in infertile men

Figure 3.6. Karyotype 46,XY,1qh+

(a) Arrow displays 1qh+

(b) Partial metaphase showing the presence of 1qh+
Figure 3.7. Karyotype 46,XY,9qh+

(a) Arrow displays 9qh+

(b) Partial metaphase showing the presence of 9qh+
Figure 3.8. Karyotype 46,XY,inv(9)(p11q13)

(a) Arrow displays inv(9)

(b) Partial metaphase showing the presence of inv(9)
Figure 3.9. Karyotype 46,XY,15p+

Arrow displays 15p+
Chromosomal analysis in infertile men

Figure 3.10. Karyotype 46,XY,22p+.

(a) Arrow displays 22p+. (b) Partial metaphase showing the presence of 22p+. 
Figure 3.11. Karyotype 46,XY, 21p+

Arrow displays 21p+
Figure 3.12. Karyotype 46,XY,t(3;6)

Arrow displays t(3;6)
Figure 3.13. Karyotype 46,XY,del(9q)

Arrow displays del(9q)
Figure 3.14. Karyotype 45,XY,t(7;14)

Arrow displays t(7;14)
Figure 3.15. Karyotype 45,XY,t(19;22)

Arrow displays t(19;22)
Figure 3.16. Karyotype 47,XXY

Arrow indicates an extra X chromosome
Figure 3.17. Karyotype 45,X

Karyotype denotes a total of 45 chromosomes.
3.4. Discussion

About 2-7% of infertile couples have a higher risk of constitutional chromosomal abnormalities which may be the cause of fertility problems [15,16]. Literature had reported that a prevalence of high chromosomal abnormalities might interfere with male infertility leading to the production of unbalanced gametes by meiotic segregation or the possible presence of interchromosomal effects [8, 17, 18, 19, 20, 21, 22, 23]. The present study has shown a frequency of 2.85% of sex chromosomal abnormality in azoospermia cases and 0.57% of autosomal chromosomal abnormalities in OAT which is in accordance with earlier reports.

A frequency of 2.76% of CA among the infertile men was reported by Marchina et al., (2007) [24] which is slightly lesser than the present study. Studies from India had shown a frequency of 10.8% of CA in 88 Indian infertile men [25]; 4.4% in 1666 couples presenting with bad obstetrics history and infertility from Bangalore, India [26], the frequency of present study is lesser than the earlier reports from India.

The frequency of autosomal chromosomal abnormality was reported to be higher in infertile men than the general population. Robertsonian translocations (RT) one of the most common structural reorganizations was frequently seen in infertile men, which occur due to meiotic nondisjunction, of which ~50% are de novo [26, 27]. Translocations were found between chromosome 13;14 [28]. Dohle et al., (2002) [29] had reported 0.66% of RT from 150 couples and Marchina et al., (2007) [24] reported 0.63% in infertile men, which supports the above findings.

Robertsonian translocations with chromosomes involving 13;14, 13;15 or 14;15 shows a clear increased risk for spontaneous pregnancy losses, viable offspring with either aneuploidy or uniparental disomy probably in the low-moderate range. Although there are no empirical data available yet, the risk for this type of genetic anomaly is probably not very high [30, 31, 32]. Govaerts and colleagues (1995) [33] had reported 5.45% of RT from one of the carrier parents; it is observable that all these chromosomal aberrations were passed on in a balanced state.
Observations in experimental animals and electron microscopic examination of meiotic profiles in human male carriers have suggested that trivalents are formed at the pachytene stage of meiosis by the RT chromosome. The two single D or G group chromosomes may interact with X/Y bivalent resulting in spermatogenic impairment or the tendency of RT to attach with sex vesicle, which possibly prevents inactivation of the sex chromosome in male meiosis that results in spermatogenic arrest [34]. Meiotic segregation in RT carriers can produce similar numbers of chromosomally normal and balanced gametes [35].

During meiosis I, trivalent is formed between a derivative chromosome composed of the long arms of two acrocentric chromosomes and both normal acrocentric chromosomes of the same pairs (homologous chromosomes). In alternate segregation, the derivative chromosome segregates in a spermatocyte II and both normal acrocentric chromosomes in another spermatocyte II, producing balanced and normal spermatozoa respectively. This segregation mode produces a translocation carrier with 45 chromosomes as the father or a chromosomally normal fetus. The other segregation modes, either adjacent or 3:0, produce unbalanced gametes. In adjacent segregation, four combinations are possible. The derivative chromosome segregates in a spermatocyte II with a normal acrocentric chromosome belonging to one of the pairs involved in the translocation, leading to a gamete with 23 chromosomes. In another spermatocyte II, there is only one acrocentric belonging to one of the pairs involved in the rearrangement; a gamete with 22 chromosomes is produced. Spermatozoa resulting from an adjacent segregation mode are nullisomic and/or disomic for acrocentrics, leading to a monosomic and/or trisomic embryo. The 3:0 segregation mode, producing a gamete with 21 chromosomes and a gamete with 24 chromosomes, is a very rare event.

Preliminary evidence suggests that sex chromosomal anomalies in male infertility may be more common than natural pregnancies that arise de novo, possibly chromosome-related or increased rate of sperm chromosome aneuploidy [36, 37, 38, 39]. The prevalence of Klinefelter’s syndrome (Klinefelter syndrome) in infertile men (azoospermia and oligospermic groups) is higher than the general population. Approximately, 80% of the cases display a higher-grade
of chromosome aneuploidies or mosaicism but still a very low rate spermatogenesis has been reported [40, 41, 42]. Nagvenkar et al., (2005) had reported 1.13% with Klinefelter syndrome, which is found to be similar to the present study [25].

Mitra et al., (2008) had reported that 50% of 14 azoospermic men presented with Klinefelter syndrome [43]. Bellovits et al., (2006) had observed three Klinefelter syndrome from 71 infertile men. This abnormality is associated with severe spermatogenic failure causing a marked reduction in testicular size and azoospermia and with high levels of FSH and LH, resulting in male infertility [44]. Although the prevalence of Klinefelter syndrome is reported to be high, Marchina et al., (2007) did not find any cases of Klinefelter syndrome in their study [24].

Sciurano et al., (2009) have reported that seminiferous tubuli with germ cells represent only a minor fraction of all tubuli in men with non-mosaic Klinefelter syndrome. Using fluorescence in situ hybridization (FISH), they showed that meiotic spermatocytes are euploid, and thus can form normal, haploid gametes [45]. Sertoli cells showed two marks for the X chromosome, meaning that they were 47,XXY. These new findings explain a high rate of normal children born after testicular sperm extraction (TESA) or ICSI when applied to Klinefelter syndrome. Ramasamy et al., (2009) showed that other medications might lead to endogenous testosterone increase seem to benefit Klinefelter syndrome men [46]; either normal or low baseline testosterone response to medical therapy (aromatase inhibitors, clomiphene or human chorionic gonadotropin) had a better chance of sperm retrieval [47].

Studies have indicated that deletions on the long arm of Y-chromosome involving a particular and consistent segment might lead to azoospermia [48] or severe oligospermia [49]. The variation in the length was due to the distal part of the long arm that is known to contain heterochromatin, the most frequent variant observed in sex chromosomes. The present study showed 1.71% of infertile men to have a deletion on chromosome Y. Cram et al., (2000) [50], reported that Yq deletion might lead to vertical transmission in the offspring, but still this should be further investigated with molecular diagnoses.
Several reports on male infertility referred to the presence of chromosomal variants. It is suggested that chromosomal variant/heteromorphism do not induce miscarriage, as the role played by variants is still controversial; a large number of variants are reported in the normal population. The most frequently occurring heterochromatic variant is inv(9) and 9qh+ which was observed to be higher in infertile men compared to normal population. The relationship between chromosomal variants such as pericentric inversions and impaired spermatogenesis is not clear [51]. Some studies reveal a direct association between inv(9)(p11q13) and infertility or repeated abortions, and significantly higher incidence of intrauterine fetal death, in which the etiology and unknown mechanisms are related to sex [12, 52].

The overall occurrence of chromosomal variants (involving autosome and sex chromosomes) in the present study was found to be in 6.84% cases, which is similar with other studies. An increase in the length of heterochromatic regions: 1qh+, 9qh+, 15p+, 21p+, 22p+, Yqh+, and inv(9p) were observed (Table 3.3); out of 6.27%, 0.57% autosomal variant were azoospermic and oligoasthenoteratozoospermia. Although inversion 9 is included in the group of variant chromosomes, contradictory information was reported about its effect on reproductive fitness in men; that a person carrying a variant chromosome has no increased risk of having spontaneous abortion, abnormal offspring, or other reproductive problem [26].

The structural aberrations (inversions) are found ten times more frequently in infertile men than the fertile men [53]. Inversion (inv) is a chromosomal rearrangement when a segment of chromosome between two breakpoints is inverted to 180 degrees into the same chromosome. Incidence varies from 1-1.5%, 0.9% and 0.1-0.3% in infertile men, oligozoospermia and azoospermia respectively [54, 55].

Y chromosome polymorphisms have been preferentially seen in azoospermia and severe oligozoospermia. ‘Long Y chromosome’ (Yqh+) and ‘short Y chromosome’ (Yqh-) are known to exist. A 3.42% of Yqh+ was found in the present study. Yqh+ has been seen to be associated with an increased risk of fetal loss [56]. However, another study did not show any relationship
between the size of the Y chromosome and the risk of abortion [57]. The contribution of Y chromosomal variants to alter the carrier’s fertility is still a controversial topic and further studies are required to understand this.

Structural rearrangements confined to a single cell were disregarded in routine cytogenetic analysis. It is uncertain whether carriers of low-level of sex chromosome mosaicism have any risk for having an abnormal child or probably negligible for the majority of fertile individuals; but there seems to be an increased risk for repeated abortions. Since low-level mosaicism is often a second abnormal cell-line involving the sex chromosomes, the significance of mosaicism (≤10% abnormal metaphase) in men (46,XY/45,X) is controversial [24, 58].

Men with a normal somatic karyotype may still have an abnormal cell line in their testes. These men are called as ‘germinal mosaics’, and it is difficult to discover them without a testicular biopsy. Studies have discovered that 1–17% of infertile men are germinal mosaics [59], so this is still a risk after a normal karyotype result, but the risks for abnormal offspring would be lower than those for non-mosaic individuals [60].

3.5. Conclusion

In infertile men with abnormal semen parameters and normal karyotype form a majority of cases may be due to unexplained/idiopathic infertility. Being a multifactorial disorder, environment and the genetic components may interact. The frequency of CA, variants and low level-mosaicism in the present study is found to be 3.42%, 6.84% and 3.42% respectively, which are much lower than the frequency (ranging between 2.1-19.6%) reported globally. However, there were no comparable observations from elsewhere in India. Therefore, it should be of critical interest to generate data from all regions of India: geographical, environmental, ethnic axis into consideration on the genetic basis of infertility.
References


