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Cytogenetic Studies of Recurrent Miscarriage- A Review

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Authors' contributions

This work was carried out in collaboration between both authors. Author YP designed the study, managed the literature searches and wrote the first draft of the manuscript. Author PK managed the manuscript design. Both authors read and approved the final manuscript.

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Review Article

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ABSTRACT

Recurrent miscarriage occurs in 1–3% of couples aiming at childbirth. It continues to be a challenging reproductive problem for the patient and clinician. Therefore, identifying a cytogenetic cause for a miscarriage may be of great significance for the management of recurrent miscarriage patients. Genetic factors in the form of chromosomal abnormalities, inherited Thrombophilia, single gene disorders and other genes involved are the main causes of recurrent miscarriage. The risk of miscarriage is highest among couples where the woman's age is 35 years or above and men's age is more than 40 years. Constitutional chromosomal abnormalities with great risk to be transmitted to offspring are rare, but their discovery is of crucial importance in prevention of spontaneous abortion and recurrent miscarriage.

Keywords: Recurrent miscarriage; chromosomal abnormality; cytogenetics.

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1. INTRODUCTION

Miscarriage is the spontaneous loss of pregnancy before viability. It is considered as 24 weeks as it is considered to be lower limit of viability. Rarely some pregnancies results in birth of baby before time. Pregnancy can be lost at an early stage of conception and present only as a positive pregnancy test and can be lost before it can be detected by ultrasound. They are referred to as biochemical pregnancy. After this stage, ultrasound might demonstrate an apparently empty gestation sac and referred to as early embryonic demise. When apparently empty gestation sac is visible in ultrasound it is called as early embryonic demise.

Recurrent miscarriage is also referred to as recurrent pregnancy loss or habitual abortion, is historically defined as three consecutive pregnancy losses prior to 20 weeks from the last menstrual period [1]. According to this definition the frequency of recurrent miscarriage is one in 300 women [2]. The American Society for Reproductive Medicine defines recurrent miscarriage as the two or more failed pregnancies [3]. As per this definition the prevalence of recurrent miscarriage is higher i.e. one in 100 women [2].

2. CAUSES AND RISK FACTORS OF RECURRENT MISCARRIAGE

Recurrent miscarriage is an extremely stressful condition for both the partners and physicians because it is difficult to find a reason behind it. Pregnancy loss is a common phenomenon. The frequency of first trimester pregnancy losses are more than that of second trimester. There are several factors responsible for early pregnancy loss such as advanced maternal age, advanced paternal age, smoking or alcohol consumption. Other causes included immunological changes and genetic changes. Genetic changes are widely reported as chromosomal abnormalities in the affected couples as well as in the fetus.

The evaluation criteria for recurrent miscarriage includes testing for cytogenetic study of couple to rule out chromosomal translocations as well as maternal testing for thyroid problem (endocrine), lupus anticoagulant and anti-phospholipid antibodies (autoimmune), endometrial or uterine abnormalities (anatomic) and in some cases single gene disorders such as inherited thrombophilia study [4].

Recurrent miscarriage is heterogeneous condition involving relationship between maternal, paternal and cumulative (placental / fetal) risk factors in pathways related to pregnancy establishment and continuation. The presence of chromosomal rearrangements can lead to unequal crossing over during meiosis which can result in gametes with unbalanced chromosomes like duplications or deletions. The clinical consequences of such imbalances usually are lethal to the developing embryo leading to spontaneous miscarriages or early neonatal deaths [5].

The major cause of recurrent miscarriages is fetal chromosome abnormalities associated with continuously increasing age of women postponing childbearing to late 30s and early. Although high maternal age is also a risk factor in recurrent miscarriage, other causes primarily force this condition as the chance of having an early pregnancy loss due to large chromosomal alterations are decreasing with an increasing number of miscarriages in a couple.

There is a two to threefold increased rate of spontaneous abortion in women attempting pregnancy at age ≥40 years and also an increased risk of chromosomal abnormalities [6].

Table 1. Common causes of recurrent early pregnancy loss

Environmental agent	→ Smoking
	→ Alcohol consumption
Endocrine factor	→ Diabetes mellitus
	→ Polycystic ovary syndrome
Maternal factor	→ Uterine anatomic Malformations
	→ Cervical abnormalities
Immunological factor	→ Antiphospholipid syndrome
Chromosomal and single gene disorders	→ Fetal chromosomal abnormalities
	→ Parental chromosomal abnormalities
	→ Thrombophilia
	→ Alpha Thalassemia Major
	→ X-Linked male lethal conditions

3. PREVALENCE OF RECURRENT MISCARRIAGE

The prevalence of recurrent miscarriage is higher that it would be expected if three miscarriages happen consecutively only by change [7]. Outcome of previous pregnancies is another

decisive factor in the risk of pregnancy loss. For young women who have never experienced a loss, the rate of a clinical miscarriage is as low as 5%. The risk increases to approximately 30% for women with three or more losses but with a previous live-born infant and up to 50% for women without a live-born infant [8].

In some studies pregnancy losses only in the first trimester i.e gestational age more than or equal to 14 weeks were included whereas in other studies second trimester pregnancy losses such as gestational age more than or equal to 24 weeks were investigated [9-14]. In some studies the gestational age of recurrent miscarriages was not clearly mentioned [15]. Therefore in current study, the review of literature includes different study populations. Hence the incidence rate of recurrent miscarriage obtained from these studies may not be comparable completely.

4. GENETIC CAUSES OF PREGNANCY LOSSES

Genetic changes are associated with pregnancy loss. It is the sudden loss of fetus before it is capable of survival outside the womb. The pregnancy loss could be clinical or preclinical. About 15-20% of clinically recognized conceptions are results in miscarriage [5,18]. The genetic information of parents is enclosed in chromosomes and fetus inherits one half of the chromosomes from the father and mother, resulting in a count of 46 chromosomes. Error in the transmission or during the division of chromosome results in the pregnancy loss.

Parental chromosomal abnormalities contribute to the 4% of the miscarriages. The rate of chromosomal abnormality is seen more in couples with the advanced maternal age where the conceptus carries the chromosomal abnormalities which are more common. The incidence of carrier status increases from around 0.7% in the general population to 2.2% after one miscarriage, 4.8% after two miscarriages, and 5.2% after three miscarriages [19].

Genetic causes of pregnancy losses are described as follows:

1. Type of chromosomal abnormalities
2. Incidence of chromosomal anomalies in Recurrent miscarriages
3. Chromosome anomalies vs. number of pregnancy losses male female distribution of abnormality
4. Fetal aneuploidy in recurrent miscarriages

4.1 Type of Chromosomal Abnormalities

Chromosomes are the basic unit of heredity transmitted from one generation to the other and carry the genetic information we possess. During the process of cell division there occurs exchange of genetic material between homologous chromosomes and sister chromatids. Though cell division is a precise

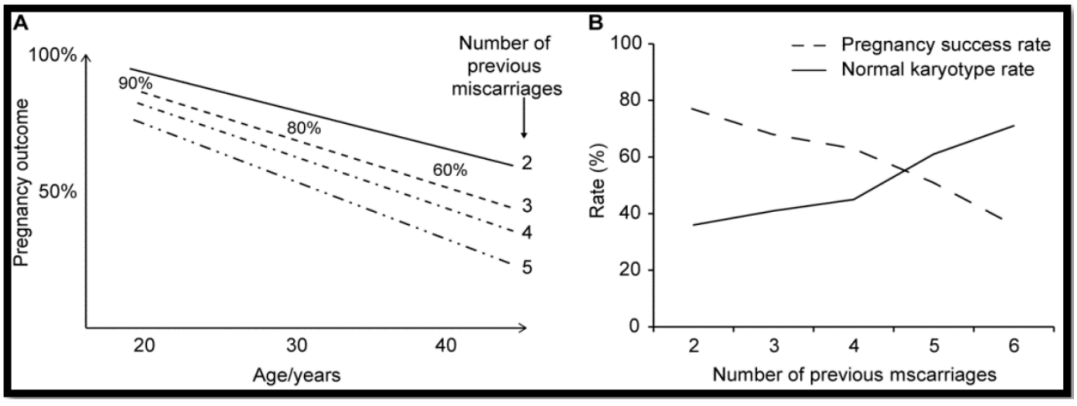

Fig. 1. Rate of pregnancy success in women experiencing recurrent miscarriages

(A) Pregnancy outcome depending on the maternal age in women with 2 or more miscarriages (adapted from Matthiesen et al. 2012) [16]

(B) Pregnancy success and the chance of observing fetal normal karyotype in women with 2 or more miscarriages and with average age of 31 years [17]

process in somatic and germ cells it is prone to errors. Such errors can lead to chromosomal aberrations and are major cause of morbidity and mortality. The mortality is usually due to various congenital abnormalities in the fetus or serious enough to cause spontaneous abortion. The morbidity can be in the form of mental retardation or a serious handicap or infertility. From the perspective of chromosomal constitution of an individual, cell division occurring in gametogenesis is important [20].

Changes that affect the structure of chromosomes can cause problems with growth, development, and function of the body's systems. These changes can affect many genes along the chromosome and disrupt the proteins made from those genes.

Structural changes can occur during the formation of egg or sperm cells, in early fetal development, or in any cell after birth. Pieces of DNA can be rearranged within one chromosome or transferred between two or more chromosomes. The effects of structural changes depend on their size and location, and whether any genetic material is gained or lost. Some changes cause medical problems, while others may have no effect on a person's health.

Chromosomal abnormalities account for about 50% of first trimester losses. There may be abnormality in the total chromosome number i.e. aneuploidy or structure. Aneuploidies may involve autosomes or sex chromosomes such as monosomy X, trisomy, triploidy, tetraploidy etc. pregnancy with trisomy are commonly associated with advanced maternal age. Trisomy 16 is the common aneuploidy in spontaneous abortions followed by trisomy 22 and trisomy 21 i.e. Down syndrome.

Cytogenetic abnormalities can be subdivided into structural chromosomal abnormalities, numerical chromosomal abnormalities and other mechanisms, such as mosaicism.

4.1.1 Structural chromosomal abnormalities

Structural chromosome abnormalities can be subdivided into deletions, translocations, inversions and duplications, but only translocations and inversions play a role in miscarriage and recurrent miscarriage [21].

Structural chromosome abnormalities results from chromosome breakage with subsequent reunion in a different configuration. About half of the structural abnormalities are inherited. These subsequent reunion configurations may be balanced or unbalanced.

The frequency of presence of at least one partner, who is a carrier of a structural chromosome abnormalities, varies from 3% to 11% among couples with a history of recurrent miscarriage [22].

Structural chromosomal abnormalities accounts for only 1-5% of all abortuses but a much higher proportion of abortuses that are recurrent. Phenotypic consequences depend on the specific duplicated or deficient chromosomal segments.

4.1.1.1 Translocations

Among couples experiencing recurrent miscarriage, the most common structural rearrangement is a translocation. In translocations, there is no loss of genetic material. Translocation is a type of chromosomal abnormality in which a chromosome breaks and a portion of it reattaches to a different chromosome. In translocation, the size of the chromosomal segment involved the frequency of the breakpoints and their positions have a vital role in reproduction. These translocations occur during meiosis and results in errors during conception. Hence carriers of balanced chromosomal translocations are phenotypically normal.

The incidence of translocation carriers in couples facing recurrent miscarriages were found to be 8.8% in a study conducted by Karaman and Ulug, 2013 [23].

Farcas et al. 2007 studied a total of 260 couples with history of repeated abortions. The age of the wives ranged from 20 to 43 years. The number of previous abortions varied from 2 to 10 abortions. The overall incidence of the translocations was 2.88 %, with 6 Robertsonian translocations (1.15%) and 9 balanced translocations (1.73%). The prevalence of translocations in males was 1.53% and in females was 4.23%. These abnormalities included 9 balanced translocations and 6 Robertsonian translocations. Among cases with abnormal karyotypes, having a translocation, the mean maternal age was 30.4 and the mean paternal age was 32.3. The mean number of abortions was 2.5 per couple [24].

Balanced translocations are known as products that erase as a consequence of meiotic recombination event between related chromosomes without loss of any chromosomal material (Fig. 2). The phenotype of the balanced translocation carriers is usually normal and may pass through several generations without detection; because of this situation they do not come to medical attention until they experience infertility or the birth of an abnormal child with an unbalanced chromosomal rearrangements or recurrent miscarriages.

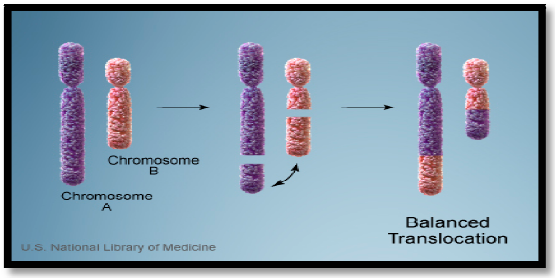

Fig. 2. Representation of balanced translocation (image adapted from genetics home references) [25]

Carriers of balanced translocations have a high reproductive risk of conceiving chromosomally abnormal embryos as a result of chromosomal imbalances that take place during meiosis, leading to recurrent miscarriages or to birth of affected offspring. The translocated chromosomes of balanced translocation carriers, pair with their matching homologous at a quadrivalent formation and imbalanced gametes result from the disjunction of these chromosomes for the segregation models at meiosis I [26].

When a parent carries a balanced chromosome abnormality, the chance of having a live birth with an unbalanced chromosome complement is usually about 1% to 15%. The exact risk depends on the specific chromosomes involved, size of the segment involved in the rearrangement, genes contained in the segment, sex of the transmitting parent, family history, and mode of ascertainment. It is estimated that the medium risk is 12% if the translocation is present in the female and 5% if it is present in males [24].

Balanced translocation carriers account for 0.08–0.3% of the normal population [27] 0.6% in infertile couples and 9.2% in cases who have recurrent miscarriages [26]. The most common chromosomal rearrangement is balanced reciprocal or Robertsonian translocation which may lead to unbalanced gene translocations in the fetus, resulting in miscarriage.

4.1.1.1.1 Reciprocal translocations

Reciprocal translocations are usually an exchange of material between non-homologous chromosomes. The resulting chromosome is called as the derivative chromosome. The length of the exchanged segment may vary from a distal segment to the whole chromosome arm with breakpoints at the centromere. Reciprocal translocations can have two or more breakpoints or can be more complex rearrangement. In general population, the frequency of reciprocal translocation in is 1 in 500 persons [28].

Balanced reciprocal translocations are usually inherited from parents. They have no phenotypic effect on individuals. The carriers of balanced chromosomal translocation are healthy individuals as there is no loss or gain of genetic material. Depending upon the nature of translocation and involved chromosomes it can cause abnormal pregnancy outcome i.e. birth defects in gamete or can cause pre-implantation and post-implantation losses [29].

In order to match the homologous segments pachytene configurations are formed during meiosis I and segregation of these occurs in four different modes. Depending upon the mode of segregation there are 16 possible combinations. First is 2:2 segregation which can be alternate or adjacent. In this type of segregation, two centromeres go to one gamete. Only 2:2 segregation mode results in balanced offspring or normal offspring. Second is 3:1 segregation in which three centromeres go to one gamete and one centromere goes to another gamete. In 4:0 segregation four centromeres go to one gamete and none of the centromere goes to another gamete. Both 3:1 segregation and 4:0 segregation modes are results in aneuploidy during conception which results in miscarriage (Fig. 3). The carriers of balanced translocations are at risk of repeated pregnancy loss, malformed child or infertility [28].

The prevalence of balanced translocation is higher in females than males, and higher still if there is a family history of a still born or abnormal live born [30]. The proposed mechanism contributing to a higher incidence of female translocation carriers is that in female carriers only one ovum matures each month. If the ovum carries an unbalanced translocation, its

fertilization will produce an abnormal zygote, most probably leading to spontaneous miscarriage. On the other hand, male carriers release millions of sperms in every ejaculation. Thus, even when gametes with an unbalanced translocation are present, they will only infrequently fertilize the ovum to produce abnormal zygotes. Thus, in males, pre-zygotic selection against unbalanced gametes may be more effective. However, there remains the possibility of the male translocation carrier contributing to recurrent pregnancy loss and therefore, chromosome study of the male should also be undertaken in such cases [27].

Although reciprocal translocations are balanced rearrangements, they are important for the offspring of carriers that have increased risk of chromosomal imbalance during gametogenesis due to unequal meiotic segregation. When one of the parents is a carrier of a balanced reciprocal translocation, a pregnancy can result in three types of offspring: a child with a normal karyotype, a child with a balanced reciprocal translocation, or a conceptus with an unbalanced karyotype that may lead to spontaneous miscarriage or live born child with malformations and mental retardation [22].

4.1.1.1.2 Robertsonian translocations

Robertsonian translocations (rob) are the most common balanced chromosomal rearrangements with an incidence of 1:1000 in general population [22,31]. Robertsonian translocations originate from the centromeric fusion of the long arm of acrocentric chromosomes either two different D group chromosomes (chromosome 13, 14 and 15) or two different G group chromosomes (21 and 22) or a D group and a G group chromosome. Usually there is a simultaneous loss of both short arms. Due to the loss of short arms which usually contains redundant DNA, the carriers have a balanced chromosomal constitution with 45 chromosomes. Carriers of Robertsonian translocations are usually phenotypically normal but often produce unbalanced gametes and have an increased risk of recurrent miscarriages.

The most common Robertsonian translocations is between chromosome 13 and 14. This D/D translocation makes up about 75% of all Robertsonian [31]. The risk of miscarriage is low in carriers of Robertsonian translocation [28]. Most of the Robertsonian translocations are inherited. The most frequent Robertsonian translocation is t(13;14). Robertsonian translocations, which are compatible with fertility in women, may be associated with sterility in men [32].

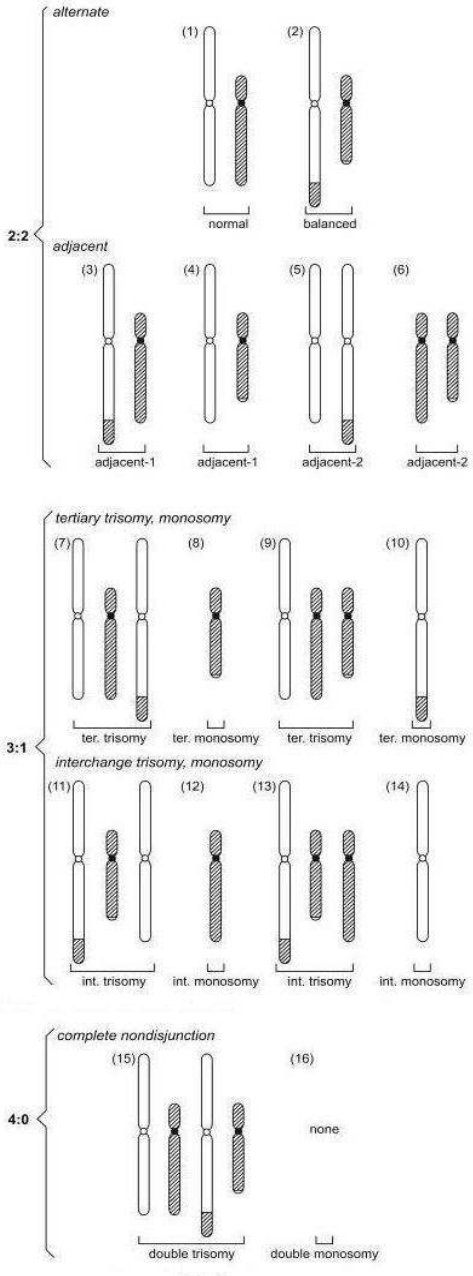

Fig. 3. Mode of segregation in a balanced translocation carrier (Total=16), 2:2 (alternate and adjacent), 3:1 and 4:0 depending upon the total number of chromosome going to one gamete. Only 2:2 alternate segregation is viable rest all results in malsegregation [31]

An unbalanced translocation occurs when a child inherits a chromosome with extra or missing genetic material from a parent with a balanced translocation (Fig. 4). The clinical consequences are very severe to the developing embryo which results in mental retardation, physical problems also recurrent miscarriages [5].

The risk of miscarriages in couples with balanced reciprocal translocation is approx 25%–50%, and with Robertsonian translocation it is approx 25%. Therefore all the couples with balanced reciprocal translocation should be strongly advised to monitor their future pregnancies by prenatal diagnosis to exclude the possibility of a chromosomally unbalanced zygote [5].

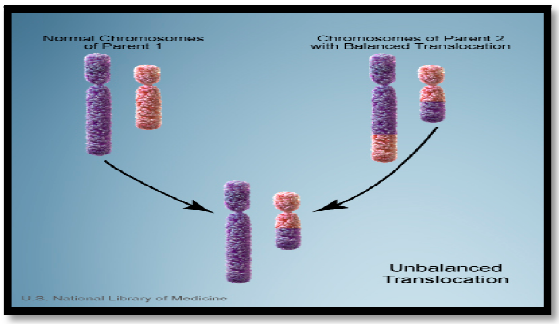

Fig. 4. Representation of unbalanced translocation (image adapted from genetics home references) [25]

Other chromosomal anomalies associated with recurrent miscarriage include chromosomal inversion, insertions and Mosaicism.

4.1.1.2 Inversions

Inversions(inv) is a chromosomal rearrangement when a segment of a chromosome between two breakpoints is inverted 180 degrees and reintegrated into the same chromosome. Inversions are of two types: paracentric (not including the centromere), in which both breaks occur in one arm, and pericentric (including the centromere), in which there is a break in each arm.

The key difference between euchromatic and heterochromatic inversions is in their respective ability to cause an abnormal phenotype. On the one hand, rearrangements of the heterochromatin represent variants of the normal phenotype, and they are never associated with phenotypic aberrations or an increased genetic risk. Euchromatic inversions are occur in genetically relevant segments of the chromosome and can thus cause phenotypic abnormalities if the breakpoints disrupt a gene or if they occur in an unbalanced form [33].

Inversions are most likely to occur during meiosis. Chances of missing sub-microscopic deletions and duplications are high in low-resolution karyotype analysis. The rearrangement may be undetected unless whole chromosome morphology is changed and critical landmark bands are shifted. Because of the high risk of recombinants for carriers of insertions, every attempt should be made to differentiate inversions from insertions.

4.1.1.2.1 Pericentric inversion

In Pericentric inversion, the inverted segment of the chromosome involves the centromeric region. It is the most frequent chromosomal rearrangement in humans with the frequency of 1.0-3.0% in the normal population [34].

Balanced inversions do not have a phenotypic effect in the majority of cases, however miscarriages and/or chromosomally unbalanced gametes can be observed in such cases [35]. The genetic risk of inversion carriers depends on the size of inverted segments, and only inversions of length more than 100Mbp would have a significant effect on fertility.

An inversion does not usually cause an abnormal phenotype in carriers, because it is a balanced rearrangement. Its medical significance is for the progeny; a carrier of either type of inversion is at risk of producing abnormal gametes that may lead to unbalanced offspring. When an inversion is present, during meiosis I, a loop will be formed. Although recombination is somewhat suppressed within inversion loops, when it occurs it can lead to production of unbalanced gametes. A pericentric inversion, on the other hand, can lead to the production of unbalanced gametes with both duplication and deficiency of chromosome segments [36,37].

4.1.1.2.2 Paracentric inversion

In paracentric inversion the inverted segment of the chromosome does not involve the centromeric region. These unbalanced chromosomes are produced by crossover. Paracentric inversion may occur in all chromosomes. About 90% of paracentric inversions are inherited, and others are of de

novo origin. Incidence of paracentric inversion in spontaneous abortions is 11.4% and 0.1-0.5% in normal population [38].

Paracentric inversions have been recognized in all chromosomes. The chromosomes most commonly reported to have paracentric inversions (>5% of the total) are chromosomes 1, 3, 5, 6, 7, 11, and 14. Less frequently identified chromosomes are chromosomes 4, 16, 17, 18, 19, 20, 21, 22, and Y [38].

Possibility of misinterpretation or detection of paracentric inversions is there as other chromosomal rearrangements in some cases because changes in the banding pattern of some chromosomes may be not easy to distinguish. Also chances of misinterpretation possible due to the similarity of the banding pattern in the inverted segment.

4.1.2 Numerical chromosomal abnormalities

Numerical chromosomal abnormalities are a type of chromosome abnormality caused by a failure of chromosome division resulting in loss or gain of chromosome. Instead of 46 chromosomes there may be 45 or 47 chromosomes which may cause health problems or birth defects such as Down syndrome (who have 47 chromosomes instead of 46), or Turner syndrome (45 chromosomes).

4.1.2.1 Sex chromosomal mosaicism

Low level of sex chromosomal mosaicism has been reported in couples with recurrent miscarriages. Gonclaves et al. [39] reported X chromosome mosaicism in 7 (4.7%) cases out of 151 women's with recurrent miscarriages.

Mozdarani et al. [35] studied a total of 221 individuals with three or more recurrent spontaneous abortions and at least three IVF/ICSI failures and found sex chromosomal abnormalities in 4 cases (1.8%).

Infertility can occasionally occur in male carriers of balanced translocations due to spermatogenetic arrest. Infertility in female carriers is rare as oogenesis is more robust process. The rearrangements may promote mitotic malsegregation or disrupt a tumor suppressor gene, and thus predispose to the development of cancer [28].

4.1.3 Structural chromosome variations

Chromosomal changes include normal polymorphic variants in addition to major chromosomal abnormalities. The term variant has been recommended for use in situations where deviations from the norm of chromosome morphology are observed (Paris Conference 1971) whereas in a supplement of the Paris Conference (1975) the term heteromorphism has been recommended to describe the chromosomes with variable bands [40]. With the advent of new banding techniques, a more specific and detailed characterization of the already known variants, as well as new variants has become much easier [41]. The term heteromorphism is used synonymously with polymorphism or normal variant. Common cytogenetic polymorphisms detected by G-banding are considered as heteromorphism and include heterochromatin regions of chromosomes 1, 9, 16 and Y and also prominent acrocentric short arms, satellites and stalks [42].

Variations of the heterochromatic regions are individually stable and frequent in the normal population. Most polymorphic variants are familial and follow Mendalian inheritance from one generation to other with a low mutation rate [41]. De novo polymorphic chromosomal variants are rarer and appear, possibly as a result of an unequal crossover between heterochromatic regions of homologous chromosomes in meiosis. It is possible due to conjugation of repeated DNA sequences. De novo heterochromatic variants are considered to be large in size and to be associated with clinical conditions.

Madon et al. [43] reported the increased frequency of variants in association with different clinical conditions such as reproductive failure, recurrent spontaneous abortions and even psychiatric disorders.

- **Variants of Chromosome 1 -** The polymorphisms of 1qh have been reported in the relationship with foetal wastage, recurrent miscarriage or malignant diseases by some authors. In inversion, inverted segment may cause synapsis failure, including asynapsis or early desynapsis, and pairing abnormalities of homolog's leading to male infertility [41]. In general, inversions of heterochromatic regions are considered not to cause phenotypic abnormalities.

- **Variants of Chromosome 9-** It is most frequent heterochromatic variant. The variant 9qh+ has been found in the association with repeated spontaneous abortions and malformed stillborn infants in some studies [18]. It has been found more frequently (8%) in children with de novo major chromosomal abnormalities than in normal newborns (0.04%) [41]. It is suggested that 9qh+ play significant roles in chromosomal non-disjunction. Large heterochromatic blocks may cause chromosome impairment and meiotic arrest resulting in infertility. Nevertheless, other studies have not found significant differences in polymorphic variants of chromosome 9 between patients and controls [41].

  The most common inversion seen in human chromosomes is a small pericentric inversion of chromosome 9, which is present in 1-3% in the general population [37].

  Pericentric inversion 9, especially complete inv(9)(p11q13) has been reported in association with reproductive failure. Inversion 9 has been considered to play significant role in chromosomal non-disjunction, and have variable effects on spermatogenesis, from azoospermia to severely altered sperm morphology, motility and meiotic segregation. In chromosome with inversion, a loop will be formed during meiosis I that can lead to production of abnormal and unbalanced gametes. Carriers of such inversion are at risk of having an offspring with unbalanced karyotype [44].
- **Variants of Chromosome 16-** In infertile males the incidence of variant 16qh+ varies from 0.9% to 1.9% (0-6 % in normal population) [41,45]. Although the incidence of 16qh- and the pericentric inversion, inv(16)(p11q11) varied in large (0.04%-23.6% and 1.4% respectively), they have not been found in infertile men [41].
- **Variants of Y chromosome** - The Y chromosome shows a wide range of variation not only between individuals but also between different populations groups. The data about clinical significance including fertility of polymorphisms of the Y chromosome are still controversial. Variant Yqh+ has been reported in association with reproductive failure.

4.2 Incidence of Chromosomal Anomalies in Recurrent Miscarriages

Genetic abnormalities can lead to impaired reproductive function in adults, cause early fetal loss, genetic diseases or even death in offspring**.** 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. The frequency of miscarriage prior to the 20th week of gestation is 15% [46].

In most healthy pregnancies, implantation usually occurs after 8-10 days of ovulation. When implantation is later than this period the ratio of early pregnancy loss is increases. Chromosomal abnormalities in the conceptus are usually the characteristic findings in cases of spontaneous miscarriages which are occurring due to problems with the pregnancy itself.

The rate of early miscarriage (<8 weeks gestation) may be even greater as some women may not recognize that they are pregnant, and the miscarriage is simply thought of as a late menstrual period. With the increasing use of home pregnancy tests, early miscarriages are now being recognized more frequently, resulting in more couples seeking evaluations for recurrent miscarriage.

Chromosomal abnormalities occur in about 50% of all products of conception from first trimester miscarriages, 5% of late pregnancy losses and 0.5% of live births [46].

Most of the chromosomal abnormalities leading to pregnancy wastage arise from errors during formation of the germ cells at gametogenesis – during meiosis or post zygotically as an error in mitotic cell division in the cleavage stage. Thus the etiology of chromosomal variability resulting in inherited chromosomal disorders is at meiosis, mitosis or genomic imprinting [31,47].

In patients with normal karyotype there are two major predictive factors of miscarriage such as number of miscarriages and maternal age. Clinical studies have shown that, women with previous pregnancy losses have a higher risk (25%) of pregnancy loss than women with previous successful pregnancies (5%). The risk of losing the next pregnancy is increases with the increasing number of previous miscarriages [12,48].

Kupka et al. found a miscarriage rate of 21% in couples with no previous miscarriage, compared with 27% with a single previous loss, and 31% with three previous losses (adapted from Garrisi et al. [12]). It is not strange for perfectly healthy couples to experience three consecutive spontaneous pregnancy losses, each for a different reason and it has been seen that more than half of recurrent miscarriages are due to non-recurrent causes. Determining the cause of recurrent spontaneous abortion are extremely difficult [48].

It has been reported that the frequency of chromosomal abnormalities in first trimester miscarriages is 50%-80% [22]. In 4% of couples suffering from recurrent miscarriage, changes in the karyotype including balanced reciprocal translocations, Robertsonian-translocations, gonosomal mosaic and inversions are found, compared to 0.2% within control couples [19].

When a history of repetitive abortions, malformative syndrome, or mental retardation is found in the family of one of the two parents, the risk of finding a structural chromosomal anomaly is significantly higher. If such rearrangements are present the chromosomes have difficulty in pairing up and dividing evenly during meiosis. Especially this is due to the fact that carriers of BRT have a risk of partial trisomy or partial monosomy for chromosomal regions involved in the translocation due to meiotic segregation [5].

In addition to clinical, environmental, and life-style risk factors, there is growing evidence that recurrent miscarriage has also genetic susceptibility. A review of initial observations indicated two to sevenfold increased prevalence of recurrent miscarriage among first-degree blood relatives compared to the background population. Population-based register studies showed that overall frequency of miscarriage among the siblings of idiopathic recurrent miscarriage is approximately doubled compared to general population [49].

In a study by Dutta et al. [5] chromosomal abnormalities in 1162 couples with recurrent miscarriage were studied. Chromosomal abnormalities were found in 78 cases i.e.3.35% cases. Among these 33 cases showed structural abnormalities were 1.41% and 44 cases showed normal polymorphic variation 1.89%. One case of numerical anomaly was also seen. Majority of abnormalities were balanced reciprocal translocations i.e. 21 cases.

Chromosomal changes including chromosomal abnormalities and polymorphic variants have been found in 2-12.5% of infertile couples [5,18,50-52]. Major chromosomal abnormalities are detested in 3.1 -7.6% of infertile couples being higher in male partners [35,53,54]. Chromosomal polymorphic variants have been found in 8.7-58.7% of infertile male and 7.3-28.3% of infertile female partners versus 32.6% and 15.2% of fertile individuals respectively [36,43].

Table 2. Incidence of chromosomal abnormalities in couples with recurrent miscarriage

Author and year	No. of couples studied	Total abnormality (Percentage)
Sider et al. (1988) [55]	232	8%
Al Hussain et al. (2000) [56]	193	7.70%
Dubey et al. (2005) [18]	742	2%
Firozabadi et al. (2006) [52]	165	12.50%
Stephenson and Sierra (2006) [57]	1893	2.7%
Cortes et al. (2009) [58]	158	7.60%
Pal S (2009) [59]	56	8.90%
Dutta wt al. (2010) [5]	1162	3.35%
Dahtory et al. (2011) [60]	73	6.10%
Durovic et al. (2012) [61]	107	9%
AL-Hassanee et al. (2012) [62]	50	6%
Khedker et al. (2012) [50]	50	6%
Shekoohi et al. (2013) [51]	68	8.3%
Rajasekhar et al. (2013) [63]	210	8.57%
Karman et al. (2013) [26]	158	8.22%
Gonclaves et al. (2014) [39]	151	9.4%
Gaboon et al. (2015) [64]	125	6.4%
Ghazaey et al. (2015) [65]	728	11.7%

Numerical chromosomal abnormalities, primarily trisomy of gonosomes and chromosomes 13,16,18,21 are the major cause of pregnancy loss and may be found in 21% spontaneous abortions [66]. The main factor that causes numerical chromosomal abnormalities is maternal age. Munne 2002 showed that the frequency of trisomy detected in amniocentesis was increased from 0.6% to 2.2% in females aged 35 to 40 years [66].

Chromosomal abnormalities, mainly balanced chromosomal abnormalities, are common in couples with recurrent miscarriages. Almost 15–20% of all clinically recognized pregnancies end up as recurrent miscarriages, out of which the contribution of chromosomal abnormalities is as high as 70%. Parental chromosomal abnormalities represent an important etiology of recurrent miscarriage; studies published elsewhere have shown a prevalence of chromosomal anomalies that varies from 2% to 8% of couples who are affected by recurrent miscarriage [5].

4.2.1 Indian scenario

Having a baby is a life changing process for the couples in Indian culture. There is no reliable estimate on the rate of abortions in India, as the registration of marriages, births and deaths are usually not complete. As mentioned earlier causes of recurrent miscarriages is a traumatic experience affecting the couples physically and emotionally.

More than ¾ population of India's contribution to the fertility determined in the age group of 15-29 years. In total 17% of fertility determines the fertility status which depends upon early child bearing by the women in the age group of 15-19 years. Obstetric behaviour and outcome of pregnancy study was carried out in teenage mothers [67]. The major obstacles in outcome of pregnancy carried out in a study are Anemia (27.5%), intrauterine growth retardation (27.5%) and hypertension (15%). Were as compared to the controls with normal outcome the ratio is 11.2%, 8.7%, and 8.7% respectively.

Serum folic acid level with recurrent miscarriage and its association with vitamin A level have been studied [68]. And various studies have been carried out on maternal infection of Toxoplasma Gondii and its relation to reproductive disorders [69].

Jalan suggests that in Indian the abortus material is not always available for chromosomal studies. Chromosomal analysis of abortus materials would be more informative to know the cause of miscarriage. The ratio is 40-50% and its comparison to chromosomal analysis to couple the abnormality was not beyond 6%. Half of the first-trimester miscarriages are caused by fetal chromosome abnormalities diagnosed by conventional techniques [70]. The overall incidence of chromosomal abnormalities indicates that chromosomal analysis of the couples with recurrent miscarriage should be essentially considered. A chromosomal anomaly finding in either of the parent can make it possible to evaluate the prognosis of future pregnancies.

4.3 Chromosome Anomalies vs Number of Pregnancy Losses and Male Female Distribution of Abnormality

Various studies on repeated pregnancy losses showed mainly translocations and inversions as major chromosomal changes. The translocations observed are mainly reciprocal translocation or Robertsonian translocations.

A study conducted by Mau et al. [36] with the 150 couples referred for genetic counselling prior to intracytoplsmic sperm injection showed the distribution of chromosomal abnormalities as follows:

Table 3. Distribution of chromosomal abnormalities showed by Mau et al. [36]

	Male	Female	Total
Total no. of individuals	150	150	300
Reciprocal translocations	4	2	6
Robertsonian translocation	4	Nil	4
Inversion	2	1	3
Sex chromosomal anomalies	7	6	13
Marker chromosome	1	Nil	1

Regarding the occurrence of incidence of structural chromosomal rearrangements in couples with two or more pregnancy losses, the rate of chromosomal abnormalities were 2-12.50% [5,18,50-52]. The chromosomal aberrations were high (17.39%) in couples with more than three abortions than that of those with two abortions (2%) [18,60].

Kochhar and Ghosh [27] studied 788 individuals with recurrent pregnancy loss and studied distribution of chromosomal anomalies among them. Chromosomal rearrangements were identified in 6.8% (54/788) cases including 5.9% reciprocal translocations, 0.7% Robertsonian translocations, and 0.1% inversions.

The study by Kochhar and Ghosh was divided into four groups as follows;

Group I- with two consecutive pregnancy losses
Group II- with three consecutive pregnancy losses
Group III- with four consecutive pregnancy losses
Group IV- with five or more consecutive pregnancy losses

- Group I consisted of 152 cases with two consecutive pregnancy losses. Chromosomal abnormalities were seen in 12 cases with the percentage 7.9%.
- Group II comprising 349 cases with three consecutive pregnancy losses, 23 (6.6%) cases were found with chromosomal abnormalities.
- Group III consisted of 236 cases with chromosomal abnormalities in 4 (7.8%) cases.
- Group IV consisted of 788 cases with 5 or more than 5 pregnancy losses and 54 cases found to have chromosomal abnormalities (6.85%).

Al Hussain et al. [56] studied a total of 193 Saudi couples with history of repeated abortions. The female age ranged from 16 to 50 years, with a mean of 30.31 years. The number of previous deliveries ranged from 0 to 13. The number of previous abortions varied from 2 to 16 abortions. Abnormal karyotype was found to be in eleven females (5.7%) and four males (2.07%). These abnormalities included 10 balanced reciprocal translocations, one Robertsonian translocation, two inversions and two cases of mosaic X-chromosome monosomy.

A study by Sider et al. [55] done a chromosome analysis of 232 couples with a history of two more pregnancy losses and found chromosome abnormality in 8% of cases (19/232). The study consisted of four groups.

- The first group with two losses consists of 99 couples with the incidence of chromosome abnormality 6% i.e. 6 cases.
- The second group with three miscarriages consists of 88 couples and 9 chromosomal abnormalities were observed (10.2%).
- The third group consists of 27 couples with the history of four miscarriages and found 2 abnormalities. The incidence rate is 7.4%.
- The last group consisted of 18 couples with five or more than five miscarriages and found two chromosomal abnormalities (11.1%).

Table 4. List of abnormal karyotype with the no of abortions, parity and maternal age (Al Hussain et al. [56])

Sr. no.	Karyotype	Parity	No. of abortions	Maternal age
1	46,XX,t(7;8)(p15;q23)	1	4	28
2	46,XY,t(5;12)(q11;p13)	1	10	30
3	46,XX,t(4;17)(p16,q24)	0	5	28
4	46,XY,t(2;11)(q24;q32)	0	3	27
5	46,XY,t(5;7)(p11;q11)	3	8	32
6	46,XX,t(7;11)(p13;q24)	1	3	27
7	46,XX,t(10;14)(p13;q14)	1	7	32
8	46,XX,t(1;3)(q22;q23)	2	3	23
9	45,XX,t(13;14)(p11;q11)	0	9	26
10	46,XY,t(2;6)(p13;p21.3)	2	9	39
11	46,XX,inv(8)(p12;p23)	0	3	19
12	46,XX[113]/45,X[4]	0	6	26
13	46,XX,t(6;11)(q23;q22)	2	4	25
14	46,XX[210]/45,X[7]	8	5	42
15	46,XY,inv(4)(p14q31.3)	1	7	28

Hahn and Kim, [71] conducted a cytogenetic study done on 18 couples with the history of habitual abortions. The maternal age ranges from 23 and 32 where paternal age was between 29 and 36. The incidence of chromosomal abnormality was 14%. The study was divided into four groups:

- Group I consisted of 3 couples with 2 miscarriages and no abnormality found in this group.
- Group II consist of 11 couples with 3 miscarriages and found two abnormalities and all were females.
- Group III consisted of 3 couples with 4 miscarriages and abnormality was found in one male and one female each.
- Group IV consisted of single couple with 5 miscarriages and found abnormality in male partner.

Mustaqahamed et al. [72] studied 30 couples with repeated pregnancy losses. Chromosome abnormalities were found in 14 subjects including 9 males and 5 females. The incidence of chromosomal abnormality was 23%.

- Group I with single miscarriage consists of 8 couples and chromosomal abnormality was found in one male and two females.
- Group II consist of 12 couples two miscarriages and chromosomal abnormality was found in five male partners.
- Group III consisted of 6 couples with history of three miscarriages and chromosomal abnormality was found in three males and one female.
- Group IV and V consist of 2 couples each with 4 and 5 miscarriages. Chromosomal abnormality was found in one female each.

Gaboon et al. [64] studied 125 couples with couples facing recurrent miscarriages. The study was divided in four groups:

- Group I consists of 23 couples with a history of 2 miscarriages. The chromosomal abnormality was found in single case (4.4%).
- Group II consist of 33 couples with history of 3 miscarriages. The chromosomal abnormality was found in 2 cases (6%)
- Group III consist of 24 couples with history of 4 miscarriages. The chromosomal abnormality was found in 2 cases (8.3%)
- Group IV consists of 45 couples with history of 5 or more miscarriages. The chromosomal abnormality was found in 3 cases (13%).

Gonclaves et al. [39] studied 151 couples with history of repeated pregnancy loss. The study was divided into 6 groups:

- Group I consists of 72 couples with history of 2 miscarriages. The incidence of chromosomal abnormality was 47.6%
- Group II consists of 57 couples with history of 3 miscarriages. The incidence of chromosomal abnormality was 37.7%
- Group III consists of 11 couples with history of 4 miscarriages. The incidence of chromosomal abnormality was 7.2%
- Group IV consists of 6 couples with history of 5 miscarriages. The incidence of chromosomal abnormality was 3.9%
- Group IV consists of 4 couples with history of 6 miscarriages. The incidence of chromosomal abnormality was 2.6%
- Group VI consists of single couples with history of 7 miscarriages. The incidence of chromosomal abnormality was 0.6%

Ghazaey et al. [65] studied 728 couples with the history of recurrent spontaneous abortion. The overall incidence of chromosomal abnormality was 11.7%. Balanced reciprocal translocations were the most frequent chromosomal abnormality found in the study (62.7%). Robertsonia translocations were found in 7 cases, inversions in 21 cases and markers were seen in 19 cases. The study was divided into 5 groups:

- Group I consist of 106 couples with single miscarriage and found chromosomal abnormality in 5 cases with the incidence of 4.7%.
- Group II consist of 376 couples with two miscarriages and found chromosomal abnormality in 41 cases with the incidence of 11%.
- Group III consist of 153 couples with three miscarriages and found chromosomal abnormality in 21 cases with the incidence of 15%.
- Group IV consist of 60 couples with four miscarriages and found chromosomal abnormality in 10 cases with the incidence of 15%.
- Group V consist of 33 couples with five and more miscarriages and found

chromosomal abnormality in 8 cases with the incidence of 21.2%.

4.4 Fetal Aneuploidy in Recurrent Miscarriages

The remarkable incompetence of human reproduction is basically the result of fetal aneuploidy. Overall, 50% –60% of fetal tissues from miscarriage material shows some type of cytogenetic abnormality. The common chromosomal abnormalities found are monosomy X, trisomy and polyploidy such as triploidy and tetraploidy [73]. Most trisomies show a maternal age effect, with the involvement of common chromosomes such as chromosome 16 and 22.

Triploid fetuses usually have a chromosomal pattern of 69,XXY or 69,XXX. It is result of dispermic fertilization. Some triploid fetuses present as a partial mole which is characterized by a large gestational sac and cystic degeneration of the placenta. Fetuses with tetraploidy karyotype pattern usually get miscarried around 4 or 5 weeks of gestation. Monosomy X is the single most common chromosomal abnormality among spontaneous abortions, accounting for 15% to 20% of all abortions.

Chromosomal abnormalities are less likely to occur in spontaneous abortions for women younger than age 36 with a history of recurrent abortion. Numeric chromosomal abnormalities, however, might be involved in both recurrent and sporadic losses. Couples who are predisposed toward chromosomal abnormal conceptions will also be at increased risk for aneuploid live-born infants. In fact, women with a previous trisomy 18 or 21 pregnancy have an increased risk for a subsequent affected fetus. Data from pre-implantation embryos support the concept of recurrent aneuploidy in women with recurrent abortion [9].

Results from germ cell development error affects couples pregnancies with the history of recurrent miscarriage or without miscarriage equally. In couples with normal karyotype, the aneuploidies results from the meiotic non-disjunction in the germ cells. The recurrences of a particular chromosomal abnormality in next pregnancies are rare in couples with recurrent miscarriages and in general population. In couples with recurrent miscarriage, the prognosis is better in aneuploid miscarriage than after euploid miscarriage [30]. The type of chromosomal abnormalities and its frequency in abortus material changes with the gestation age at the time of miscarriage and maternal age.

The frequency of parental chromosomal abnormalities is higher in couples with a recurrent miscarriage history (2% –5%) than in the general population (0.2%) [74].

5. CONCLUSION

While increased incidence of aneuploidy as compare to control in embryos from women with recurrent miscarriage, the frequency of chromosomal abnormalities in products of conception from women recurrent miscarriage cases is lower than sporadic miscarriage. This suggests that aetiologies other than cytogenetic study occur more frequently in women with recurrent miscarriage than with sporadic miscarriage.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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