CHAPTER I

INTRODUCTION

Infertility is a cause of enormous emotional and psychological strain amongst the afflicted couples. It has been estimated that approximately 15% of married couples are infertile. It is estimated that globally, 60–80 million couples suffer from infertility every year, of which probably 15–20 million are in India alone. Infertility affects both men and women equally. Therefore, an investigation into the causative factors of infertility amongst men and women is highly desirable (Dada et al. 2003; Hellani et al. 2005; Khaleghian & Azimi 2006).

Patients with fertility problems are known to be a clinically heterogeneous group, as it is known that a variety of factors can perpetuate infertility. Generally, infertility can result from hormonal disturbances, increased maternal or paternal age, obesity, infectious disease, immunological or psychological factors, surgery, or defined abnormalities in the gametes, such as azoospermia or oligoasthenoteratozoospermia syndrome (OAT) (Manvelyan et al. 2008).

Infertility is defined as Primary Infertility if no pregnancy has ever occurred in the subjects, or secondary, where there has been a pregnancy and a subsequent miscarriage. The maximum probability of conceiving during a menstrual cycle is only about 40%. One third of conceptions do not result in the delivery of a baby (Zinaman et al. 1996). The vast majority of continuing pregnancies result in the birth of a healthy human being who will, eventually, pass his or her genes on to the next generation.

Miscarriages—most common complication of pregnancy, affecting approximately 15% of all clinically recognized pregnancies in the general population are the inevitable byproduct of such a process. They are common and often remain unexplained, even after investigation. The World Health Organization (WHO) and the American Society of Reproductive Medicine (ASRM) has defined a miscarriage as the loss of a fetus or embryo weighing ≤ 500g, which would normally be at 20-22 complete weeks of gestation (WHO 1977). When two or more consecutive miscarriages occur, the condition is termed recurrent pregnancy loss (RPL) or Repeated Spontaneous Abortion (RSA) or habitual abortion. They are heartbreaking and a source of distress for women, their partners and their families too and in such a case it is likely to seek professional help, in the hope that a cause and a cure
will be found. The exact frequency of miscarriages is, however, unknown as miscarriages frequently occur before the woman is aware of her pregnancy. It is estimated that more pregnancies are lost spontaneously than are actually carried to term (Rai & Regan 2006; Stephenson & Kutteh 2007).

1.1 Repeated spontaneous abortion

Recurrent miscarriage is ill-defined, probably because of the uncertainty, complexity and heterogeneity surrounding the condition (Block et al. 1998). The classic definition of RSA is the loss of 3 or more clinically recognized pregnancies spontaneously during early gestation. However, some investigators included two or more miscarriages in series (Stephenson et al. 1998; Quenby & Farquharson 1993) others did not clarify whether the miscarriages were consecutive (Wilson et al. 1999). Furthermore some authors only included those with first trimester miscarriages (Clifford et al. 1996), whereas others included losses in the mid-trimester or did not specify whether or not second trimester miscarriages were included (Kolho et al. 1999). Furthermore, it is essential to specify the types of pregnancy loss, i.e. whether or not the pregnancy loss occurred after fetal cardiac activity was identified. Bricker & Farquharson (2002) considered the pregnancy loss as embryonic loss if it occurred before fetal cardiac activity was identified and as fetal loss if it occurred after fetal cardiac activity was identified. However, other authors may classify the type of loss differently; for example, it has been proposed that there are three different types of loss: preclinical, demise <6 weeks; embryonic loss, demise at >6 weeks but <10 weeks gestation; fetal loss, demise at >10 weeks but <20 weeks gestation (Stephenson et al. 2002). Until a classification is universally accepted, it is important to clearly define the stages at which the loss occurred, which are possible only if serial ultrasound examinations are carried out early in the pregnancy according to current guidelines (Gynaecologists 1995). Therefore, when comparing results from different investigators, it is important to understand that there may be different definitions and populations involved (Li et al. 2002). However, The American Society for Reproductive Medicine defines RPL (Repeated pregnancy loss) as two or more failed pregnancies, which have been documented by either ultrasound or histopathological examination. They suggest that some investigation must be done after each miscarriage, with a thorough evaluation after three or more losses.
1.1.1 Occurrence of spontaneous pregnancy loss
Spontaneous pregnancy loss has a surprisingly common occurrence. It is estimated that fetal viability is achieved only in 30% of all human conceptions, 50% of which are lost prior to the first missed menses (Edmonds et al. 1982). In humans, approximately 25% of implanted embryos are resorbed within 7-14 days after attachment to the uterine endometrium (Baines & Gendron 1993; Comings 1978). The loss of clinically recognized pregnancies prior to the 20th week of gestation occurs at a frequency of 15% (Warburton & Fraser 1964). The physical, emotional and financial toll of pregnancy loss is large. The emotional issues surrounding pregnancy loss become magnified exponentially when miscarriage occurs on a repetitive basis. Statistically, 4-6% of all women attempting pregnancy will experience at least two miscarriages, and about 1-2% will have 3 or more miscarriages (Feinberg & Van Deerlin 1997). Some studies suggest that clinical intrauterine pregnancy losses occur in 12-14% of all pregnancies (Regan et al. 1989; Wilcox et al. 1988) and can be attributed to random factors (often fetal de novo chromosomal abnormalities). The number of women with RSA for non-recurrent reasons will decline exponentially with the number of previous miscarriages, because their risk of a new miscarriage in their fourth or fifth pregnancy is still only 14% compared with the 35-50% risk in the whole group of RSA patients with a similar number of previous miscarriages (Cauchi et al. 1995). Patients with a systemic problem will comprise an increasingly larger fraction of all RSA patients with an increased number of previous pregnancy losses. Spontaneous pregnancy loss can be physically and emotionally taxing for couples, especially when faced with recurrent losses.

1.1.2 Evaluation of recurrent pregnancy loss
The challenge for the clinicians is to differentiate sporadic miscarriages (that occur at random in different women rather than over and over again in the same woman) from RSA. Self-reported losses by patient may not be accurate. In one study only 71% of self-reported clinical pregnancy losses could be verified in hospital records (Christiansen et al. 2005). For the purpose of determining whether evaluation of RPL is accurate, a pregnancy is defined as clinical pregnancy when it is documented by ultrasonography or
histopathological examination (Stray-Pedersen & Stray-Pedersen 1984). Ideally, a clinical evaluation may proceed following two first trimester pregnancy losses.

1.1.3 Etiology of recurrent spontaneous abortion
Repeated Spontaneous Abortion is one of the least understood pathological process inspite of one of the most common symptoms (Meka & Reddy 2006). There are numerous factors that may cause RSA, but the underlying problem often remains undetected. Although much work has been done to identify the underlying mechanisms, the cause of miscarriage can be identified in only about 50% of cases. The known causes of RSA include chromosomal and metabolic abnormalities, uterine anomalies, and immunologic factors (Li et al. 2002; Rai & Regan 2006). Even though RSA is a heterogeneous condition and the progress in identifying causative factors has been slow, the repetitive pregnancy losses in some couples and the high percentage of unexplained RSA indicate that there are specific underlying causes yet to be found (Bick et al. 1998). The diagnosis of infertility and its underlying mechanism require multidisciplinary approaches, including physiological, biochemical, cytogenetic, and molecular studies.

1.2 GENETIC FACTORS CAUSING INFERTILITY
Genetic factors are a major cause of clinically recognized miscarriages. In the following sections, the currently reported genetic causes associated with miscarriages are described. About 1 in 150 babies is born with a chromosomal abnormality (Bull 2001; Carey 2003). These disorders are caused by either errors in the number or structure of chromosomes, which usually result from an error that occurred when an egg or sperm cell was developing. Babies may be born with too few or too many chromosomes. In some instances, a piece of a chromosome may be missing or the chromosomes may be rearranged (Morava et al. 2002). However, it is still unknown why these errors occur. Nonetheless, these errors can cause a variety of birth defects ranging from mild to severe. Many children with a chromosomal abnormality have mental and/or physical birth defects. Understanding chromosomal structure is a key to understand the wide range of problems associated with chromosomal abnormalities.
1.3 CHROMOSOMES

Chromosomes are tiny string-like structures in cells of the body that contains genes. Humans have about 19000 genes that determine traits like eye and hair color (Ezkurdia et al. 2014). They also direct the growth and development of every part of the body. Each person normally has 23 pair of chromosomes or 46 in all. Every human inherit one chromosome per pair from our mother and one from our father. Each chromosome has a p and q arm; p is the shorter arm and q is the longer arm. By convention, normal chromosomes are always considered to have the p – arm at top. The arms are separated by a pinched region known as the centromere (Figure 1.1).

![One Chromosome](https://example.com/one_chromosome)

**Figure 1.1 Structure of a chromosome**

The body is made up of individual units called cells. Human body has many different kinds of cells, such as skin cells, liver cells and blood cells. In the center of most cells is a structure called the nucleus. This is where chromosomes can be found.

The typical number of chromosomes in a human cell is 46 - two pairs of 23 - holding an estimated 25,000 genes. One set of 23 chromosomes is inherited from the biological mother (from the egg), and the other set is inherited from the biological father (from the sperm).
1.4 STUDY OF CHROMOSOMES

In order for chromosomes to be seen with a microscope, they need to be stained. Once stained, the chromosomes look like strings with light and dark "bands" and their picture can be taken. A picture, or chromosome map, of all 46 chromosomes is called a karyotype (Figure 1.2). The karyotype can help identify chromosome abnormalities that are evident in either the structure or the number of chromosomes. To help identify chromosomes, the pairs have been numbered from 1 to 22, with the 23rd pair labeled "X" and "Y." The first 22 pairs of chromosomes are called "autosomes" and the final pair is called the "sex chromosomes." The sex chromosomes in an individual determine that person's gender; females have two X chromosomes (XX), and males have an X and a Y chromosome (XY).
1.5 CAUSES OF CHROMOSOMAL ABNORMALITIES

Chromosome abnormalities usually occur when there is an error in cell division. It is not known why these errors occur.

**There are two kinds of cell division.**

**Mitosis** results in two cells that are duplicates of the original cell. In other words, one cell with 46 chromosomes becomes two cells with 46 chromosomes each. This kind of cell division occurs throughout the body, except in the reproductive organs. This is how most of the cells that make up human body are made and replaced.

**Meiosis** results in cells with half the number of chromosomes, 23 instead of the normal 46. These are the eggs and sperm. Sperm and egg cells are different from other cells in the body. These cells have only 23 unpaired chromosomes. When an egg and sperm cell join together they form a fertilized egg with 46 chromosomes. But sometimes something goes wrong before fertilization. An egg or sperm cell may divide incorrectly, resulting in an egg or sperm cell with too many or too few chromosomes. When this cell with the wrong number of chromosomes joins with a normal egg or sperm cell, the resulting embryo has a chromosomal abnormality.

In both processes, the correct number of chromosomes is supposed to end up in the resulting cells. However, errors in cell division can result in cells with too few or too many copies of a chromosome. Errors can also occur when the chromosomes are being duplicated.

1.6 TYPES OF CHROMOSOME ABNORMALITIES

A chromosome anomaly, abnormality, aberration or mutation is a missing, extra, or irregular portion of chromosomal DNA. It can be from an atypical number of chromosomes or a structural abnormality in one or more chromosomes. A chromosome anomaly may be detected or confirmed by comparing it with the normal karyotype of the species via genetic testing. Chromosome anomalies usually occur when there is an error in cell
division following meiosis or mitosis. There are many types of chromosome anomalies. They can be organized into two basic groups, Numerical and Structural Anomalies.

1.6.1 Numerical Chromosomal Abnormalities
Numerical abnormalities occur when there is a different number of a chromosome in the cells of the body from what is usually found. So, instead of the usual 46 chromosomes in each cell of the body, there may be 45 or 47 chromosomes. Having too many or too few chromosomes may cause health problems or birth defects. The term "trisomy" is used to describe the presence of three chromosomes, rather than the usual pair of chromosomes. For example, if a baby is born with three #21 chromosomes, rather than the usual pair, this baby would be said to have "trisomy 21." Trisomy 21 is also known as Down syndrome. Other examples of trisomy include trisomy 18 and trisomy 13. Again, trisomy 18 or trisomy 13 simply means there are three copies of the #18 chromosome (or of the #13 chromosome) present in each cell of the body, rather than the usual pair.

The term "MONOSOMY" is used to describe the absence of one member of a pair of chromosomes. Therefore, there are a total of 45 chromosomes in each cell of the body, rather than 46. For example, if a baby is born with only one X sex chromosome, rather than the usual pair (either two X's or one X and one Y sex chromosome), baby would be said to have "monosomy X." Monosomy X is also known as Turner syndrome.

1.6.2 Structural Chromosomal Abnormalities
Structural chromosomal aberrations can result in genetic disease due to trisomy and/or monosomy of chromosomal segments. These aberrations may be de novo events or may be inherited from carrier parents. Structural abnormalities are formed by chromosomal breakage or unequal crossing over which result in deletions, ring chromosomes, duplications, translocations, insertions and inversions. A single break in one chromosome will produce a terminal deletion, whereas two breaks in a single chromosome can result in an interstitial deletion, a ring chromosome or an inversion. Two breaks in two different chromosomes can produce structural changes including Reciprocal and Robertsonian translocations. Unequal crossing-over can result in duplications or deletions. Chromosome rearrangements are considered balanced if disomy is maintained for all of the autosomes.
and a normal complement of sex chromatin is present, even if the positions of the homologous segments on the chromosomes have been changed.

In contrast, when chromatin is lost or gained in the process the rearrangement is said to be unbalanced. Unbalanced constitutional rearrangements are generally associated with developmental delay or intellectual impairment, birth defects and poor growth, whereas balanced rearrangements often have no effect on physical or intellectual development. Structural chromosome rearrangements that are present at conception affect every cell and are referred to as constitutional (Anon n.d.).

A standard nomenclature has been developed to describe each of the types of abnormality found in human chromosomes. The current version was developed by the International Standing Committee on Human Cytogenetic Nomenclature and adopted in 2013 (Schaffer & McGowan-Jordan 2013). It is accepted throughout the world as the definitive work for describing and designating both constitutive and acquired chromosomal abnormalities.

1.6.2.1 Deletions
Abnormalities in which a portion of chromatin from a single chromosome is lost are called deletions. Deletions result in a partial monosomy and are, therefore, unbalanced rearrangements. Single breaks cause terminal deletions with a subsequent loss of the chromosome end. When two breaks occur in the same arm of a chromosome, interstitial deletions are formed by a loss of the chromatin between the breaks and a rejoining of the remaining segments. Deletions that are large enough to be visible to the eye using light microscopy represent the loss of many genes that are physically located in the same band or region of the chromosome, and result in monosomy for that portion of the genome. For many loci, this represents a haplo-insufficiency in function and is often severe enough to cause death of the embryo. Deletions that survive to birth are associated with a very high risk of birth defects and intellectual impairment.

1.6.2.2 Duplications
Duplications are unbalanced rearrangements that result in partial trisomy. Compared with deletions, duplications tend to be somewhat milder in effect, but they share many of the same clinical features. Duplications are believed to result primarily from unequal crossing
Segmental duplications can be oriented in two ways: direct or inverted. Direct duplications retain the same order of gene loci and chromosome bands in relation to the centromere as the parent chromosome, whereas inverted duplications exhibit a complete reversal of loci and bands contained in the duplication. Duplications on one chromosome produce partial trisomies when paired with a normal chromosome in a diploid cell. Partial trisomies can also be caused by translocations or through recombination in inversion heterozygotes. These are referred to as duplications despite the difference in the mechanism of formation. One example of common chromosome duplication is an inverted duplication of a segment of the long arm of chromosome 15, which is generally observed as an extra dicentric chromosome. The phenotype of patients with this chromosome is highly variable and dependent upon the size of the duplicated segment, the parent of origin, and the presence or absence of the critical region.

1.6.2.3 Translocations
Translocations involve breaks in two different chromosomes with an exchange of segments. In humans, there are two major types of translocation: reciprocal translocations in which there is no visual loss of chromatin and Robertsonian translocations in which the long arms of two acrocentric chromosomes are joined with loss of the two short arms. Ascertainment of both reciprocal and Robertsonian translocations is often through multiple miscarriages, unbalanced progeny or infertility.

1.6.2.4 Reciprocal Translocations
Reciprocal translocations are characterized by an exchange of chromatin between different chromosomes. A single break occurs in each chromosome, and the noncentric segments are exchanged without the visible loss of any chromatin (Figure 1.3). However, the two new derivative chromosomes may have very different morphology depending on the breakpoints. The carrier of a reciprocal translocation generally has no phenotypic effects due to the rearrangement except for possible reproductive abnormalities including infertility, spontaneous abortions and abnormal offspring. Pairing of homologues at meiosis is altered in translocation carriers. Rather than normal pairing as bivalents, the two
derivative chromosomes and their two normal homologues pair to form a cross-shaped quadrivalent at pachytene with each homologous segment pairing with its counterpart. Pairing and segregation take place after DNA replication, so each chromosome consists of two chromatids and, thus, at each point, the quadrivalent consists of four chromatids. In most cases, two chromosomes move to one daughter cell and two to the other; in rare situations, three chromosomes segregate together, leaving one to move alone.

Figure 1.3 Reciprocal translocation event between chromosome 4 and chromosome 20 (Gardner et al. 2011).
1.6.2.5 Robertsonian translocations

Robertsonian translocations are unique types of whole arm translocations that result from ‘centric fusion’ of the long arms of two acrocentric chromosomes with loss of the short arms, thus reducing the number of chromosomes by one (Figure 1.4). They are named after W. R. B. Robertson, who was an insect cytogeneticist and studied numerical chromosome changes in several orthopteran populations (Lewis & Robertson 1916). The formation of a Robertsonian translocation may actually result from breaks in the short arm, in the long arm or within the centromere of the two chromosomes that form the ‘fusion’ product. Depending on the position of the breaks and exchange of chromatin segments, the resulting derivative chromosome may be either monocentric or dentric. Robertsonian chromosomes formed of two homologous long arms (e.g. a chromosome composed of two chromosome 14 long arms) may be the result of a U-type exchange between sister chromatids or two homologous chromosomes, or may actually be an isochromosome with identical arms formed by a mis-division of the centromere. Participation in Robertsonian translocations is not equal among the 10 human acrocentric chromosomes. Unbiased ascertainment data from amniocenteses or consecutive newborn surveys found that a 13; 14 translocation is the most common Robertsonian translocation, followed by a 14; 21 translocation (Hook & Cross 1987; Therman et al. 1989). However, many families are ascertained through children with Down syndrome (trisomy 21), Patau syndrome (trisomy 13), Prader–Willi syndrome or unspecified intellectual impairment, and, therefore, Robertsonian translocations that involve chromosomes 13, 15 or 21 will show an increase in these series. Other ascertainment biases may be due to detection of a Robertsonian translocation carrier through a history of multiple miscarriages or infertility. A carrier of a Robertsonian translocation will not generally show any physical effects until reproduction. Then, as in reciprocal translocations, pairing at pachytene involves both the normal homologues and the translocation chromosome. However, in the case of Robertsonian translocations, there are only three chromosomes involved; thus, a trivalent is formed at pachytene. Segregation from the trivalent results in the production of six types of gametes. Two of these are normal and the other four will produce trisomies or monosomies when fertilized by a normal gamete. The concepts may be viable, depending on which acrocentric chromosomes are involved. Trisomy for chromosomes 13 and 21 are compatible with life,
whereas trisomy for the other acrocentrics (i.e. 14, 15 and 22) will virtually all be lost as spontaneous abortions. All the conceptions with monosomies will also be lost prenatally. For female carriers of both reciprocal and Robertsonian translocations, there is an increased risk for abnormal offspring as well as an increased risk for miscarriages due to inviable products of conception. The male translocation carrier has an increased risk for oligospermia or complete azoospermia and often is ascertained through investigation for infertility.

![Figure 1.4 Robertsonian Translocation](image-url)
1.6.2.6 Inversions

Inversions are formed by two breaks in the same chromosome with exchange of the two ends. Inversions are thus essentially formed in the same manner as translocations except that the breaks and exchange occur in the same chromosome. Two different types of inversion are found. One is a pericentric chromosome in which one break occurs in each arm of the chromosome (Figure 1.5) and, thus, the centromere is included in the inverted segment. This changes the banding patterns and may also change the shape of the chromosome due to movement of the centromere. Alternatively, a paracentric chromosome is formed when both breaks occur in the same arm and, therefore, the centromere is not included in the inverted segment. This alters the banding patterns, but not the shape of the chromosome. Both pericentric and paracentric inversions can be carried in the heterozygous state. Like translocation carriers, there is generally no phenotypic effect on inversion heterozygotes due to the inverted gene order of one homologue, except as a result of abnormalities in meiosis.

Figure 1.5 Paracentric and pericentric inversion
Like translocation carriers, there is generally no phenotypic effect on inversion heterozygotes due to the inverted gene order of one homologue, except as a result of abnormalities in meiosis. Here, as in other heterozygotes for structural rearrangements, difficulties in pairing and segregation arise at the first meiotic prophase during pachytene when homologous pairing and recombination take place. In this instance, the inverted segment forms a loop to maximize pairing of homologous loci between the inverted and normal homologues. The inversion loop structure is formed after the chromosomes have replicated so that the bivalent is composed of four chromatids, two normal and two inverted strands. Abnormal gametes are formed only when an unequal number of recombination (crossing-over) events occur within the loop structure. As a result of crossing-over, the recombinant chromatid has both a duplicated segment and a deleted segment, i.e. duplication of the terminal segment of the short arm with deletion of the terminal segment of the long arm or vice versa. Since only two of the four chromatids in a bivalent participate in a single cross-over, any recombinant event produces only two recombinant chromatids; but also present are one normal chromatid and one inverted, but balanced, chromatid. It should be noted that the terminal segments which are duplicated and deleted from crossingover are the parts of the chromosome distal to the breakpoints and are, therefore, the segments outside the loop. Thus, the larger the segment between the breakpoints (i.e. the closer the breakpoints are to the telomeres), the larger the loop, and the more likely that recombination will occur within it. At the same time, the distal segments that are duplicated and deleted will be smaller. Consequently, it will be more likely for the recombinant gamete to result in a conceptus that will be abnormal, but viable. In contrast, the smaller the segment between breakpoints, the less likely it is that a cross-over will take place in this region. But the recombinant products that are formed are less likely to come to term because of the larger duplicated and deleted segments and, instead, result in miscarriage. The major difference between pericentric and paracentric inversions involves the position of the centromere in the recombinant products. Since the region within the inversion loop remains balanced, the recombination products of the pericentric inversion each retain a single copy of the centromere and can, therefore, disjoin normally during mitosis. In contrast, because the region outside the inversion loop is either duplicated or deleted, the recombination products from the paracentric inversion receive either two copies or no
copies of the centromere, neither of which is compatible with long-term survival. On rare occasions, recombination products with a single active centromere have been reported from paracentric inversions, which allow the embryo to survive.

### 1.6.2.7 Inversion of Chromosome 9
The most common form of inversion which is encountered in human chromosomes is a pericentric inversion of chromosome 9 (inv 9), that occurs in 1%-1.65% of the general population (Cheong et al. 1997). In the Asian population, the incidence of this anomaly is projected to be lower than that in other ethnic groups. Various studies have estimated it to be in the range of 0.25% (Teo et al. 1995).

### 1.6.2.8 Rings
A portion of a chromosome has broken off and formed a circle or ring. This can happen with or without loss of genetic material. Most chromosome abnormalities occur as an accident in the egg or sperm. Therefore, the abnormality is present in every cell of the body. Some abnormalities, however, can happen after conception, resulting in mosaicism, where some cells have the abnormality and some do not. Chromosome abnormalities can be inherited from a parent (such as a translocation) or be "de novo" (new to the individual). This is why chromosome studies are often performed on parents when a child is found to have an abnormality (Anon n.d.).

### 1.7 DIFFERENT TYPES OF BANDING METHODS FOR DIAGNOSIS OF CHROMOSOMAL ABERRATIONS

#### 1.7.1 G-Banding
The karyotyping protocol generally involves one or other type of banding to visualize and identify the chromosomes in a better way. G-banding is obtained with Giemsa staining after the digestion of chromosomes with the enzyme trypsin. Trypsin produces a pattern of light and dark bands on the chromosomes. The dark regions are that of heterochromatin whereas the light bands are that of euchromatin. Since each chromosome has a distinct pattern of banding these chromosomes can be identified easily by this technique.
1.7.2 Q-Banding
Q-banding is obtained by the fluorescent dye quinacrine. The pattern of bands is very similar to that of G-banding. It is especially useful for distinguishing the Y chromosome which is the smallest human chromosome. This method requires a fluorescence microscope.

1.7.3 R-Banding
R-banding is the reverse of G-banding. The dark regions are that of euchromatin (guanine-cytosine rich regions) and the bright regions are heterochromatic (thymine-adenine rich regions). R- Banding is used for the identification of bright region (Telomeres).

1.7.4 C-Banding
C-banding stains only constitutive heterochromatin, which usually lies near the centromere. This banding is commonly used for identification of centromere and location of centromere.

1.7.5 High-resolution banding
The degree of difficulty in the analysis of the karyotype is depends upon the number of bands that can be identified (resolved). Aneuploidies (for e.g. trisomy and monosomy) can be readily evaluated at low band resolution (i.e.350–550 bands) whereas the visualization of micro-deletions and cryptic translocations requires high resolution banding. High-resolution banding techniques are designed to allow more than 550 bands levels of chromosomal analysis.

1.8 MOLECULAR CYTOGENETICS FOR DIAGNOSIS OF CHROMOSOMAL ANOMALIES

1.8.1 Fluorescence in situ hybridization
FISH (fluorescence in situ hybridization) is a cytogenetic technique used to detect and localize the presence or absence of specific DNA sequences on chromosomes. FISH uses fluorescent probes that bind to only those parts of the chromosome with which they
show a high degree of sequence complementarity. Fluorescence microscopy can be used to find out the location of fluorescent probe bound to the chromosomes. FISH is often used for finding specific features in DNA for use in genetic counselling, medicine, and species identification. FISH can also be used to identify and locate the specific RNA targets in cells. It can help in determination of patterns of gene expression within cells and tissues. There are 3 types of probes used in FISH (i) locus specific probes that bind to the specific locus in the chromosome (ii) Centromeric probes which are PCR amplified repeated DNA sequences (iii) oligonucleotides specific for repetitive DNA in heterochromatin blocks or centromeric regions of individual chromosomes. Whole chromosome probes are actually collections of smaller probes each of them specifically binding to a different sequence along the length of a given chromosome. These probes mixtures are often used to detect deletions or translocations in chromosomes.

1.8.2 Multicolour FISH

Sophisticated methods have been developed that permit the simultaneous detection of all 23 human chromosomes for eg. spectral karyotyping (SKY), multiplex FISH (M-FISH), and cross-species colour banding (RX-FISH). In SKY and M-FISH, different dyes are used to label each chromosome with a unique colour. RX-FISH employs labelled probes from primates for hybridization with human chromosomes. These probes produce coloured banding pattern that is unique for each chromosome.

Each of these techniques provides a useful tool for evaluating complex chromosomal abnormalities in humans and are valuable for establishing the genetic basis of infertility.

1.8.3 Microarray comparative genomic hybridization (CGH)

Microarray comparative genomic hybridization (CGH) testing is useful for the detection of small genetic imbalances (gains or losses of chromosomal material), also known as genomic copy number changes, which may not be detectable by conventional cytogenetic and/or FISH techniques. Some of the diagnostic techniques routinely employed for the diagnosis of infertility have been listed in above table
1.9 ROLE OF CONVENTIONAL AND MOLECULAR CYTOGENETIC TECHNIQUES FOR DIAGNOSIS OF INFERTILITY

Conventional cytogenetic analysis (karyotyping) provides an overview of all chromosomes giving vital information on aneuploidies such as Klinefelter (Figure 1.6) and Turner’s syndrome as well as major structural alterations, such as translocations, which are common causes of abortions. Routine or conventional chromosome analyses require sterile viable tissue sample. Since it analysis involves culture of cells and arresting the cells in metaphase followed by different methods of visualization through chromosome banding. The common G banding techniques can achieve a resolution greater than 10 Mb. High resolution bandings used for an improved resolution of 3-5 Mb. Alterations such as sub-microscopic or molecular defects that are smaller than 3 Mb cannot be detected by these methods.

The diagnosis of molecular defects is carried out by molecular cytogenetic testing also known as fluorescence in-situ hybridization (FISH) which can be employed to assess the anomalies present in specific DNA segments by fluorescence microscopy. Metaphase FISH can be used to diagnose position and copy number changes, deletions and duplication of specific chromosomal segments for which the DNA probes are employed. FISH can be employed to cells in interphase which avoids the requirement of culture of cells before analysis. Interphase FISH can reveal numerical anomalies or rearrangements involving specific DNA segments. This test has been used in addition to standard cytogenetic analysis for prenatal diagnosis. In addition to locus-specific FISH probes, other targeted probes include whole-chromosome painting probes, allow the detection of all 24 chromosomes (22 autosomes and the X and Y chromosomes) in 24 unique colour combinations (Schröck et al. 1996; Speicher et al. 1996).

Moreover; the cause of infertility remains obscure in as high as 10% of cases (Hamada et al. 2012). Therefore there is an increasing need to diagnose the causative mechanisms of infertility in such idiopathic cases.
Sex chromosome abnormalities are the most frequently observed conditions in male infertility. Klinefelter syndrome has been reported as the most frequent chromosomal abnormality by many of the researchers. Turner Syndrome is commonly associated with primary infertility in female patient (Figure 1.7).
In approximately 5-7% of couples with RSA one or other partner (more commonly the woman) possesses abnormal chromosomes which they repeatedly pass on to the fetus (Wang et al. 2012). The abnormality is usually not in the number of chromosomes, but in the way in which they are arranged. The commonest such re-arrangement are balanced translocation, inversion and even some cases mosaics are also reported. Although Robertsonian translocations are rare yet they are also present in certain cases. Obviously
there is currently no cure for the chromosomal abnormality in the parent, but when such a parental chromosomal abnormality is identified, a referral to a clinical geneticist is offered to get advice on the future prospects, and the need for prenatal tests to detect the abnormality in any future pregnancy, as some abnormalities may be compatible with the birth of a live but incapacitated baby. The chances of a successful pregnancy in the future will depend on the specific type of chromosomal abnormality.

1.10 POLYMORPHIC VARIANTS OF CHROMOSOMES

Polymorphic variants of chromosomes are generally considered as ‘normal’ as they vary in heterochromatin regions which are non-coding regions in the genome. Polymorphic variants on non-acrocentric chromosomes usually occur in the paracentric heterochromatin on the long arms of chromosomes 1, 9 and 16, the short-arm regions of D and G group chromosomes, and the distal heterochromatin of the Y chromosome. However, a high frequency of polymorphic variants has been reported by many researchers (Madon et al. 2005). The studies regarding involvement of polymorphic variants in perpetuating infertility have been inconclusive. Sequence analysis of chromosome 9 has revealed a high degree of polymorphism with much intra-chromosomal and inter-chromosomal duplication. It also contains greatest amount of heterochromatin. Novel studies have discovered that heterochromatin regions can become transcriptionally active in response to some environmental stress like heat shock (Kreps et al. 2002; Mahalingam et al. 2003; Matsui et al. 2008). Therefore it can be said that all of polymorphic are not normal. It has motivated us to study the role of inv(9) a common anomaly found in infertile couples which is up till now considered as normal polymorph.

The idiopathic secondary infertility is again a cause of concern for the couples seeking children through assisted reproduction technologies. The majority of first and early second trimester abortions are a result of abnormal karyotype in the fetus. The karyotyping analysis on products of conception (POC’s) is able to find the cases where more detailed studies are required. The parents maybe carriers mutations and therefore may require counseling future pregnancies. However, products of conception (POC’s) undergo rapid autolysis and become contaminated by normal vaginal flora. Also it is difficult to separate
the deciduous maternal tissue from the sample. It was one of the objectives of the present study to carry out the karyotyping analysis on products of conception to find out the cytogenetic anomalies in the fetuses.