CHAPTER – I

INTRODUCTION
Human chromosomes were observed during the late nineteenth century, where the behavior of chromosomes was investigated rather than their number, morphology, and relation to cell lineage. In the 1890s, with the emergence of the chromosome theory of heredity, cytologists began to pay attention to the chromosome number. However, from 1890s to 1920s, reports on human chromosome number varied from 8 to over 50, most frequent counts being 24 for the diploid cells and 12 for the haploid cells.

Hans von Winiwarter was the first one to insist on the side-by-side pairing of chromosomes in meiosis. He reported 47 chromosomes in human spermatogonia and 48 in oogonia (Winiwarter 1912). His conclusion on man and his sex determination mechanism was XX/XO.

The discrepancy between Winiwarter's count of 48/47 and the prevailing belief of 24 caused speculation. T.S. Painter 1922 dispelled many doubts by ascertaining the diploid number of man as 48 or 46. He later revised
his opinion from 46 to 48 in 1923 and determined the sex
of man as XX/XY.

It was Tijio and Levan who were unable to find
the expected 48 chromosomes instead they observed 46,
in 1955. In 1956 they observed and concluded from a
lung tissues of four fetuses the diploid number of man
as 46. Charles Ford and John Hamerton 1956, in the
spermatocytes of three patients observed 23 bivalents
instead of 24, and it become therefore obvious that the
chromosome number of man was 23 bivalent or 46.

In the past 2 decades the development and refine-
ment of cytogenetic techniques have contributed important
information on the chromosome basis of human reproduction.
Chromosome banding methods reveals the precise detection
of chromosome rearrangements and their parental contribu-
tion at conception.

Natural and man-made environmental hazards,
to which the human population is increasingly exposed,
may produce genetic damage, Sorsa 1986. Testing of
nuclear weapons to nuclear energy, medical uses of X-
rays both for diagnostic and therapeutic purposes, many
groups of chemically active substances used in the
chemical industry, in medicine and in agriculture are
the recognized sources of man-made hazards. Assessment
of these levels to genetic damage in human, male and
female level is of great importance, Zenzes et al. 1985.
PLATE 1. A NORMAL FEMALE KARYOTYPE. (CONVENTIONALLY STAINED). [950 magnification]

PLATE 2. A NORMAL MALE KARYOTYPE. (CONVENTIONALLY STAINED). [950 magnification]
Changes of number and structure at chromosome level is detectable by the banding methods. On the contrary if these changes occur at gametogenesis or fertilization, which, obviously are a great cause of human reproductive failure, are manifested in either sterility, low fertility and a very high rate of mortality among human conceptuses, Carr 1971, Jacobs 1972; Boue et al. 1975; Carr and Geddes 1977; Bond and Chandley 1983.

With advent of cytogenetics techniques it became possible to identify with great precision, certain structural chromosomal alterations which remained unrecognised previously such as small translocations, deletions, inversions, inapparent chromosomes, and many others. These techniques have helped in identifying more subtle chromosome disorders.

There is much interest in the origin of human chromosomal abnormalities because of their great frequency and importance as a cause in abortions, birth defects, infertility in male as well as females and mental retardation. In fact gross human chromosome abnormalities are not rare. More than 25% of human abortuses lost before eighth week of pregnancy have abnormal karyotypes.

Chromosome errors are known to be important causes of some human congenital anomalies, of most spontaneous abortions, and also of infertility but not
until the last few years have reliable estimates of their frequency been available. Ever since Lejeune et al. 1959 reported the first chromosome aberration in humans, after which an impressive number of investigations have been carried out showing the great complexity of the human karyotype. The investigations hence done were based on chromosome morphology and autoradiographic data.

One of the primary causes of chromosomal abnormalities particularly the numerical ones, is chromosomal non-disjunction. It is said to occur more commonly with advancing maternal age. Numerous ideas have been advanced to explain chromosomal non-disjunction in older mothers such as infection of oocyte with microorganisms, persistence of nucleolus in cell division, autoimmunity, radiation, deterioration of mitotic apparatus in oocyte, decreased number of synapsis in eggs released late in life and delayed fertilization of ovum.

There have been several studies performed regarding the aetiology of structural chromosomal aberrations as agents causing these chromosomal rearrangements are known. Irradiation, radioactive elements and many human viral infections are known to cause breakages and rearrangements in chromosomes. There has also been evaluation of drugs which induces breakages.
Chromosomal disorders can be grouped into:

(A) Autosomal chromosomal aberrations of both the types structural and numerical aneuploidy.

(B) Sex chromosomal aberration.

(C) Chromosome and cancer.

(D) Chromosome aberrations in infertility and subfertility in males.

(E) Chromosome aberrations in infertile females.

(E) Chromosomal aberrations in spontaneous abortions miscarriages and still birth.

(G) Radiation, viruses and chemical clastogens causing chromosome aberrations.

**Cytogenetic Basis for the Determination of Sex**

If nondisjunction occur during the meiotic production of the ova and spermatozoa the individual resulting from subsequent fertilization has an abnormal genetic endowment which is uniform throughout the body (eg, XXX). On the other hand, if non-disjunction occur in a zygote at a mitosis subsequent to fertilization, a mosaic pattern is formed in which cells of the individuals vary in genetic make up (eg. XX becomes XO/XX). These may be uniformly distributed in mosaic pattern throughout the body or apparently may be localized in areas or organs.

**Chromosomal Sex**

Sex is primarily determined at the moment the
Diagrammatic representation of reduction division of the ovum and spermatozoon before and after fertilization, showing normal distribution of autosomes (A) and sex chromosomes (SC).
ovum is fertilized, determined by the type of sex chromosome supplied by the spermatozoan. The chromosomal pattern, even the sex chromosomal pattern, varies in different animals but the nucleus of every cell of human body normally carries 46 chromosomes arranged in 23 pairs. One of these pairs, the sex chromosomes is mainly concerned with sex, the remaining 22 pairs are designated as autosomes. In the female the two sex chromosomes are similar, XX, the upper arms of X is much shorter than lower. In male they are dissimilar, XY, the Y being much smaller in structure than X.

Mature ovum carries 22 autosomes and one X sex chromosomes, on the other hand the mature spermatozoa, carry either an X or a Y chromosomes and therefore there are two types of sex chromosomes in male spermatozoa. If one type fertilizes the ovum it contributes an X chromosome which results a female zygote, whose karyotype is expressed as 46, XX, if the Y chromosome is contributed to the ovum the operative outcome is male with the karyotype of 46, XY.

During cell division the chromosomes normally splits longitudinally, one half of each going too each of two daughter cells. When cleavage does not take place the phenomenon is termed non-disjunction; this is not uncommon, especially during the first meiotic (reduction) division leading to the formation of a mature ovum or
Illustrations of normal and abnormal division of chromosomes

(A) across their centromere producing regular division;
(B) across their centromere producing isochromosomes.
spermatozoan. The result is an oocyte which may contain either two X chromosomes or, no sex chromosome at all, and a spermatocyte with either XY or no sex chromosomes. When fertilization takes place the outcomes which are possible are zygotes whose chromosome make-up is 45XO, 45YO, 47XXX and 47XXY when oogenesis is affected; and 45XO, 45XO, 47XXY and 47XXY if spermatogenesis is at fault.

Non-disjunction involves the ovum more frequently than the spermatozoan and are of its possible theoretical outcome an embryo with a chromosome pattern of 45YO, has never been found. This, it is assumed, is because such an arrangement is incompatible with the life of a cell or early embryo, Jeffcoate 1983.

During mitotic division of the fertilized ovum errors in the distribution of the chromosomes, even if their original complement is normal, can still occur. Non-disjunction is again possible or, if cleavage takes place, all the resulting products can pass to one daughter nucleus to give it a 48 XXXY or 48XXYY complement. This is known as duplication.

Sometimes in the course of cell division, all or part of a chromosome may be lost (by anaphase lag deletion or fragmentation) one or two of the arms of an X for example, may disappear or, on the other hand, become displaced and link with an autosome (translocation).
Even translocation of part of a Y to an X chromosome the so called X-Y interchange, is described. Another possibility is for chromosomes to divide across their centrosomes instead of longitudinally to produce isochromosomes.

Errors of these kinds, affecting only one or two cells at a very early stage of segmentation of the ovum, are reproduced in their offspring and the final outcome is two or more cell lines is the foetus. These can be found throughout the whole body or each may be localized or be dominant in particular tissues. This phenomenon is called mosaicism and the affected individual a 'mosaic'. How for mosaicism is present in all men and women is unknown but, in minor degrees, it may be universal. Mosaicism of a gross degree is not uncommonly found in men and women suffering from aberrations in sex differentiation and reproductive function. The possible combinations are endless. A few examples are 46XX / 45XO, 46XY / 45XO, 46XX / 46XY, 46XX / 45Xf (f meaning a fragment) and 46 XY/47 XXY.

The effects on the genital apparatus and phenotype depend on the relative number of the different types of cells, that is, on which cell line is dominant, and on their tissue distribution. Thus, if the primitive gonad is predominantly 45/XO it is not likely to develop or function even though most other tissues in the body
are 46/XX; if it is 46/XY it will become a testis despite the fact that elsewhere the dominant cell line is 45/XO.

The earlier in embryogenesis that any of the abnormalities in the sex chromosome pattern arise, the more widespread and serious is their effect. Amongst communities of European stock, 3-4 per 1000 live babies are born with clearly recognizable sex chromosome aberrations. This, however, understates the incidence since many affected foetuses especially those with XO patterns are aborted.

**Sex Chromatin Pattern**

The number of X chromosomes in the nuclei of various cells can roughly be judged by the sex chromatin pattern. When more than one X chromosome is present the nucleus has an additional deposit of chromatin. In epithelial cells this is disposed ecentrically and is called the Barr body after its discoverer. In neutrophils it appears as a "drum stick" appendages to one of the lobes of the nucleus, Jeffcoate, 1983. This chromatin mass is found whenever two or more X chromosomes are present in the nucleus and irrespective of the Y complement. A cell which carries it is said to be chromatin positive one which does not carry it is, called chromatin negative. A cell with only one X chromosome 45XO is chromatin negative, while
any extra X would be accordingly called chromatin positive.

The Barr body first appears in trophoblast cells at about the twelfth day, and in the tissues of the foetus itself by the eighteenth day. It is also detectable in the nuclei of the cells of the amnion and this offers a means of diagnosing the sex of the foetus if amniocentesis is carried out in pregnancy.

The Chromosomal Sex Drive

The Y chromosome gives the all powerful and positive drive towards sex differentiation and in particular, determines the nature of the gonads. It carries at least two genes which ensure that a gonad develops into a testis. The development of an ovary depends not so much on an XX chromosome complement but on the absence of the Y, Jeffcoate 1983.

When the chromosomal direction to the gonads is confused, as, it may in certain states of mosaicism, a possible result is the development of both testicular and ovarian tissue. These being either separate or in the form of an ovotestis. This condition is a true hermaphroditism. Affected individuals may be phenotypically male or female. In the former they often have gynaecomastia in later life, and in latter case they often manifest virilism.
As a result of errors in nuclear division, such as duplication in the zygote, there are men with two or more Y chromosomes and with karyotypes such as 47XYY, 48XYYY and 48XXYY, and mosaic patterns containing these, such men often suffer from the 'YY' syndrome which is characterized by excessive tallness, aggressive and anti-social behaviour, and criminal tendencies with or without mental subnormality. Their genital development and fertility are usually normal.

The possible aberrations of sex chromosome numbers and types, and their combinations in mosaics, are endless, but their effects are always in line with the principles stated above. Thus, a 48XXXY individual is likely to present klinefelter's syndrome, as are the mosaics in which the 47 XXY cell line is dominant. A woman with a 48 XXXX karyotype is likely to have the clinical features of the triple X syndrome, Jeffcoate 1983.

Men with an XYY sex chromosome constitution may even have XY/XYYY mosaicism in their gonadal tissue. Recently a study has analysed sperm chromosomes complements in a 27-year old XYY man, Martin and Benet 1986, he was oligospermic with large number of immature cells. His wife had chromosomal constitution 46XX but had first trimester spontaneous abortions.
Male individuals who are carriers of balanced chromosomes rearrangements have an increase risk of recurrent fetal wastage, by production of gametes with unbalanced structural rearrangements. The direct assessments of the frequency and type of imbalanced sperm complements and their segregation have practical applications for the genetic counselling of the parents.

**Sperm Chromosome Abnormalities in Infertile Men**

Chromosome abnormalities make a significant contribution to male infertility either by spermatogenic impairment or by increasing levels of abortion through the production of genetically imbalanced spermatozoa.

Chromosome abnormalities responsible for reproductive failure can occur during gametogenesis or as heritable chromosome imbalance. The former are namely aneuploidies of the sex chromosome and may have severe consequences for gonadal development. These are mainly represented by the 47, XXY karyotype, followed by 47, XYY karyotype. Inherited chromosome imbalance renders males subfertility or infertility by two different mechanisms.

**Infertile Individuals**

Once it became established that specific sex-chromosomal aneuploidies were associated in man with particular syndromes leading to infertility, it became a priority in human cytogenetics to screen groups of
individuals attending infertility clinics in order to establish the frequency of such abnormalities within the subfertile population, Chandley 1979.

In one of the earliest surveys, Ferguson-Smith et al. 1957, examined the buccal smears of 91 men with azoospermia or a sperm count of less than 1000000/ml who were attending an infertility clinics in Glasgow. Ten patients, six azoospermic and four severely oligospermic, were found to be chromatin positive and were suffering from Klinefelter's syndrome. Thus it was concluded from this particular study that this particular syndrome accounted for 11 per cent of all high-graded subfertility cases amongst men individuals.

Chandley et al. 1975, observed a similar frequency of 13 per cent; has since been found following chromosomal investigations in 121 azoospermic men attending a subfertility clinic in Edinburgh. Approximately 0.1 per cent, of XXY frequency of the individuals at birth were estimated, Jacob et al. 1974.

Investigations were later initiated in various parts of the world to ascertain the contribution made to male infertility, not only by sex chromosomal abnormalities but also by abnormalities of autosomes, Kjessler, 1966; McIlree et al. 1966a, b; Philip et al. 1970; Dutrillaux et al. 1971; Laurent et al. 1973; Luciani 1973; Koulischer and Schoysman 1974; Chandley et al. 1975.
Subfertile males patients attending infertile clinics were, Chandley et al. 1975 selected for chromosomal analysis because they were either azoospermic or oligospermic, whereas in some cases analysis were carried out on unselected groups. Different authors have given different criteria in defining a true chromosomal abnormalities in relation to male infertility or subfertility. In some surveys, minor chromosomal variants, such as enlarged satellites or large and small Y chromosomes or autosomes have been included as abnormalities along with structural rearrangements and aneuploidies. In yet others, where, meiotic studies were also made, anomalies seen at meiosis have been assessed together with the abnormalities and variants found in somatic cells.

Kjessler 1972 presented cytogenetic data on 1263 male partners of sterile couples presenting at an infertility clinic in Uppsala, Sweden and obtained, an over-all incidence of chromosomal abnormalities of 6.6 per cent, which included several karyotypes classified as being "of unknown significance" and minor anomalies of Y chromosomes. The incidence of constitutional chromosomal abnormalities, chiefly aneuploidies, translocations and other structural re-arrangements but excluding variants, among 1599 subfertile males was 2.2 per cent, in a survey carried out in Edinburgh by Chandley et al. 1975
A comparison of chromosomal abnormalities among the normal adult male population with the incidence of various abnormalities amongst new born males at Edinburgh revealed a total abnormalities among the subfertile group as three times more common and sex chromosomal four times more common, the XXY karyotypes occurring eight times more often. Heterozygotes for reciprocal translocation were found five times more often in the subfertile population.

Cytogenetic studies performed in 110 infertile males revealed 8 subjects with chromosomal abnormalities, 4 of these had Klinefelter's syndrome and 1 duplication of Y chromosome. Reciprocal translocation involving autosomes were detected in 3 subjects and 1 subject was observed with Robertsonian translocation involving chromosomes 14 and 21, Ghosh et al. 1986.

Cytogenetic surveys of males attending infertility clinics have revealed numerical and structural re-arrangements of the autosomes at a frequency of 4 to 15 per cent, Ghosh et al. 1986. Thus, certain karyotypes which are known to have a low frequency in the general population, appear in an infertile males with an increased frequency. Association of etiological for the infertile status in the detection of chromosomal error in an infertile male can be an indicator, and may also be of great help in the management of the individual concerned.
The incidence of chromosomal abnormalities has been estimated to 0.5 per cent, Ghosh et al. 1986, in the new born population. Nevertheless, several studies have indicated an increased occurrence of chromosomal abnormalities in women with relation to spontaneous or recurrent abortions and primary or secondary amenorrhea.

Before the period when banding techniques for human somatic chromosomes had been developed, meiotic studies were sometimes used to diagnose chromosomal abnormalities that could not be precisely identified by reference to the somatic karyotype alone. Now, with the advent of new and improved methods of staining for the indentifications of human mitotic chromosomes, the diagnostic value of a meiotic study has been somewhat reduced, though importance in basic information about meiosis in elucidating the manner in which chromosomal abnormalities behave in the germ line.

Human pregnancy wastage exemplified by miscarriage, still birth, neonatal and infant death occurs due to a major factor of chromosomal abnormalities, particularly during the first three months of gestation. In general, in sibships were both parents are chromosomally normal, about 15 per cent of clinically recognized pregnancies terminate in spontaneous abortions.

In the study by Boue et al. 1975 over 60 per cent of 1498 spontaneous abortuses less than 12 weeks
old, studied had abnormal karyotype. When these were analysed according to the type of anomaly, nearly 95.1 per cent were shown to be numerical and 3.8 per cent structural, abnormalities mainly resulted from error of cell division at meiosis, Beatty 1978.

Frequency of trisomy among the liveborn shows a very marked relationship to maternal age. Paternal non-disjunction, however, many account for 1/5th to 1/3rd of viable cases of trisomy 21 in man. When spontaneous abortion occurs repeatedly, however, a problem of infertility does arise, Chandley et al. 1975 suggests consideration to the behaviour of reciprocal translocation should be observed.

At the onset of abortion studies, it became obvious that the distribution of types of chromosomal anomalies observed in abortions differed from that in newborns. Aberrations, such as XO, were present in newborns as well as in abortions others, for example triploidies lead almost always to miscarriage and were compatible with birth of a living child only in exceptional cases, Vogel Motulsky 1982, others such as trisomy 16 were exclusively observed in aborted fetuses.

A research era in human cytogenetics began in the mid-1950s with progress in the development of human tissue culture technique. The improvement of these methods to analyse human chromosome and the observation
that numerical chromosome mutations and structural aberrations were linked with disease in man, initiated a new branch in human genetics, namely clinical cytogenetics.

The work done during the last 3 decades on chromosome analysis in adults, newborns and abortuses permits an estimate of the load of chromosome mutations in man. The results of different studies of population cytogenetics are summarized as follows Sankaranarayanan 1979: 150,000 spontaneous abortions occur per 1,000,000 conceptions. About 50% of them are chromosomally abnormal. Of 850,000 live births 17,000 die perinatally and about 6% are carriers of a chromosome mutation. Of the 833,000 surviving children 5,165 are chromosomally abnormal. From these estimates it can be calculated that 6 of 1,000 newborns carry chromosome anomalies and at least 80 of 1,000 zygotes are chromosomally abnormal. These values are the minimal value, Sankaranarayanan 1979, as the clinically unrecognizable preimplation and early postimplation loss is not included in these calculations.

Jacobs 1972, compared the karyotype of newborns with chromosome anomalies and their parents and observed that 100 per cent of the aneuploidies of the sex chromosomes and autosomes and, 25 per cent of the structural aberrations were new mutations. Basler 1987, from the
two mentioned data estimated that at least 4 of 1,000 newborns carry a new genome or chromosome mutation. These mutations are called 'spontaneous' mutations, the inducing mechanisms are unknown for its occurrence. It was postulated that a part of these new mutations in human germ cells may be induced by exogenous agents, including drugs and chemicals, Luer's 1955 a, b; Barthelme B 1956; Rohrborn 1965.

Induced germ cell mutations are eliminated mainly during oogenesis or spermatogenesis and at various stages of embryogenesis. Frequently, these mutations are believed to manifest themselves as abortions, sometimes as genetic diseases.

Spontaneous abortion is generally defined by national and state laws, and therefore its definition is a subject to considerable variation. Novitski, 1977 defines spontaneous abortion as the natural termination of pregnancy before the fetus has reached an age of 22 weeks or a weight of 500 gms. The spontaneous loss of the fetus after this period is a still birth.

The estimate of the frequency of spontaneous abortions in the population depends on the source of the data. Estimates based on interviews with women suggests a figure close to 15 per cent, but this is undoubtedly an underestimate, because most women may not be aware of early embryonic losses. Studies of the
early stages of pregnancy in women normal in fertility show that approximately 30\% of all fertilized eggs are clearly physically abnormal and incapable of further development. This additional 30\% represents fertilized eggs that would have been lost prior to three weeks of pregnancy, that is, probably before the women themselves would have been aware of its existence. This gives a total 45\% for spontaneous loss of very early embryos.

Nearly half of the embryos and fetuses spontaneously aborted are morphologically abnormal. It seems quite likely that an additional percentage are abnormal, to some degree not obvious by ordinary inspection. From this point of view, then, it would appear that in most cases early spontaneous abortions have a beneficial effect in preventing grossly abnormal fetuses from coming to term.

Spontaneously aborted fetus are the result of nondisjunction during meiosis, Carlson 1984, and these aborted fetuses have abnormal chromosomal number. Experience of spontaneous abortion is basically undergone by almost 1/5th of all fertile woman, which means, that as many as five percent of all gametes have an abnormal chromosome number. Fortunately the most serious type of nondisjunction is destroyed at an early stage of development, Carlson, 1984.
Nondisjunction during sex cell formation occurs more frequently in older females. Those who accept abortion for clinical reasons, there happens to be a technique of prenatal diagnosis called amniocentesis. After 16 weeks of fertilization which happens to an early pregnancy period, some of the fetal cells floating in the protective fluid around the fetus can be removed by amniocentesis, if, in case the culture reveals an abnormal chromosome, parents are obviously informed of it, who naturally would choose to abort the fetus. Such prenatal diagnosis of a pregnancy should be done at the age of 32 or 35 and above, when chances of nondisjunction are there, Carlson 1984.

A form of chromosome abnormality that seems to be hereditary is an occasional findings. Parents who have translocated chromosomes may seem normal phenotypically but 1/3rd of their children may have an excess or deficit chromosome. This unbalanced genes if does not abort the embryo is otherwise liable to adopt severe birth defect including lethal and semilethal. Parents with the family history of many spontaneous abortions or occasional birth with congenital abnormalities should prefer chromosomal study before having children, Carlson, 1984.

Ghosh et al. 1983 examined in 100 couples with repeated spontaneous abortions as compared to 100 control
couples. Incidence of long Y chromosome was found to be more common in couples with repeated spontaneous abortions as compared to the normal controls, a significant difference in the distribution of minor chromosome variants was found between the two groups. Literature reviewed so far establishes a regardiing the fact that the chromosomal firm relation to infertility. There-

study deals with the nature of distribution of qualitative and quantitative chromosomal structure among persons who suffer the impact of infertility due to, spontaneous abortions, primary and secondary amenorrhea in females and infertility due to azoospermia, subfertility due to oligospermia in male individuals.

In the process of studying the differences regarding various aberrations in chromosomal constitution among the infertile individuals, karyotyping has been implemented, performing the latest techniques. The results obtained from this study has been compared on some occasions with the study done by others on which the data was available.
REFERENCES


Boeue et al. (1975); Retrospective and prospective epidemiological and prospective epidemiological studies of 1500 karyotyped spontaneous human abortions. Teratology, 12:11-26.


Luers H. (1955b); Zur Frage der Erbgutschadigung durch tumortherapeutische cytostatica. Z. Krebsforsch, 60: 528.

Martin R.H., Benet J. (1986); Analysis of Sperm Chromosome Complements in a 47, XXY male. 7th Int. Congr Human Genetics, Berlin, Sept. 22-26, 1986, p. 149.
McIlree M.E., Tulloch W.S. and Newsam J.E. (1966a); Lancet, 1, 679-682.


