A Study of C-Band and Satellite Chromosomal Association in Azoospermia and Oligospermia

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We, the examining committee, certify that we have read this thesis, and have examined the student in its contents, and that in our opinion is adequate as a thesis for the degree of doctor of Philosophy in Biology (Zoology).

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DEDICATION

To my wife...

and to my daughter.....

Nadeen.

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Abbreviations

AZF: azoospermia factor.

CBAVD: congenital bilateral absence of vas deference.

CFTR: cystic fibrosis transmembrane conductance regulator gene.

DAZ: deleted in azoospermia.

FISH: fluorescent in situ hybridization.

mbp: million base pairs.

mt DNA: mitochondria DNA.

NOR: nucleolar organizer region.

RRM: RNA recognition motifs.

SA: satellite association.

Abstract

The work described in this thesis was undertaken at the University of Babylon between July ١٩٩٨ and July ٢٠٠١ under the supervision of Prof. Dr. Akeel A. Yasseen and Prof. Dr. Ismail Ejam. Except where indicated by references, it is the original work of the author, and has not submitted for any other scientific degree.

A total of two-hundred and seventy–seven patients with a clinical diagnosis of infertility have been subjected to cytogenetic screening. The lymphocyte of two-hundred and twelve patients out of this total and one hundred control fertile groups were successfully cultured. The patient's ages ranged from ١٩-٥٤ years.

A – Giemsa stain and chromosome analysis:-

١- Out of a total of two – hundred and twelve infertile men only twelve patients showed an obvious chromosomal aberration, which account to ٥.٧٪.

٢- Out of one –hundred twenty azoospermia, only four patients had abnormal karyotype which account to ٣.٢٪ .

٣- Out of eighty- eight oligospermia only eight patients showed an obvious chromosomal aberration, which account to ٩.١٪.

٤- Four Azoospermia patients showed ٤٦,XXY male mitotic karyotype which was associated with klinefelter`s syndrome. No other abnormalities were detected.

٥- Seven oligospermia patients revealed a mosaic ٤٦,XY/ ٤٧,XXY male mitotic karyotype.

٦- One oligospermia patients showed ٤٦,XX/٤٥,XY,t(D/D) male mitotic karyotype.

B- Autosomal C- band polymorphism:-

١- The size of C- band, the occurrence of inversion, C- band symmetry versus asymmetry which is located on chromosome numbers ١, ٩ and ١٦ have been studied more extensively.

٢- There was a significant increase in C- band size of chromosme numbers ٩ and ١٦ among infertile patients as compared to normal fertile men (C- band quantitative study). ($P < ٠.٠٠١$).

٣- There was a significant increase in C- band size (level ٣) of chromosome ١ among infertile group compared to a normal control group.

٤- There was a significant increase in the frequency of C- band heteromorphisms among infertile patients compared to normal control group ($P < ٠.٠١$).

٥- With regard to pericentric inversion, there was no significant difference between the infertile group compared to a normal control group ($P > ٠.٠٥$) .

C-Y- chromosomes variations:-

١- According to Y/F criteria, it has been found a high frequency of small size Y chromosome was observed among the infertile group as compared to a normal fertile group ($P < ٠.٠٥$) .

٢- There was a strong correlation between the C- band which is located on the distal portion of the Y- chromosome, the euchromatin region and the total length of the Y- chromosome.

D- Satellite Associations:

١- There was a significant increase in the frequency of satellite association among the infertile group compared to a normal control group.

٢- The associated frequency of chromosome numbers (١٣-١٤) was more frequent among the oligospermia than the other associated chromosomes.

٣- The associated frequency of chromosome number (١٣-١٣), (١٣-١٤), (١٣-١٥), (١٣-٢٢) among the oligospermia were distributed at non-random pattern ($P < ٠.٠٠٥$)

٤- With regard to azoospermia patients, the high frequency of (١٣-١٤) associated chromosome was recorded. The two-by-two other associated chromosomes, were distributed at a random pattern (P > ٠.٠٠٥) .

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Introduction

١.١ General Introduction:

Chromosomal abnormalities are the cardinal feature of men with infertility. It takes a rough estimate of being between ٢٪ to ٢٠٪ (Vogt, ١٩٩٥), and it is some five times higher among infertile men than in the general population (Vogt, ١٩٩٥).

So far two main categories of chromosomal anomalies must be distinguished. The first is due to aneuploidy in autosomes. The second is due to the sex chromosomal aneuploidy or balanced structural rearrangement . Indeed, the majority of anomalies, which have been observed among sterile males attending infertility clinics, are of the second category.

Furthermore, Lang *et. al.*, ١٩٩٢ stated that approximately ١٠٪ of patients with clinical symptoms of male infertility have chromosomal aberration in their somatic cells.

Various extensive studies revealed that about ١١٪ of men with severe sterility factors, as azoospermia, had chromosomal abnormalities compared to about ٢٪ among oligospermic patients (Vogt *et al.*, ١٩٩٥). But the bulk of these abnormalities were associated with sex chromosomal anomalies, while autosomal anomalies were found only among oligospermic patients (Matsuda *et al.*, ١٩٩٢; Yaseen *et al.*, ٢٠٠١).

In Iraq, it has been found that the chromosomal anomalies among infertile patients occurr in a frequency of about ١٢.٥٪ (Yasseen *et al.*, ٢٠٠١ a; Yasseen *et al.*, ٢٠٠١ b).

١.١.١ The Normal Human Karyotype

Recall that ٢٢ of the ٢٣ pairs of human chromosomes occur in the cells of both sexes, These autosomes are numbered from ١ to ٢٢ according to length (with chromosome ١ being the longest) .The two remaining chromosomes – The sex chromosomes - are not numbered. The largest one is the X chromosome and the smaller one is the Y chromosome . Male cells normally contain one X and one Y chromosome , whereas female cells contain two X chromosomes.(Vogt,١٩٩٥).

The haploid autosomal length (HAL) is the total amount of chromosomal material present in a haploid set of autosomes. The relative lengths of human autosomes decrease with increasing chromosome number. For example, chromosome ١ (the longest) is ٨.٤٪ of HAL, while chromosome ٢١ (the shortest) is ١.٩٪ of HAL. Why is the shortest chromosome called ٢١ rather than ٢٢ ? This one exception is due to a historical fluke involving the extra chromosome present in Down Syndrome individuals, the chromosome was originally named ٢١ in the mistaken believe that it was the next – to – shortest autosome .Later, with improved techniques, Cytogeneticists discovered that it was really the shortest autosome and should be called ٢٢. But by this time, the error was so entrenched in the scientific literature that it was easier to accept the inconsistency in the relative length than to change the number.

Before human autosomes were distinguishable by banding method, they could only be arranged according to the length and centromere position into seven groups (A-G). Within each of these groups, the centromeric position is quite similar. For example, groups A and F contain the four most metacentric chromosomes; groups D and G consist of acrocentric chromosomes ; and the chromosomes in groups B,C,and E are submetacentric. This convention is often retained even in newer banded caryotypes (Mange and Mange, ١٩٩٩).

It is often convenient to describe an individual's karyotypes with cytogenitic shorthand rather than by a picture. At the simplest level, this description is merely the total number of chromosomes and the sex chromosomal complement, thus, a normal female is designated ٤٦,XX and normal male ٤٦,XY. The fact that the bands appear much fuzzier in a photograph than in a diagram point up the need for great technical expretise in analyzing karyotypes (Dutrillaux et al.,١٩٨٢).

١.١.٢ Sex Chromosomal Anomalies:

It is highly accepted that the sex chromosome is more likely to be involved in abnormality. The majority of reports showed that ٤٧, XXY and ٤٦, XX karyotypes lead to spermatogenesis defect. Indeed, the translocation between two sex chromosomes has the same deleterious effect. On the other hand, inversion, structural polymorphism of Y- chromosome, ٤٧, XYY and ٤٥, X٠/٤٦XY karyotypes were associated with infertility, and had influence on infertility ranges from normal fertility to azoospermia (Lang *et al.,* ١٩٩٠).

١.١.٢.١ Klinefelter Syndrome: ٤٧, XXY Males:

In the ١٩٤٠, Harry Klinefelter and his colleagues at Massachusetts general hospital described a syndrome of sex chromosome aneuploidy in human male (Jeqvir, ١٩٨٦). Later, cytogeneticists Patricia Jocobs and J. A. Strong (١٩٥٩), showed that male with Klinefelter syndrome usually, but not always, have the ٤٧, XXY karyotype.

The signs almost always observed in these males are very small testis, infertility and underdeveloped secondary sexual characteristics, and tend to be tall and may (or may not) exhibit mild mental deficiency and/or some breast development.

Klinefelter syndrome is not rare in general population .Its overall frequency is ١ in ٦٠٠ to ١,٠٠٠ live born males (Nielsen *et al.,* ١٩٩١), and

about ١ in ٣٠٠, among spontaneous abortion. In sub-populations of tall men (Over ١٨٣cm), the frequency of male with Klinefelter syndrome may be as high as ١ in ٢٦٠, and perhaps about ١ in ٢٠ male patients that were seen in infertility clinics has Klinefelter syndrome (Mang *et al.* , ١٩٩٩).

Klinefelter syndrome occurs randomly within families. The extra chromosome is thought to arise from non disjunction with slightly more than half (٥٦٪) of cases occurring during agenesis in the affected man's mother.

Experts disagree on whether or not there is a significant maternal or paternal age effect (Robinson and Chapelle, ١٩٩٧), where the non-disjunction of the sex chromosomes occurs during gametogenesis in either father or mother. However, the non-disjunction in the father can give rise to a XY spermatozoa, where as a non disjunction in the mother may result in an XX egg, consequently, on fertilization with Y-bearing sperm will result in a XXY zygote (Fraser and Mayo, ١٩٧٥).

More recently, (Mang and Mang, ١٩٩٩) stated that Klinefelter variant occurred in about ٨٠-٨٥٪ of males with Klinefelter syndrome and exhibited the usual ٤٧, XXY karyotype. While the remaining type called Klinefelter's variance where some are karyotypically normal, and about ١٠٪ have abnormal karyotypes that are variant, from ٤٧XXY, including ٤٨XXXY, ٤٨XXYY, ٤٩XXXXY and ٤٩XXXYY. Klinefelter syndrome involving single cell lines of ٤٨ or ٤٩ chromosomes seems to be associated with more extreme problems of all sort, like severe mental retardation.

Klinefelter syndrome is easily diagnosed by barr bodies on a buccal smear, however, the buccal smear test can give no useful information about the autosomal complement of an individual. for this reason a full chromosomal investigation is required (Chandly, ١٩٨٣).

All these unusual karyotypes such as karyotype with ٤٩ or ٤٨ chromosomes, arise from two non-disjunction events, for example, a ٤٨, XXXY zygote can arise in several ways, as: a Y bearing sperm fertilizing an XXX egg; or XY sperm fertilizing an XX egg; or an XXY sperm fertilization an X-bearing egg. Often the pattern of inheritance of X-linked gene, or special DNA sequences called RFLPs can be distinguished among the various possibilities (Mang *et al.*, ١٩٩٩).

However, all men with Klinefelter's syndrome are azoospermic, but in some cases this karyotype may be found in men with oligospermic (Paulsen *et al.*, ١٩٦٨), so the sterility is the rule in men with this disorder. Nearly ١٠٪ of these patients, have chromosomal mosaicism (٤٦,XY/٤٧, XXY) which, they have less severe features of Klinefelter syndrome and may be fertile (Dale, ١٩٩٥). Recent report stated that a Klinefelter's syndrome was associated with a ١٣/١٤ translocation, such a rare occurrence most probably due to the de novo arrangement of chromosomes related to the advanced age of both parents during conception (Gul and Sayli., ١٩٩٤).

١.١.٢.٢ Poly Y Karyotype (٤٧, XYY karyotype):

Sandberg and his colleagues discovered the first XYY male by chance as the father of a child with Down syndrome.

It occurrs at a frequency of about ٠.٢٪ of all men, this chromosomal constitution resulted from paternal non-disjunction during meiosis II, producing YY sperm. (Jacobs *et al.*, ١٩٦٥).

Few years later several reports were made, but they did not begin to attract wide interest. Until ١٩٦٥, Jacobs and her colleagues by studying the chromosomes of ١٩٧ mentally subnormal males with dangerous, Violent, or criminal tendencies. They found seven males with ٤٧, XYY karyotype with a rate of (٣.٥٪). Many studies have followed; by testing tall-institutionalized male and finding additional ٤٧, XYY was made. The incidence of XYY

karyotype is in the order of ١ in ١,٠٠٠ males, but among tall males, it is perhaps, ١ in ٣٢٥.

Advance reports revealed that as compared to the X-chromosome, poly Y karyotype containing more than ٢Y chromosomes are relatively rare. Also only few cases of XYY karyotype with or without mosaicism have been reported, moreover, all such patients with poly Y karyotype revealed behavioral disturbances and azoospermia (Teyssier and Pouses, ١٩٩٤).

١.١.٢.٣ XX Disorder (Sex Reversal Syndrome Sex Reversal and the SRY Gene:

The basic question of what determines the sex in human being seems to be answered by the cytogenetic evidence of a Y in the male instead of a second X. The male phenotype, thus, is triggered by the presence, of Y chromosme while the female by the absence of a Y (Ohno, ١٩٧٠).

The role of the Y-chromosome in maleness determination has, in fact remained unchallenged for long time because even in the presence of several X's it causes a male phenotype.

Exceptions to this rule was the rare cases of sex reversal (most notably ٤٦, XX male and ٤٦, XY female) which opened the door to geneticists to study an elusive SRY gene (Weissenbach, ١٩٩٥). The patients are phenotypically males and they have feature of infertility, azoospermic, and some adults show some of the clinical feature associated with Klinefelter males (Zakharia and Krauss, ١٩٩٠).

Indeed, the mutations or rearrangements of the various genes associated with sex determination are causes of sex reversal of males and females.(Zakharia and Krauss, ١٩٩٠).

Many years ago it was proposed that the sex reversal could occur when a tiny but critical bit of Y-chromosome is lost (from ٤٦, XY females)

or gained (by ٤٦, XX males). The missing or extra piece was presumed to carry the gene for Testis Determining Factor (TDF), (Ferguson Smith *et al.*, ١٩٩٠).

The human Y-chromosome, is roughly one third of the size of the X-chromosome (Lahn and Page, ١٩٩٧). It carries about ٣٠ genes and gene families, compared to the many known genes on the X. Despite their great dissimilarity, the Y and X-chromosomes pair during meiosis, this is because the tips of their short arm share a tiny region of homology (Dolon and Muller , ١٩٩١).

During male meiosis, synaptonemal complex and one chiasma always form in this region, resulting in regular exchange of material between the tips of Xq and Yq. In ٤٦, XY males the homologous region is present in duplicate (rather than singly as with regular X-linked loci), and any genes within are inherited as they are autosomal hence the term pseudoautosomal region, PARI (PARI contains nine known genes) (Lahn and Page, ١٩٩٧).

Normal pairing between the X and Y, but without regular crossing over, also extends into the neighboring non pseudoautosomal (and genetically non homologous) region, often just beyond the centromere of the Y chromosome. Very rarely, an illegitimate crossover occurs in this later region, resulting in transfer of the TDF encoding locus, to the X-chromosome and loss of that locus from Y-chromosome in that particular spermatocytes if the resulting sperm fertilizes a normal egg, a ٤٦, XX male or a ٤٦, XY female will be conceive.

Researchers focused their research on the TDF encoding gene by analyzing and comparing the DNA, from a several dozen ٤٦, XX males (with tiny piece of a Y-chromosome attached to one of their two Xs) (Dolon and Muller, ١٩٩١). And a few ٤٦, XY female, (with a tiny deletion of their Y-chromosome). Both types of abnormalities were located in the

same minute region of the Y-chromosome where the gene for testis-determining factor must reside.

In ١٩٨٧, an international team of scientists reported that they had finally found the gene for testis-determining factor, but the excitement was short-lived.

Further research indicated that this new gene, called ZFY (for zincfinger on the Y), did not meet all the necessary criteria, for one thing, it turned out to have a homologous on the X-chromosome.

Three years later a group of British investigators identified a near by gene, located very close to the pseudoautosomal boundary, which they called SRY (sex-determining region on Y-chromosome). SRY codes for a transcription feature closely resembling some DNA binding proteins known to turn other genes off. (Mang and Mang, ١٩٩٩).

Additional evidence also points to SRY as the testis-determining feature:

(١) Essentially the same Y-chromosome DNA sequences is detected in all males, but not in females, in a wide variety of mammals.

(٢) In XY embryos of mice, the SRY gene is expressed only in cells of the gonadal ridges, and at a time just before testis should begin to form.

(٣) In mice, the SRY DNA sequence is present in male determining region of normal Y chromosome (with a tiny deletion) that has lost it’s male determining ability.

(٤) If mouse SRY gene sequences are transplanted into XX zygotes, some (but not all) of these prospective females development.

(٥) In human, at least two-sex reversals ٤٦, XY woman were found to have mutations (rather than cytologically observable deletion) within a key part of their SRY genes.

Although they seem to have found the long-sought (master switch) that activates a cascade of steps leading to maleness, scientists sound a

strong note of caution: not all sex reversal cases can be explained by these findings.

Indeed, most ٤٦, XX males lack any detectable Y chromosome DNA. Certainly, non Y-linked mutations must affect later steps in the cascade of events leading to the development or non-development of gonads, but they have not yet been clearly identified. Thus, to understand the complex process of sex determination in both males and females, researchers will have to find and analyze the other genes. This means that the researchers have to find and analyze the other gene-known to exist on both sex chromosomes and autosomes, that have a functional role in sexual development (Zhang *et al.*, ١٩٩٨).

Other types of sex reversal have also elucidated the functions of additional sex determining genes, for example mutations of SOX٩ cause compomelic dysplasia (Kamp, bending; melos, “limb”), a usually lethal form of dwarfism. Infants born with this condition have severe abnormalities of the long bones (including bowing) (Muscatelli *et al.*, ١٩٩٤).

In addition to these major defects, they also exhibit what is called autosomal sex reversal ٧٥٪ of the ٤٦, XY individuals develop as females or hermophrodites. Furthermore, defect in the DAX١ gene have provided crucial information about it’s normal developmental role. Males born with mutations or deletions of DAX, have a condition, called adrenal hypoplasia. Uunderdevelopment of their adrenal glands causes a severe and ,if untreated potentially (lethal deficiency of certain adrenal hormones), male reach the age of ١٤ will also exhibit hypogonadism (Muscatelli *et al.*, ١٩٩٤; Zhang *et al.*, ١٩٩٨). This under development of their gonads at the time of puberty is due to the later effects of DAX١ mutations on the hypothalamus and pituitary glands, reducing their output of gonadotropic hormones. A different condition dosage sensitive sex reversal, results from

a rare duplication of the locus. Males possess a normal Y-chromosome and an X-chromosome with two copies (rather than the usual single copy) of DAX١ develop, as phenotypic female transgenic mice carrying two copies of DAX١, reveal the anti-testis function of this locus (Swain *et al.*, ١٩٩٨).

١.١.٢.٤ ٤٧, XXX Male:

Bigozzi and his colleagues in ١٩٨٠ firstly reported rare cases of genetic condition in male phenotype. By cytogenetic analysis using Q and R band techniques revealed ٤٧, XXX karyotype in both lymphocytes and fibroblast, while abuccal smear showed ٢٠٪ X-chromatin positive nuclei were found at the same time. Bigozzi and co-workers explained that these patients originally XXXY and after testicular differentiation was triggered, mitotic non disjunction may produce two stem lines, one containing the Y-chromosome. Furthermore, it is believed that Y line is probably eliminated during the cell division (Dutrillaux *et al.*, ١٩٧٣).

١.١.٢.٥ Noonan's Syndrome (Male Turner syndrome):

Botlon and his colleagues (١٩٧٤) were the first group, who reported the important genetic condition that corresponds, to Turner's syndrome (Noonan's Syndrome). Few years later Gal found the ٤٥, X٠ karyotype is usually combined with maleness (Gal *et al.*, ١٩٨٧). Clinical feature of most males affected with this syndrome was diminished spermatogenesis and sterility as general. Several studies on karyotype analysis revealed that the sex chromosome anomalies such as X٠/XY mosaicism represent a partial sex chromosome monosomy (Dale, ١٩٩٥).

Harmerton *et al.*, ١٩٧٥ mentioned that the incidence is estimated between ٠.٠١٤٪ to ٠.٤٪ (Harmerton *et al.*, ١٩٧٥).

Furthermore Richard and Stewort, (١٩٧٨) recorded a case of X٠/XY karyotype associated with non fluorescing Y-chromosome, whether

terminally deleted or non fluorescing owing to an altered chemical state, predisposes of anaphase legging and non-disjunction.

١.١.٢.٦ Y - chromosome Anomalies:

١.١.٢.٦.١ Y chromosome Deletion:

Tiepolo and Zuffardi (١٩٧٦) were the first to propose that Y chromosome deletions interfer with male fertility. Those author's observed terminally deleted Y chromosomes in the karyotype of six sterile males with azoospermia. The deletion includes the large heterochromatin block in the long Y arm (Yq١٢) and an undefined amount of the adjacent enchromatic part (Yq١١). Since that time, nobody considered the presence of genetic activity in heterochromatin, it was postulated that genetic Y factors are important for male germ cell development one located in Yq١١. They were defined azoospermia factor (AZF), as their deletion correlates to the sterile male phenotype azoospermia, which means that no mature sperm cells were selectable in patients seminal fluid. Few years later, the presence of AZF in Yq١١ was confirmed by numerous studies of the cytogenetic level (Sandberg, ١٩٨٥) and at the molecular level (Anderson *et al.*, ١٩٨٨; Bardoni *et al.*, ١٩٩١; Vogt, ١٩٩٥).

This first becomes possible after the deletion at different interstitial microdeletion in Yq١١ not visible in the microscope but detectable by molecular deletion mapping (Ma *et al.*, ١٩٩٢; Vogt *et al.*, ١٩٩٢; Vogt *et al.*, ١٩٩٣). Under the microscope, the metaphase chromosome with microdeletion still displays a normal banding pattern because deletion, not larger than ٢Mb of DNA is hidden due to the high packaging, density at the chromatin fiber at metaphase.

Vogt (١٩٩٨), reported that chromosome deletions in Yq١١ seem to occur frequently as de novo mutation events in men with idiopathic azoospermia or severe oligospermia. However, the molecular level extensions of these deletions are variable. They can be large and therefore

visible under the microscope or small, not visible under the microscope, and contain deletion of one or more DNA loci recently mapped in apparently consecutive order along the Yq١١ chromosome region.

The results of ٢٠ extensive microdeletion screening programs have now corroborated the prevalence of the deletion of three non-over lapping DNA regions in proximal, middle and distal Yq١١, which were designated earlier as AZFa, AZFb, AZFc.

١.١.٢.٦.٢ Polymorphism of Y chromosome:

Taillemite *et al.*, (١٩٧٨) reported that Y chromosome polymorphism has a close association with male infertility. Furthermore, it was proposed that ring Y chromosome seems to be unusual abnormality and has been recorded in child with ambiguous external genitalia.

Taninch and his colleagues (١٩٩١) demonstrated later that the dicentric Y chromosome has close association with sterile male. While, Kohler and Vogt (١٩٩٤) showed a case of dicentric human Y chromosome with interstitial deletion of repetitive DNA.

Furthermore, Dutrillaux *et al.*, (١٩٨٢) in their excellent review of chromosomal factor in the infertile male, have found many cases of pericentric inverted Y chromosome, whereas other studies have stated a case of pericentric Y chromosome with minute deletion involving mine loci within the most distal part of the euchromatin region of the long arm (Iwameto *et al.*, ١٩٩٥).

Mang and Mang (١٩٩٩) showed that males heterozygous for a ring chromosome are less fertile. In most cases of known ring chromosome inherited, it was transmitted by the mother.

١.١.٢.٢ Genosome-Autosome Translocation:

Mang and Mang, (١٩٩٩), reported that sex chromosome aneuploidy is much more benign than autosomal aneuploidy. The reasons lie partly in the paucity of genes on the Y, but mostly in dosage compensation (lyonization) of the X chromosome.

Recall that inactivation begins at an X inactivation center containing the XIST gene and spreads in both directions through the X chromosome.

١.١.٢.٢.١ X- Autosome translocations:

X-autosome translocation is less frequent than Y-autosome translocation. The review showed a case of t(X;٢١) (Croquette and Fourlinie, ١٩٨٠), few years later Dutrillaux et al (١٩٨٢) detected a case of t(X;١٢) in sterile man.

There is some explanation for this situation. There are two X chromosome segments, one carrying the XIST locus and the other lacking it. The later cannot be inactivated. Also, in X-autosome translocations with XIST bearing segment, the inactivation can spread to the adjacent autosomal region. Consider the following two inactivation alternatives for cell containing balanced X-autosome translocation:

١-If both translocated segments (i.e. the XIST-bearing segment and the XIST-less segment) remain active, then the intact (un translocated)X is inactivated and the genome is functionally balanced. That is, the cell has one intact inactive X and, although it has been speard into two portions, the equivalent of one whole active X.

٢-If the XIST-bearing translocated segment gets inactivated, the intact X must then remain active. But the translocated XIST-less segment of the X is also active so that the cell has two active cell allels rather than just one for every locus on this segment of the X.

An embryo with an X autosome translocation will randomly contain both types of cells (type ١ and ٢). Those embryos with substantial fraction of type ٢ cells cannot survive unless the doubly active segment of the X is very small. But if all or nearly all the X type ٢ cells die, the embryo can survive, although it may show phenotypic abnormalities. Nearly all of the males and about half of the female translocated carriers will probably be infertile, and for fertile females, the risk of structural or functional aneuploidy in a live born child could be ٢٠-٤٠٪. Mang and Mang (١٩٩٩).

١.١.٢.٢.٢ Y – Autosome translocation:

It's well established that Y-autosome translocation is rare condition. There are, two types of translocations, the first translocation of genetically inert long arm involved, the autosome is usual acrocentric chromosome. In such cases Chandly *et al.*, (١٩٧٥) have been detected a case of Y; ٢٢ translocation, in following years, Retif *et al.* (١٩٨٤) reported a case of ٤٥, X, t(Y;٢١), the translocation carrier may be phenotypically unaffected. Other type, where long arm is translocated to nonacrocentric chromosome, the almost result have been obtained by Lobez Pajares *et al.* (١٩٧٩) who also noticed a translocation between an autosomal and Y chromosome ٤٥, t(Y;١٠), shortly after that, Croquette and fourlinie, (١٩٨٠) described Y; autosome translocation including t(Y;٧). The observed phenotypic abnormalities may be associated with breaks in autosomes euchromatin. Whereas transloction of the short of the Y almost always leads to infertility and other abnormalities. Because the short arm contains the testis determining region, more specifically SRY gene X-Y translocation are rare and give rise to ٤٦,XX male if translocated Y segment contains the SRY gene.

١.١.٢.٣ Autosomal Anomalies:

It's well assumed that autosomal abnormality to be some eight times higher in infertile men than the normal population (Lang *et al.*, ١٩٩٠).

Below are the most common types:-

١.١.٢.٣.١ Robertsonian's Translocation:

It has been detected that autosomal abnormalities mainly consist of balanced translocation (Piovani *et al.,* ١٩٩٠). Robertsonian translocations are a common structural abnormality in humans (Page *et al.,* ١٩٩٦). It occurs in a frequency of nearly ١ in ١,٠٠٠ newborns, (Hook and Hamerton, ١٩٧٧). Furthermore, it is about ٧٥٪ of these join together chromosomes ١٣ and ١٤, consisting the most common sign of rearrangement in human race (Gardner and Suther Land, ١٩٩٦).

Translocation among two acrocentric chromosomes consisted of the centromere, as are the two largely hetrochromatic tips. The tiny chromosome so formed is usually lost. The loss has no phenotypic consequences, since, as long as other a crocentric's short arms are present no essential materials, are lost But the longer translocated chromosome contains the full complement of essential genes from the two chromosomes. A gamete with this balanced chromosome, when combined with a normal gamete, gives rise to a balanced heterozygote, the resulting individual has a total of only ٤٥ chromosome, yet it is phenotypically normal. So far Dutrillaux, in his review of chromosomal factors in the infertile male, found that the majority of this specific translocation constituted by t(١٣q;١٤q). Also, it has been noticed ٤٥, XY, t(١٥;٢١); ٤٥, XY, t(١٣;١٤) and ٤٥, XY, t(١٤;٢١) (Retief *et al.,* ١٩٨٤).

In addition, it has been detected by Bourrouillou *et al.,* (١٩٨٥) several cases of ٤٥, XY, t(١٣;١٤); ٤٥, XY, t(١٣;١٥) and ٤٥, XY, t(١٤;٢١). More recently Yasseen *et al.,* ٢٠٠١ reported a case of t(١٣;١٤) in Iraq out of ٦٤ infertile patients . The frequency of Robertsonian translocation in Iraq is about ٣.٧ % .

١.١.٢.٣.٢ Reciprocal Translocation:

It is the rearrangement of chromosome parts that involves the exchange of two centric pieces that reattached to two centric, and no chromosomal material becomes lost, the translocation is reciprocal. Because all of the genetic materials is still present but in different arrangement, heterozygote for such a translocation is balanced (Chandly *et al.,* ١٩٧٥).

The phenotype is usually normal, but the fertility may be reduced (De –Braekeleer and Dao, ١٩٩١). In general population, about ١ person in ٦٢٥ carries a reciprocal translocation. So that it's considered as important factor known to influence the spermatogenesis. In review of literature on mitotic studies in infertile man ,it has been proved that the reciprocal translocation is on of the most common of these rearrangement (De-Braekeleer *et al.,* ١٩٩١), furthermore, Chandly *et al.,* (١٩٧٥) stated many cases of reciprocal translocation in sterile men including t(٣;١٩); t(٧;١٧); t(٩,١٤); t(١٣;١٨) and t(٩;١٠).

A few years later, Dutrillaux *et al.,* (١٩٨٢), also detected cases of reciprocal translocation between different autosomes including t(١١;٢٢); t(٦;١١); t(٦;٢٠); t(٥;٨); t(١٠;١٤). In addition, were recorded cases of t(١;٤); t(٧;١٥) by Retief *et al.,* (١٩٨٤), whereas, Bourrouillou *et al.,* (١٩٨٥) have found cases of t(١;٧); t(٢;٧); t(٣;٥); t(٩;١٧); t(٩;٢١); t(١٠;١١) and t(١٢;١٣). More recently Piovani *et al.,* (١٩٩٠) detected a case of t(٤;١٥) with an common break-points involved in the translocation.

١.١.٢.٣.٣ Autosomal Inversions:

When two breaks occur in one chromosome and the intervening segment gets inverted before the broken ends region, the resulted aberration is known as an inversion. The reversed segment may or may not include the centromere.

Instead, its presence is detected by unusual segregation patterns in organisms heterozygous for the inversion. Its frequency might be as high as ١-٢٪. The particular chromosomes involved and the locations of breakpoints appear to be highly nonrandom. (Gardner and Sutherland ١٩٩٦), reported in their review that the inversion variants not involving centromeric heterochromatin have also been described for chromosomes ٢ the most common ٣,٢, and ١٠. Furthermore, pericentric inversion was recorded to affect chromosomes ١,٧ and ١٠ at frequency of about ٠.٣٠٤٪ (Dutrillaux *et al.*, ١٩٨٢). At the same time Abramsson *et al.*, (١٩٨٢), estimated that autsmoal inversions ranging between ٠.٠٤٪ and ١٥.٧٪. Whereas, few years later, Bourrouillou *et al.*, (١٩٨٥) have detected cases of pericentric inversion affecting chromosome ١,٢,٣ and ٥.

Recently, Lange *et al.*, (١٩٩٠) stated cases of this abnormality affecting chromosome ١,٣,٥,٦,٩ and ١٠. Complete gene-by-gene pairing during synapsis of an inversion heterozygote requires an inverted chromosome and it's normal homologue to form a loop during prophase I. What happens then depends on (١) whether or not crossing over occurs within the inversion loop (٢) whether or not the centromere is included within the inversion. When no crossovers occur within the inversion loop, no unbalanced chromosomes are formed and there is no effect on fertility. (Lange et al., ١٩٩٠).

But when crossing over occurs within the inversion loop, whether or not it includes the centromere, the two crossover chromatids end up with both duplication and deletions. In addition, crossover chromatids (from inversions that include the centromere) may be either dicentric or acentric. Dicentrics breaks and acentric are lost, resulting in highly unbalanced gametes and zygote death, (Mang and Mang ١٩٩٠).

The overall effect on fertility varies with the frequency of crossing over within the inversion. Thus, larger inversion (with more crossing over) may give rise to more defective gametes and greater infertility.

١.١.٢.٣.٤ Supernumerary Marker Chromosome:

The supernumerary marker chromosomes (mar) are the most common autosomal anomalies found in man. The occurrence is one person in ١,٥٠٠. Individuals with an extra structurally abnormal chromosome may or may not be associated with phenotypic effects. Such chromosomes, whose origins are often difficult to identify, are expected by FISH and molecular methods.

Webb, (١٩٩٤) reported that approximately ٥٠٪ of supernumerary chromosomes are inverted duplication ١٥. Furthermore, Dutrillax *et al.*, (١٩٨٢) reported that frequency of this abnormalities among subfertile men is about ٠.١٪. Moreover, the previously aformationed study of Chandly *et al.*, (١٩٧٥) have detected various types of supernumerary marker chromosome including ametacentric chromosome which was slightly smaller than G group, a tiny ring chromosome and a submetacentric chromosome so slighlty smaller than G group. Furthermore, other results have recorded by Jaafar *et al.*, (١٩٩٤), who found ametacentric supernumerary marker chromosome in oligospermic male. More recently, a mosaicism form was detected in azoospermic infertile male, this chromosomal analysis revealed a karyotype ٤٥,X/٤٦,X^{+} mar (Terada *et al.*, ١٩٩٥).

١.١.٣ Molecular Bases of Male Infertility:

١.١.٣.١ Introduction:

As a matter of fact the molecular bases of male infertility is not a linear order of genetic events, it is most probably due to a complex genetic network that function in three main developmental pathways: A-male germ – line development, B-male gonad development, C-and male somatic

development, consequently primary genetic switch signals should exist for linking the different genes networks and/or for starting them. There is some evidence that such switch signal is concentrated on the sex chromosomes (Vogt, ١٩٩٧).

Accordingly, the alteration of their signals may alter different sperm parameters and spermatogenesis in general. Indeed all the signals which are responsible for spermatogenesis are the result of genes expression either directly or indirectly, (Vogt, ١٩٩٦). Accordingly any changes in genes alter the sperm parameters. The changes on genes may result from mutation which are either point mutation or structural rearrangement (deletion, duplication). Gene mutations which are thought to disturb sperm parameters and consequently the male fertility, they also called molecular genetic sterility factors (parameters). These are divided into three according to :

١-٢ Sterility factors (parameters) caused by mutation in genes: Whose function is known to be restricted to germ line.

١-٣ Sterility factors caused by mutation in genes: Whose function is essential for development of gonads.

١-٤ Sterility factors caused by mutation in genes: The function of which takes place also in non gonad somatic cells, and where sterility is manifested as effect of the dysfunction of this genes in gonad cell.

١.١.٣.٢ Sterility factors caused by mutation in genes: Whose function is known to be restricted to germline.

Human genes with a specific expression pattern in the germ line are assumed to be the most important genes for spermatogenesis and their function expected, for example, during spermatogonic proliferation cycle, for meiotic synaptonemal complex formation, for timely regulated differentiation of spermatogonia to spermatocytes, to spermatid, to spermatozoa and for the renewal of spermatogonia from a permanent germ-cell stem population. They are expressed in sertoli cells, leyding cells,

interstitial cells and germ cells. Expression in different cell types and changes in their expression pattern during the process of germ-cell development are expected. Spermatogenic disruptions by mutation in germ-line genes can have a complex history and are generally difficult to detect because the genotypical haploid spermatids are phenotypically diploid.

This means that recessive and dominant mutations in spermatogenesis genes can not be distinguished and explained. However, why mutations in human germ-line genes disrupting spermatogenesis are not so much known despite our increasing knowledge about their homologous in animal (Elliot *et. al.,* ١٩٩٧) (Jones *et al.,* ١٩٩٦). Indeed, there are some exceptions, the most common genes mapped related to:-

١.١.٣.٢.١ Azoospermic Factors:

Azoospermia factors are more likely to be important factors of male infertility after excluding the obvious urological reasons and the effect of Klinefelter's syndrome.

Tieplolo and Zuffardi, ١٩٧٦ described a large microscopically visible deletion of Y chromosome in chromosomal spread of infertile men, this description leads to detection of the chromosome rearrangement in band q١١ of the Y chromosome in sterile men suggested the presence of one or more genes in this region that control spermatogenesis (AZF, Azoospermia factor).

Few years latter, Vergnaud *et al.,* ١٩٨٦, reported that molecular analysis of patients carrying chromosomal rearrangement permitted the definition seven intervals within the Y chromosomes. Andersson and his co-workers (١٩٨٨) assigned the AZF loci to interval ٦ at band Yq ١١.٢٣. This later region is subdivided into six-sub intervals (A, B, C, D, E and F) . Recently, there are different Y genes cloned from intervals ٦. At the same

time it has been argued that no less than three AZF loci exist, they are AZF a, AZFb, and AZFc.

Vogt (١٩٩٧), were first to demonstrate DFFRY gene mapped to AZFa local in distal Yq١١. The Drosophila fat factors-related Y (DFFRY) gene,which has an X homologus (DFFRy), is expressed in different tissue (Jones *et al.,* ١٩٩٦), and encodes a protein supposed to contribute to the ubiquitin mediated degradation of protein by antagonizing the function of proteasomes. Proteasomes are multimeric protein degradation complex ubiquitin as a recognition signal. Enzymes such as the DFFRY/DFFRX protein release the ubiquitin peptide chain from proteins inhibiting thus their degradation. If DFFRY contributes to the function of AZFa, testis-specific is form of its transcript and/or protein sequence is expected.

Furthermore studies reported that RBM gene family mapped to AZFb locus in distal Yq١١ (Vogt *et al.,* ١٩٩٦, Elliot, ١٩٩٧). Elliot and his colleagues ١٩٩٧ reported that RBM proteins were shown to be present in the nuclei of premeiotic germ cell; this suggests their involvement in nuclear metabolism of newly synthesized testis RNA of this phase of human spermatogenesis. Because after deletion of AZFb these proteins are absent, most functional RBM gene copies must be located in the Y regions designated as AZFb. However mutations in one RBM gene causing the AZFb pathological phenotype are not yet known. The AZFb phenotype might therefore be caused only by deletion of all RBM genes and/or additional deletion, of other spermatogenesis genes within this Yq١١ region not yet identified.

During the same period, Reijo *et al.*, (١٩٩٥); Vogt *et al.*, (١٩٩٦), found that DAZ gene family cloned from locus AZFc in distal part Yq١١. Habermann *et al.,* (١٩٩٧) showed that many DAZ proteins were present in late spermatids and in sperm tails, but their function is not known. Men with deletions of DAZ can produce mature motile sperms, although in low

numbers and in rare cases DAZ deletions can be inherited (Reijo *et al.*, ١٩٩٦); (Vogt *et al.*, ١٩٩٦).

The most common genes mutations belong to this group are listed below:

١.١.٣.٢.٢ Cyclin Genes Mutations:

Wolgemuth and Co-workers in ١٩٩٥ reported that genes involved in regulation of cell cycle are supposed to be involved in spermatogenesis.

Protein phosphorylation was recognized as an important mechanism for controlling cell cycle progression. An example are the cyclin genes, which were subdivided into different groups A, B, C, D, E, F, G, H depending on their time of function (Wolgemuth *et al.*, ١٩٩٥).

Cyclins are expressed in G٢, B cyclins are expressed during DNA synthesis. Accordingly, during spermatogenesis cyclin genes are expressed mostly during the premeiotic phase.

١.١.٣.٢.٣ Mitochondrial DNA Mutations:

It has been known that the mitochondrial dysfunction is well interfered as a factor possibly implicated in infertility (Cummins *et al.*, ١٩٩٤), (Kao *et al.*, ١٩٩٨).

Mitochondrial ATP is thought to be essential for sperm motility (Zevian *et al.*, ١٩٩٧).

In fact high quantities of energy would be depleted by sperm during their long rapid swim up female genital tracts. The mitochondrias in the sperm mid-piece are the energy generator and have been considered as the combustion engine of the spermatozoa.

So far, it has postulated that ultrastructural abnormalities of mitochondria have been observed in the azoospermia subject patient

compared to controls, suggesting a link between reduced energy production and poor sperm function (Kao *et al.*, ١٩٩٨).

It is well established like most human cells, that spermatozoa also contain copy of mitochondrial DNA (mtDNA) in addition to the nuclear DNA, so each human and animal cell contains ٧٢-٨٠ mitochondria in the mitochondrial sheath of mid-piece (Kao *et al.*, ١٩٩٨). Furthermore, it is found that human mtDNA is a (١٥٦٦٩) bp. circular double-stranded DNA molecule.This extrachromosomal genetic system contains genes coding for B poly peptide essential for respiration and oxidative phosphorylation, and rRNAs, and set of ٢٢ tRNAs that constitute the protein synthesis machinary in mitochondria. Moreover the human mtDNA is a naked compact DNA molecule without protection of histones, besides, replicates rapidly without proof reading and efficient DNA repair system, and thus a mutation rate ١٠-١٠٠ times higher then that of muclear DNA. More recently, molecular analysis of mitochondrial disease revealed distinct mutations in mtDNA that are associated with or responsible for the pathogenesis of these diseases (Kao *et al.*, ١٩٩٥).

These mutation include rearrangement of mtDNA (duplication and deletion), point mutation in mitochondrial protein synthesis genes and point mutation in the mtDNA structural gene (Pang *et al.*, ١٩٩٩).

Furthermore, there are more than ٢٤ mutations mtDNA have been identified and proved to be associated with human diseases (Wallace, ١٩٩٤), (Kao *et al.*, ١٩٩٥). Investigated mtDNA deletion associated with diminished fertility and motility of human sperm, furthermore, they examined the relationship between the proportion of ٤٩٧٧ bp. Deleted mtDNA to total mtDNA and impaired sperm motility, indeed, they have demonstrated that relationship.

A few years later (Kao *et al.*, ١٩٩٨), found two novel types of (٧٣٤٥) and (٧٥٩٩) bp. deletions in the mtDNA of spermatozoa with poor motolity, also, they found that the (٧٣٤٥) and the (٧٥٩٩) bp. deletions of mtDNA were more frequent in the spermatozoa with low motility.

١.١.٣.٢.٤ cAMP Responsive Element Binding (cREB) and cAMP Response Element Modular (cREM):

The other important gene products controlling spermatogenesis are the cAMP response element binding (cREB) protein and the cAMP element modular (cREM). They are expressed in germ cells and sertoli cell in a cyclical fashion depending on signals induced by the pitutary gonadotrophic hormones FSH (Follicle steroid hormone) and LH (Lutenizing Hormone) (Walker and Habener, ١٩٩٦). These hormones are important during sexual maturation of the testis and during spermatogenesis and thus link the genetic network of gonad development to that of spermatogenesis (Vogt, ١٩٩٧).By alternative promoter usage and alternative exon splicing events, different protein product is translated functioning as transcription activator or repressor for distinct target gene. The complex mechanisms for the regulation of cREB/cREM expression in testis suggest that the functional adaptation of their target genes is critical for proper development of the male germ cell along its whole pathway.

Masquilier and his colleagues demonstrated that cREM gene in human was mapped to the short arm of chromosomes ١٠ [١٠p١٢.١ – p١١.٢] (Masquilier *et al.*, ١٩٩٣).

١.١.٣.٢.٥ Protamine Gene Mutations:

Hecht (١٩٩٠) observed that the condensation of the germ cell chromatin was induced by germ line specific nuclear protein so that mature sperm lack histone, but contains basic proteins bound to DNA; they are called protamin (Hecht, ١٩٩٠).

Other workers reported that these proteins gradually replace the histon from DNA of postmeiotic spermatid nuclei which is prerequistes for its higher compaction in developing sperm heads (Smith *et al.,* ١٩٨٣), (Vogt *et al.,* ١٩٩٦).

Indeed, the proportion of protamine in sperm obtained from infertile male was lower than in the fertile males, (Belokopytova *et al.,* ١٩٩٣).

During the same period, other important molecular study was made suggested that some cases of human male infertility may be due to mutation within protamine p٢ gene (Miller *et al.,* ١٩٩٤), and also found that the monitoring expression of protamine p٢ genes in human, ejaculate spermatozoa can be identified as molecular marker correspond to male infertility.

١.١.٣.٣ Sterility Factors caused by mutations in genes, whose function is essential for development of gonads:

Human genes with a specific expression pattern in male gonads, those genes union to be functional in human male gonad development, the disruption at these genes leads to sterility because it produce intersexual phenotype often with large variation of expression.

١.١.٣.٣.١ Androgen Gene Mutations:

Androgen resistance AR syndromes or (testicular feminization reifenstein, infertile male syndrome). This syndrome is caused by mutations in the AR gene, (Griffin *et al.,* ١٩٩٥).

Laspadu *et al.,* (١٩٩١) reported that the most important point mutation causing these syndromes is clustered in the hormone binding domain and no amino acid substitutions have been identified in the N – terminal region although the region is essential for the function of ARs as well.

Amplification of the Glutamine repeats in exon ١ seems to induce spinobulbar muscular atrophy. This syndrome is characterized by progressive degeneration of anterior motor neurons and a late onset of mild

androgen resistance. AR mutations that are found specifically in reifenstein or infertile male syndrome have not yet been reported. Moreover, mutations in the ٥α - reductase ٢(SRD٥A٢) gene are also reported as causative agent of androgen resistance syndromes. They can be differentiated from AR mutations by measuring the level of dihydro testosterone (DHT), (Griffin *et al.*, ١٩٩٥).

١.١.٣.٤ Sterility factors caused by mutation in genes the function of which takes place in non gonad somatic cells:

Multiple somatic disorders causing disruption of spermatogenesis, which molecular genetic linkage analysis has mapped some of them to a specific chromosomes region indicating the location of at least one gene within this region responsible for observed pathological phenotype. Usually this phenotype is doubtful whether a somatic gene phenotype defect linked to infertility has dominant or recessive inheritance pattern.

١.٢ Mechanism of Chromosome Banding:

١.٢.١ Introduction:

J.C. Caspersson and his colleagues (١٩٦٨) showed selective, discrete regions of fluoresce by applying quinacrine to chromosomes of a number of plant and mammalian species. This result has opened the door to a new era of cytogenetics studies and given a powerful tool to worker in this field. Many reports have followed, including the recognition of human chromosomes and the application of this methods to clinical problems Caspersson *et al.*, (١٩٧٠); Vosa (١٩٧٠).

During the same period other important cytogenetic advances were made, Pardu and Gall (١٩٧٠) investigated the location of mouse satellite DNA using in situ hybridization of radioactivity labelled nucleic acid to chromosomes. Then Arrighi and Hsu (١٩٧٥) reported the location of the heterochromatin in human chromosomes. Their method required denaturation of the chromosomal DNA with alkali. The amount of

heterochromatin was not the same in all chromosomes. The centromeric regions of chromosomes Nos. ١,٩,١٦ and the long arm of Y have a large amount of heterochromatin. A few months later another technique was developed which had the advantage of banding the chromosome arms. This was carried out by a modification of Arrighi and Hsu's C – banding technique with the use of Giemsa stain.

Drets and Shaw, (١٩٧١); Patil *et al.* (١٩٧١); Schnedl, (١٩٧١)a,b ; Sumner *et al.*, (١٩٧١); and Seabright, (١٩٧١). All these used modified to produce

G – banding on chromosomes. These three banding techniques were called Q,C and G banding respectively (Paris Conference ١٩٧١).

١.٢.١.١ Mechanism of C-banding:

Yunis *et al.* (١٩٧١) and Arrighi and Hsu (١٩٧١) were the first to demonstrate C-banding of chromosomes. Their techniques were developed directly from those used by Pardue and Gall, (١٩٧٠) to localize satellite DNA in centromeric heterochromatin by in situ hybridisation. Because of this it was generally assumed that the dense staining of centromeres observed in C-banding was due to rapid reannealing of stellite DNA at the centromere. DNA in the arms was thought to reanneal more slowly and hence to stain less intensively. Chapelle *et al.,* (١٩٧١) tested this hypothesis using acridine orange (AO) which causes double-stranded DNA fluoresce green and the single-stranded DNA fluoresce red, after treatment with ٠.٠٣ – ٠.٧ M NaOH the color of all metaphase chromosomes changed from green to red which indicate denaturation, but after subsequent incubation with ٢X SSC for ١-١٦ hours the chromosomes fluoresce green which means that renaturation has taken place. The centromeres fluoresced intensely but the arms only weakly and this was taken to show that renaturation had occurred more rapidly in the centromeres than in the rest of chromosomes;

this was confirmed in other laboratories, Schnedl, (١٩٧١), Mace *et al.*, (١٩٧٢), Bobrow and Madan, (١٩٧٣).

However a number of subsequent experiments proved clearly that denaturation and renaturation are not the explanation of the C-banding mechanism, Comings *et al.*, (١٩٧٣) also used (AO) fluoresce to examine whether or not C-banding was a result of rapid renturation of satellite DNA. They were able to show that several different treatments would produce C-banding irrespective of whether or not the centomeric DNA was single or double stranded at the end of the procedure. They claimed that DNA denatured in site reannealed very rapidly both in centromeres and arms, and that the prolonged treatment in SSC which was regarded as a renaturation period and in fact, caused DNA denaturation. Arrighi *et al.*, (١٩٧٤) pointed out that the heterochromatin of Chinese Hamster was deficient in highly repetitive DNA sequence and therefore the highly repetitive or satellite DNA is not always present in C-band hetrochromation. Comings, (١٩٧٨) showed that double – stranded DNA does not bind greater amount of dye than single – stranded DNA.

Moreover Mckenzie and Lubs, (١٩٧٣) found that slides exposed to NaOH, whether followed by ٢X SSC incubation or not, produced at least some C-banding in metaphase chromosomes.

Furthermore, slides treated with HCl and ٢X SSC (heat), without any NaOH step, gave clear C-bands. They suggested that DNA/ protein interaction rather than DNA denaturation-renaturation is the key factor in C- banding. Comings *et al*,(١٩٧٣) reported that ٦٠٪ the labeled DNA was removed after the C-band procedure and was extracted from non C-band regions only.

Pathak and Arrighi, (١٩٧٣) also found that the loss of DNA during C-band procedure was from euchromatic regions.

They used ³H – thymidine to label the DNA of metaphase chromosomes and by autoradiography showed that the loss of DNA had occurred in the non- banded regions.

Indeed, the above conclusion brought attention to the role of protein/DNA interaction in C-band positive regions. Chromosomal proteins are divided into two classes; the basic histones and the acidic and neutral proteins usually referred to as the non-histones.

Histones may be removed from chromatin by treatment with acid. Fixation with methanol: acetic acid removes only some of the histone present (Dick and John, ١٩٦٨). Commings *et al.*, (١٩٧٣) used hydrochloric acid to remove histones from chromosomes. This had no effect on the production of C-bands suggesting that histones are not involved in the mechanism of C-banding. However Pothier *et al.*, (١٩٧٥) suggested that histones were not completely removed from the chromosomes even after

treatment with fixative or ٠.٢N HCl for four hours because positive reactions could still be obtained with anti – H_4 antibodies. This is not variance with the report of Comings and Avelino, (١٩٧٤) who found by using electrophoresis on fixed mouse nucleic treated with ٠.٢N HCL that histones were completely removed.

However, the suggestion that histones are relatively unimportant in C-banding led many workers to concentrate on the role of DNA non-histone interactions.

Burkholder and Weaver ,(١٩٧٧) suggested that the DNA – particular protein association might be involved in C-banding mechanism. Protection of DNA from digestion by DNase ١ was used to study the degree of association between DNA and chromosomal proteins in condensed and extended chromatin fractions isolated from Chinese hamester liver. They found that the extended chromatin was more sensitive to DNase ١ . This is

due to differences between the two in regard to protein binding. Histones and non-histone proteins both protect DNA from digestion but tightly associated non-histone proteins are responsible for differential sensitivity of condensed and extended chromatin to DNase ١.

Matsukuma and Utukos, (١٩٧٧) suggested that the centromeric heterochromatin in the mouse was associated with the non-histone protein. They used dansyl chloride which binds to polypeptides and reflects protein content in the chromosomes after banding. The bright dansyl – fluorescent areas of chromosomes after HCl or NaCl treatment occupied the same regions C-bands. This was taken as evidence that C-bands represent concentration of non-histone proteins.

١.٢.١.٢ C-band Heteromorphism:

Introduction

Th constitutive heterochromatin or C-band represent perhaps the most notable chromosome heteromorphisms because of its variation in length. As a matter of fact, the chromosome variations related to c-band were known even before the discovery of banding technique. However, the c-band made it possible to determine with a degree of certainty of all their variability. By this new staining method, it is now possible to subdivide a specific region into sub-unit, each showing on independent variation. Accordingly, the term chromosome variation heteromorphism is recommended (Pairs conference ١٩٧١ supplement ١٩٧٥).

١.٢.١.٣ C-band Classifications:

In the supplement (١٩٧٥) of the Paris conference (١٩٧١) the use of five classes was suggested to identify the C-band length variability. There is no objective definition for the classes when subjective or arbitrary relative forms of classification are used. Indeed the data from different workers have very few points of comparability.

The determination of variants often considering only the exceptionally long, was mainly used at the beginning of the study. But it is always helpful for certain kinds of analysis, such as segregation (Phillips, ١٩٧٧) or selective effect (Jacobs *et al.,* ١٩٧٥), because the variants can be identified beyond any doubt, and c-bands have a detectable function or selective effects which can be determined more easily in carrier of extreme variants.

Furthermore, all data which have been obtained from various studies showed a representative studies of C-band variability using subjective or relative classification. Buckton *et al.,* (١٩٧٦) tried to show a more conservative tendency, and the normal class includes more than ٩٠٪ of the bands. Muller *et al.,* (١٩٧٥) used the٢١q region as relative C-band length and obtained a different distribution from Verma *et al.,* (١٩٧٨), who used the ١٦p as a reference size. But Berger *et al.,* (١٩٧٩) with the same method of that used by Verma found difference between the result and their data were distributed in only three classes, similar to classification "small-normal large", Sofuni *et al.,* (١٩٧٩). They also used the ١٦p for the relative classification, which data are not exactly comparable with other data.

The results suggested a possible population difference. However, Caralli found similar results in length and variability in a well controlled quantitative study of C-band heteromorphism in Japanese and Caucasian sample living in Brazil (Erdtmann *et al.,* ١٩٨٢).

In view of the above, it seems likely that the agreement standard C-band classification is still not finished yet. Nevertheless, individual criteria based on the criteria highlighted by other investigations which give us some clue on the basis that one can use in the study or comparing of C-band polymorphism between different people.

١.٢.١.٤ Localization on p and/or q (C – bands Inversion):

Besides the well known size variation, the C-bands can also be used to represent position heteromorphism. Excluding the Y-terminal C-bands, all centromeric heterochromatin have a pericentric localization. The C-bands of chromosomes ١,٩ and ١٦ are generally located on q, though the pair ١ and ٢ may also show part or all of the heteromorphism heterochromatin on p, which is commonly classified as pericentric inversion.

Gosden *et al.,* (١٩٨١) and Donlon and Magnis (١٩٨١), using diverse staining methods, detected that the heteromatin on ٩p may show different staining affinities from that on ٩p. Mattei *et al.*, (١٩٨١) suggested that ٩h has two regions, also noted by Buys *et al.,* (١٩٨١): one pericentric, similar to that of other chromosomes, stains with normal, stains with normal C-band techniques but not with Giemsa ١١ and DA/DAPI, and the other region, specific to chromosome ٩, shows the stain specificity to Giemsa ١١ and also dyes with other c-banding method. They suggested that all partial inversions of ٩h result from on an increment of pericentric heterochromatin on ٩p and are not due to inversion, and that if $^{9}ph^{+}$ makes an inversion, it seems like a partial inversion with C-banding by Barium hydroxide using Giemsa methods but “total inversion” with Giemsa ١١ and DA/DAPI (Mattei *et al.,* ١٩٨١). Gosden *et al.,* (١٩٨١) also detected a partial inversion, with DA/DAPI staining heterochromatin on p and q. This is a genuine partial inversion of ٩h according to Mattei *et al.,* (١٩٨١), but it seems to be rare to date, since there has been no independent evaluation of the variability of the two heterochromatin regions of ٩h.

Perhaps it is possible to detect more types of heteromorphism by a precisely performed evaluation of the two regions. Some rare types of heteromorphism such as the partial inversion of ٩h, confirmed by Giemsa

١١ or DA/DAPI staining, can be used as population markers (Gosden *et al.*, ١٩٨١).

The detection of heterochromatin on p or q in principle is independent of subjective factors, but the reported results showed very large differences. Some of the divergences can be explained as real population differences, but the main cause is probably methodological factors. As outlined above, the C-band on ٩ may show different staining affinities and minor laboratory variations in the diverse steps of the technique can be made responsible for whether or not a specific region is well stained (Mattei *et al.*, ١٩٨١).

Another problem concerned with position identification of the heterochromatin is the very compact C-blocks with a dubious or unrecognizable centromeric location.

The use of well-extended chromosomes with a clearly identified centromere as the use of specific centromere marker (Eiberg, ١٩٧٤) can improve the analysis when the heterochromatin is located on p and q. Abe *et al.*, (١٩٩٥) reported a modified (C-banding using barium hydroxide by Giemsa) CBG method in which the centromere dots are recognized.

If we exclude the acrocentrics, whose short arms show very complex variation, and the Y, whose variable C-band is terminal, then chromosome ١٦ is the only one that has no C-band position heteromorphism (Phillips, ١٩٨٠).

According to data of Phillips (١٩٨٠), the most analyzed C-bands, those of chromosomes ١,٩ and ١٦, are the least variable in localization. Some pervious studies on chromosomes ٣ and ١٩ yielded data discordant with those reported by (Phillips, ١٩٨٠; Crossen ١٩٧٥; Soudek and Stroka ١٩٧٨; Mikelsaar *et al.*, ١٩٧٨).

If the results of Phillips (١٩٨٠) can be reproduced by well-established method, they will increase considerably the usefulness of C-band, as genetic markers.

The size of C-bands on p can also be classified into different types. The most used classification for heterochromatin on p is partial total for the chromosomes ١ and ٩. Also in use are relative classification (Muller *et al.*, ١٩٧٥; Verma *et al.*, ١٩٧٨). Comparing the heterochromatin on p with the heterochromatin on ٩: but by this method the equal size of a c-band on p can be classified diversely depending on the size of C-band on q. Less attention is given to the other chromosomes, Phillips (١٩٨٠) did not differentiat the inversion of heterochromatin. Grossen (١٩٧٥) classified the heterochromatin on chromosome ١٩ into four types: (I) small centromeric dot, (II) on p, (III) on q, (IV) on p and q.

١.٢.١.٥ Chromosome Aberrations and C – band:

There is a large body of work to suggest a positive relation between C-band polymorphism and different chromosomal aberrations. Holbek *et al.*, (١٩٧٤) found a higher frequency of $^{١}qh^{+}$ and $^{٩}qh^{+}$ in parent of aborted fetuses with chromosome aberration than in parent of those with out, further more investigations, found that $^{٩}qh^{+}$ occurred more frequently in the parents and relatives of individuals with chromosome aberration (Nielsen *et al.*, ١٩٧٤). These data suggest a greater risk of chromosomes aberration in the carrier of C-band heteromorphisms. Lopetequs (١٩٨٠) in controlless and inconclusive study detected a larger C-band in Japanese of parents of ٢١-trisomics than in normal.

Wang and Hamerton, (١٩٧٩) detected a higher frequency of $^{٩}ph^{+}$ in trisomics group than in normal newborns, but no length differences has been recognized. Furthermore, other workers showed that ٢١-trisomics and the mentally retarded with normal chromosomes showing no differences in C-band length and position (Funderburg *et al.*, ١٩٨٠).

Later many studies have followed, Ford and Lester (١٩٧٨) Found a higher frequency of mitotic non-disjunction in carrier of ٩qh^{+} than in normal and in fertile males without these heteromorphisms.

During the same period, Shabtai and Halbreet (١٩٧٩) reported a greater rate of chromosomes breakage in cells of individuals with positive C-band of chromosome ١qh^{+}, ١ph^{+} and ٩ph^{+} and ٩qh^{+} .

On the other band, another type of analyses was made by Schmid *et al.*, (١٩٧٥) and the result showed that the acrocentric chromosomes are preferentially attracted to centric heterochromatin of chromosomes ١,٩ and ١٦ and the larger C-bands demonstrated larger attraction. However, Driscoll *et al.*, (١٩٧٩) detected the similar result but less degree of association which is found in meiosis, but they did not relat it to the sizes of C-band.

Furthermore, the acrocentric chromosomes which are frequently involved in association are also involved in non-disjunction and translocation's. If the larger C-bands determine a higher attraction or association rate, as Schmidle *et al.*, (١٩٧٥) have reported, then larger heterochromatic segments might predispose to chromosome anomalies.

In addition, many authors reported a possible relationship between patients carrier C-band positive and some of congenital malformation, Gardner *et al.*, (١٩٧٤) suggested a possible prodisposition of ١qh^{+} carrier to apatau/nekellike syndrome. Habrech and Shabtai results (١٩٧٦) were consistent to certain extent with this suggestion.

Furthermore, Kunze and Mau, (١٩٧٥) reported that in ٢٣ patients with congenital malformation and mental retardation all have at least one heterochromatic variants. In spite of the importance of this problem, these finding are undependable because of their error of methodology. Similarly, very little can be deduced from papers describing the association of

congenital malformations with C-band variants in selective samples (Leonard *et al.*, ١٩٧٥; Pescia *et al.*, ١٩٧٧).

However, the facts that the C-band variants are common, and the classification of C-band often is subjective, further the congenital malformation with unknown etiology are more frequent, may all easily lead to misinterpretation.

Brown *et al.*, (١٩٨٠), by using an objective measurements of C-band, in a well-controlled sample of ٣٨ abnormal and ٣٩ normal newborns their parents, failed to establish any differences. More studies of this type are needed to reveal the possible relation between C-band and phenotype.

١.٢.١.٦ Reproductive fitness and C – band:

Some other publications claimed an association between c-band variants and abortion (Patil and Lubs, ١٩٧٤; Boue *et al.*, ١٩٧٥; Tsengh *et al.*, ١٩٧٦), but these conclusions were uncertain due to perhaps methodological problem. The controlled studies of Hemming and Barns (١٩٧٩) and Ward *et al.*, (١٩٨٠) failed to find an increase in C-band heteromorphism in female with repeated abortion.

During the same period, other applications of C-band was made, by some workers, Patil and Lubs, (١٩٧٧) and Nielsen (١٩٧٨) found a higher abortion rate (approximately twice as high) in mothers of boys with Yq+ than in mothers of boys without Yq+ .On the other hand, it is interesting to compare precise Y measurements with their abortive effect to determine whether specific type of the Y is associated with higher rate of abortion.

Beltran *et al.*, (١٩٧٩) detected a positive correlation of the Y euchromatin variation between ١٨ fathers and their newborn sons with multiple congenital anomalies, but they failed to find similar significant result between ٢٧ fathers and their normal sons. It is possible that the

variation in euchromatin of the Y play the main role in their deleterious function.

On the others hand, the role of chromosome aberration as one of the most important causes of abortion is well documented. As well as the reason for reproductive failure or abortion is often unknown, as the reason for C-band heteromorphism. The reproductive fitness considered in general or in specific aspects is good parameter to evaluate the selective effect of genetic polymorphism trait.

One of the important study that has been carried out by Jacobs *et al.*, (١٩٧٥) who analyzed the reproductive fitness of ٣١ probands with extreme c-band variants and their first degree relative without these variants as controls. They calculated in two ways: The first method was based on live birth, fetal and infant deaths, and generation times; The second was based on segregation analysis. The first method of calculation showed significantly lower reproductive fitness of the carriers when compared with the control, but the second resulted in no significant differences was detected. These differences between two methods of calculation ways depending by this author, as well as the unexpected low fertility of the carriers variants need to be analyzed further in order to come to a final conclusion?

Dellachapella *et al.*, (١٩٧٤) analyzed the segregation and abortion rates of ten families with pericentric inversions of the heterochromatin but failed to find a selective effect of these variants. In spite of the possibility of chromosomes imbalance resulting from normal and c-band inverted chromosome (Howard-Peebles, ١٩٧٨; Daniel, ١٩٨١), in fact this probability has proved to be very low and has yet been detected.

١.٢.١.٧ The function of Constitutive Hererochromatin:

As a matter of fact there is very little information that can be considered as a demonstration of heterochromatin function. The C-bands contain a large a mount of repetitive DNA of obscure function. The aspect

that leads us to inquire about this problem is the great amount of DNA and the spectacular heteromorphism of the C-bands. Many studies showed a great population and evolutionary stability of C-band heteromorphisms. Ibramov *et al.,* (١٩٨٢) did not find any statistically significant difference in the frequency of the C-band classes in four mongolian groups living under different ecological conditions. Erdtmann *et al.,* (١٩٨١), detected rare slight differences in size of autosomal C-band in five Indian tribes living in north and in south of Brazil and obtained only small difference between Indians and Caucasians. Similar results were obtained by Cavalli for the Japanese and Caucasians samples from Brazil (Erdtmann *et al.*, ١٩٨٢).

In addition many other workers reported a smaller C-band average for chromosomes ١,٩ and ١٦ in blacks than in Caucasoids from Brazil; However, Lubs *et al.*, (١٩٧٧) and Verma *et al.*, (١٩٨١) obtained similar frequencies in c-band classes of Blacks and Caucasoids from USA.

From interpopulation comparisons, C-band seems to maintain a constant amount of constitutive heterochromatin. A significant interpairs compensation can be confirmed by the negative correlation between c-band average length on pair and sum of the other pairs.

The interpopulation homogeneity and the interchromosomal compensation of C-band sizes strongly support the constitutive heterochromatin having a biologically important function. It has been proposed that the heterochromatin has the passive role of bodyguard, i.e., it is used by the cell as bodyguard to protect the vital euchromatin by forming a layer of dispensable shield on the outer surface of the nucleus, Mutagen, clastogenes, or even viruses attacking the nucleus must first make contact with constitutive heterochromatin which absorbs the assault, thus sparing the euchromatic genes damage, unless the detrimental agents are over powering (Hsu, ١٩٧٥).

This hypothesis can be tested with cells from individuals with extreme amounts of heterochromatin, or also with animal of related species and different amounts of constitutive heterochromatin.

The preferential and frequent breakage on the heterochromatin regions of chromosomes ١,٩ and ١٦ in individuals with combined immune deficiency (Fryas *et al.,* ١٩٨١), may considered to be consistent with bodyguard hypothesis; perhaps other pathological conditions involving chromosome breakage, as the Bloom syndrome, can provide additional information about this problem.

Mitomycin C is the most important mutagenic agent that increased breakage of the centromeric regions of chromosomes ١,٩ and ١٦ in normal cells (Schapp *et al.,* ١٩٨٠), but this mutagen did not effect the cells from patients with Blooms syndrome (Kuhn, ١٩٧٨). A possible explanation is that in this syndrome an altered order chromosome in the interphase nucleus may cause an altered response to the mutagen mitomycin C.

Based on the experiments described here it is thought that C-bands represent specific regions of strong association between DNA and non-histone proteins. This association minimizes the loss of DNA during the various C-banding procedures and thus the bands subsequently stain heavily with Giemsa.

١.٢.١.٨ Mechanism of G-banding:

A major question which accompanied the development of G-banding techniques; is whether the bands already present on the chromosomes and simply require enhancement, or are they induced by the technique itself ?

A considerable body of evidence has been produced which states that treatment of chromosomes by any of the G-banding techniques is unlikely to induce the banding de novo.

Yunis and Sanches, (١٩٧٣) reported typical G-banding pattern by staining chromosomes prepared by standard techniques with ١/٨٠ or ١/١٠٠ dilution of Giemsa. The same chromosomes when stained with ١/١٠ Giemsa/phosphate buffer showed uniformly staining chromosome arms. Mckay, (١٩٧٣) observed bands similar to G – band in the fixed mitotic chromosome of mouse ٢ cells without any further treatment. Comings and Okada, (١٩٧٥) using electron microscopy pointed out bands of condensed chromatin in unstained preparation also the chromatin of the chromosomes were shown to be highly condensed. An important observation was reported by Okada and Comings, (١٩٧٤) who found that chromosomes no.١ and no.٢ of the chinese hamester showed a correlation between their G – banding pattern in mitotic chromosomes and the position of chromomeres in meiotic pachytene bivalents. This was established by comparison between untreated Giemsa stained pachytene bivalents and metaphase chromosomes.

The short arm of metaphase chromosome no.١ possessed seven bands stained intensively including the telomere and the centromere. These bands correspond to heavily stained chromomeres in the pachytene bivalent. The long arm had ten bands which again corresponded to chromomers in the bivalent. The interband regions were also found to match poorly stained regions in pachytene bivalents. Luciant *et al.,* (١٩٧٥) showed quite congenitally that there is a considerable correlation between the chromomeres of bivalents and described G – bands for human chromosomes.

From the above findings, one can conclude that G – bands are already present in the mitotic chromosomes and need enhancement to make them clear. Another set of experiments deal with the mechanism of this enhancement. Burkholder (١٩٧٤) and (١٩٧٥) noted that unstained chromosomes which were with trypsin when viewed by electron

microscopy showed interbands with less densely packed chromatin fibers and bands with highly packed chromatin fiber while the untreated chromosomes were uniformly electron dense. The interbands appear to have changed as a result of the trypsin treatment. This is unlikely due to loss of chromatin as Comings *et al*. (١٩٧٣) reported that G – banding removed less than ٩.٠٪ of chromosomal DNA. The most likely explanation of Burkholder's results is that the appearance of the interband regions is the result of rearrangement of the chromatin fibers by trypsin.

The bands described by Burkhoder were of much less intensity than these seen after Giemsa staining. Similarly banding when feulgen was used instead of Giemsa. Comings, (١٩٧٥) using thionine also found very weak bands. This suggests that Giemsa itself may play a role in the enhancement of the bands.

Giemsa stain is composed of methylene blue and its oxidation products, azure A, B and C combined with eosin. Except for eosin, all the dyes are thiazins which have two benzene rings bound together by a nitrogen or sulphur atom. The number of methyl groups varies, methylene blue has four, azure B three, azure A two, azure C one, thiazine none. The mode of action is that the positively charged thiazine due stacks on the negatively charged phosphate group of DNA (Comings, ١٩٧٥).

This is associated with a decrease in the optical density and a shift in the peak of absorbency to lower wavelength (metachromasia). Accordingly there is a change of color from light blue to a dark magnata.

Comings and Avelino (١٩٧٤) showed that when pure DNA was treated with excess dyes the (r) value (moles of bound dye/mole of nucleotide) was ١.٠ indicating that all the phosphate groups were bounded because of the excess of the dye molecules. In the unfixed chromatin the (r) value was ٠.٦ indicating that about ٥٠٪ of the phosphate groups were bounded whereas in fixed chromatin the (r) value was ٠.٨ which means that

phosphate groups were able to interact with to dyes. This may be due to some loss of histone during methanol / acetic acid fixation. When fixed chromatin is treated with saline – citrate at ٦٠ C for ٣ hours (as in some G – banding techniques) the (r) value drops from ٠.٨ to ٠.٥٥).

Comings (١٩٧٨) proposed a hypothesis to explain both the reduced r value and production of discrete bands. The hypothesis states that since G – banding procedures are known to be mildly denaturing to proteins such treatments may cause non – histone proteins in the interband regions to be denatured. The denaturation would in turn prevent more of the phosphate groups in DNA from binding with the thiazin dyes and thus reduce both the (r) value and the intensity of staining in the interbands. Such denaturation may also be the cause of the dispersion of chromosome fibers which Burkholder observed in the interband.

It is clear the mechanism of G – banding is such less well understood than that C – banding. The position is made more complex by the fact that Q – bands appear to be almost identical with G – bands although no pretreatment is involved in this case and the intensity of quinacrine fluoresce is dependent on the DNA base ratios (Ellson and Barr ١٩٧٢). Two other factors should also be noted in relation to G – banding:

(i) Although DNA loss from chromosomes during the G – banding procedure is small, it may be specific in location and play a role in banding.

(ii) The physical condition of the chromosomes may affect their banding for routine G – banding slides are usually stored in a dry atmosphere for more than one week. During this time the chromosomal structure may collapse. It has been suggested (Gromley and Ross ١٩٧٦) that such collapse may be important in banding.

In conclusion, G – banding procedures appear to affect interbands rather than the bands, which probably represent either individual chromosomes or groups of chromosomes. The fibrillar structure of the interband region is affected and this results in failure of the DNA to stain probably due to non – specific blocking of phosphate groups by denatured.

١.٢.١.٩ Satellite Association:

It has been observed that certain chromosomes tend to occupy positions in the mitotic cells close to one another (Miller *et. al.,* ١٩٧٧). The most obviously associated chromosomes are numbers ١٣,١٤,١٥,٢١ and ٢٢ that are often linked together by their short arms. This phenomenon is called satellite association.

Acrocentric association frequency varies between individuals and the basis of this variation is thought to produce an extensive polymorphisms for associating ability in each homdogous class of nucleolus-organizing chromosome. (Ying Yip *et al,* ١٩٨١). A broad range of criteria, sometimes subjective, has been used to determine the associations, a clear definition of the term, therefore is lacking (Rodman *et al.*, ١٩٧٨).

All previous studies have been designed with consideration that the satellite regions of human acrocentric chromosomes are frequently located near one another in metaphase preparations, and that relationship referred to satellite association (Miller *et al.,* ١٩٧٧). Indeed, particular attention has been directed toward the question whether and why the involvement of different acrocentric chromosome is non-random (Hansson and mikkelsen., ١٩٧٨) and toward the correlation between satellite association tendency and the incidence of anomalies involving acrocentric chromosome (Ohno *et al.,* ١٩٦١). However, number of studies have shown that the acrocentric chromosomes of human metaphase complement are involved in non-random association and there is a correlation between satellite association

tendency and the incidence of anomalies involving acrocentric chromosomes (Miller *et al.*, ١٩٧٧).

This observation is in agreement with the suggestion of Ohno *et al.*, (١٩٦١), who reported that acrocentric associations might be related to Robertsonian translocations, while (Ferguson Smith *et al.*, ١٩٦١) suggested that satellite associations might have an influence on both mitotic and meiotic non-disjunction, which given rise to zygotes with numerical chromosome abnormality.

The emphasis in previous studies has been on the definition of specific chromosome pairs or groups that form associations (Miller *et al.*, ١٩٧٧). However, it has been considered that the characteristic tendency of each chromosome to be associative (Rodman *et al.*, ١٩٧٨) contributes to an overall pattern of spatial relation ships of the entire complement.

Retrospectively, its considered that chromosome association to be detectable at metaphase as relices of spatial proximity at interphase (Rodman *et al.*, ١٩٧٨), and may be postulated to result from the following:-

١-**Functional homology:** The only known chromosomal activity in which functional homology would require spatial proximity of multiple sites is that of the nuclear organizer region (Ferguson-Smith, ١٩٦٧).

٢-**Molecular homology:** It has long been considered, that DNA regions of similar nucleotide sequence tend to associate and that tendency is greatly accentuated if the sequence is highly repetitive and clustered (Mayfield and Ellison, ١٩٧٥).

٣-**Inter chromosomal connecting fibers:** It has been seen that inter chromosomal connectives are attached to the centromere regions of two acrocentric chromosomes (Moore and Albueb, ١٩٧١).

٤- **Homologues Association:** It’s known that pairing of homologues in somatic cells, with the degree of the fidelity of meiotic pairing, to occur

only in genesis of polytene chromosome (Rodman *et al.*, ١٩٧٨). Furthermore well known phenomenon and occasional evidence of somatic crossing over in mammalian cells (Gibson, ١٩٧٠). This would suggest that the capacity for pairing of homologous chromosomes, that manifested and essential in meiosis, may be retained in mitotic cells.

٥-**Methodologic artifact:** In addition to the intrinsic bases for chromosome association, some consideration must be given to the possible artifactual manifestations of association arising from the method of cytological preparation in which the chromosomes are disloged from their in situ position and dispersed (Rodman *et al.*, ١٩٧٨).

Indeed, the results of the mitotic investigations were often inconsistent, some workers reported random association and other non-random involvement of the acrocentric chromosomes (Guichaoua *et al.*, ١٩٨٦). Likewise, several investigators found that there is no correlation between the distribution of Robertsonian translocation and the distribution of acrocentric chromosomes to associate depends on the activity of the nucleolus organizing regions (NORs) (Zankl *et al.*, ١٩٨٠).

It is clear that the nucleolus organizer regions (NORs) on acrocentric chromosomes are associated with the secondary constrictions on the short arms and has been shown by in situ hybridization techniques to be the sites of ١٨s and ٢٨s rDNA (Ironside and Faed, ١٩٧٩).

Furtherly, Miller *et al.*, (١٩٧٧) suggested that only those NORs that were functionally active during the preceding interphase are stained by the specific silver techniques and also suggested the amount of Ag^{-} staining of NORs on acrocentric is correlated with the frequency of their participation in satellite associations (Miller *et al.*, ١٩٧٧). These two characteristics, associative tendency and NOR activity, are inheritable in a stable Mendelian Fashion (Zakharov *et al.*, ١٩٨٢).

On the other hand, the variable dispersion of the metaphase chromosomes in the cytological method could have a randomizing effect (Rodman *et al.,* ١٩٧٨). Therefore, on the premise that persistent and extreme doseness reflects the in situ pattern rather than the effect of dispersion, only the shortest interchromosomal distances of each metaphase were considered to represent association.

Furthermore, an association was considered to be present of the satellite ends of two (or more) acrocentic chromosomes may lay within ١.٢ micrometer of each other (Ying Yip and Fox, ١٩٨١). It's also known that the frequency of satellite association can vary in the same individual according to different physiological conditions, such as the amount of the thyroid hormones (Nilsson *et al.,* ١٩٧٥), as well as in cell cultures depending on the different culture conditions (Zang and Back, ١٩٦٩). Other factors, such as the different techniques involved in chromosome spreading and preparation, can also have marked effect on the frequency of satellite association (Nankin, ١٩٧١). On the other hand some authors have indicated that aging, may bring changes in activity of acrocentrics (Mattei *et al.,* ١٩٧٦), whereas, other studies suggested that the possibility of activity of acrocentrics is not necessarily affected by age (Nikolis *et al.,* ١٩٨١).

١.٢.١.١٠ Satellite Association in Some Pathologic Conditions:

There is a complicated relationship between satellite association, NORs, and some pathologic conditions. The results of other studies have been conflicting. It has been found with trisomy ٢١ that the number of associations is significantly higher in these trismoic cells than those in cells of normal individuals (Roserkranz and fleck, ١٩٦٩).

Furthermore, reports had suggested that satellite association involving acrocentric chromosomes, and specifically association involving chromosome No. ٢١, were seen more frequently in parents of Down's syndrome patients than in controls (Hansson and Mikkkelsen, ١٩٧٤), leding

credence to the idea that satellite associations play a significant role in non-disjunction events leading to down syndrome, (Jacobs and Mager, ١٩٨١), The same observations were made by Hansson, (١٩٧٥), who found a highly significant increase in satellite associations involving chromosome ٢١ in the parent in whom the no-disjunction was known to have present although they also found a significantly increased association frequency for certain chromosomes, including chromosome ٢١, in the parents in whom the non-disjunction had not occurred. Other investigations, however, did not find any increase in satellite association in parents of Down's syndrome patients (Taysi, ١٩٧٥). Indeed, the association, involving chromosome No. ٢١, was significantly higher in trisomy-٢١ individuals (Rosenkronz and Fleck, ١٩٦٩). Though Yip and Fox ١٩٨١ reported the absolute frequency of acrocentric association was lower in trisomy ٢١ individuals than disomic controls but the relative involvement of chromosome No. ٢١ **(**after correction for the trisomic state**)** was higher than in normal controls (Yip and Fox, ١٩٨١).

On the contrary a negative correlation has been found in patient, with trisomy ١٣. This phenomenon was interpreted as resulting from decreased activity of the NORs in cells with supernumerary acrocentric chromosome which lessened the tendency to build up large association (Zankle *et al.*, ١٩٧٩) and, further, these results seem to indicate different involvement of the acrocentric chromosomes in the nucleolus organization in trisomies ١٣ and ٢١ (Zankle and Zang, ١٩٧٩).

In cystic fibrosis, it has been observed that phytohemagglutinin (PHA) stimulated lymphocyte from CF patients, which exhibited a higher frequency of satellite association (Ravia *et al.*, ١٩٨٥). However, this fact seemed to develop a diagnostic method for early identification of cystic fibrosis.

It has been reported that hyperthyrodism patients exhibited a higher number of chromosomal associations than those usual, a reduction of the

association frequency after the patients were treated for there conditions (Nilsson *et al.*, ١٩٧٤). Furthermore, it has been found significantly increased association frequency of chromosome ١٤ and ٢١ hyperthyroid patients, which decreased after treatment. Whereas, (Zankle *et al.*, ١٩٨٠) did not confirm that increased frequency of chromosome ١٤ and ٢١ in hyperthyroid patients. Also, higher stability of chromosome ٢٢ was observed after treatment of hyperthyroid patients (Zankle *et al.*, ١٩٨٠).

More recently, there is a study of activity of satellite association and polymorphisms of Ag^- stained nucleolus organizer regions (Ag^+ NORs) in lymphocytes from woman with cervical uterine cancer, it has been found that average number of (Ag^+ NORs) was higher in woman with adenocarcinoma than in controls. Also, the most frequent association is in patients controls (Cortes-Gutierrez *et al.*, ١٩٩٧).

However, it has been interpreted that the frequency of satellite association and of polymorphism, of (Ag^+ NORs) could be related to the neoplastic process.

١.٣. Background to the Present Study

Chromosomal abnormalities are not uncommon among men with infertility. It is estimated at between ٢٪ to ٢٠٪ and it is some five times higher in the infertile men than the general population. It has been revealed that there are two ways by which chromosome disorder can be influencing fertility. Firstly, they can disturb the development of the testis leading to a serious impairment of its normal function. Such disorders are usually caused by abnormality of sex chromosomes (Chandley et al., ١٩٧٥). Secondly, autosomal anomalies may result in disruption of the normal process of cell division that occur during the development of the gametes. Furthermore, it has been reported that over ١٠٪ of human males with a history of infertility suffer from oligospermia and azoospermia of unknown cause (Hargreane , ١٩٩٠). Idiopathic oligospermia is another cause of male

infertility, affecting about ٣-٤٪ of men. After the first description of large microscopically visible deletion of the Y chromosome in chromosomal spreads of infertile men (Tiepolo and Zuffardi , ١٩٧٦) the efforts of many geneticists lead to the characterization of several discrete tracts on the Y chromosome that contain genes probably involved in male gamete maturation (Simoni et al., ١٩٩٨).

On the other hand the frequency of satellite association has been studied in somatic cell metaphases by many authors (Patil and Lubs ,١٩٧١; Cook, ١٩٧٢, Nakagome, ١٩٧٣; Galperin –Lamaitre et al. ١٩٧٧). Their aims were to specify the involvement of acrocentric associations in the occurrence of chromosomal non-disjunction and Robertsonian`s trans location.

A high frequency of specific acrocentric associations has been considered as a predisposing factor to meiotic and mitotic non-disjunction (Ferguson-Smith et al., ١٩٦٤). Matti et al. (١٩٧٦) also suggested that the unequal frequency observed in the distribution of Rabertsonian`s translocation constituted an argument supporting the view that the association between acrocentric chromosomes do not occur at random. Furthermore, satellite association has recently been associated with infertility (Yasseen et al., ٢٠٠٠, ٢٠٠١a,b).

Based on the above information and despite the large body of work which has carried out the etiology by which the sex chromosomes and, to a lesser extent the autosomes lead to oligospemia or a zoospermia are still to be revealed.

Accordingly the present investigation is designed to:

١- Estimate the contribution of chromosomal abnormality among infertile men by conducting a cytogentic analysis to a large number of men who attended infertility clinics.

٢- Assess the contribution of C-band polymorphism on male infertility. This approach is born in mind on the basis of the fact that the gene responsible for the spermatogenesis is located near the heterochromatin region of the Y- chromosome. Thus, any change in the size of the heterochromatic region may have direct or indirect effect on the genes responsible for sperm production .

٣- Evaluate whether the large C- band which is usually located in the centromeric regions of chromosomes number ١, ٩ and ١٦ in comparison with the rest of the chromosomes may have a positive or negative relationship with infertility.

٤- Assign the most frequent associated chromosomes that might predispose the chromosomes to centric fusion and might have indirect effect on spermatogenesis by applying G-banding technique.

Chapter Two

٢. Materials and Methods:

During two-year period a total of two-hundred and thirty infertile male patients and one hundred normal control were subjected to the present study. All patients were assessed by consultant urologist and by clinical laboratory tests.

٢.١ Patients selection

٢.١.١ Azoospermia Patients Characteristics :

One hundred twenty four patients were elected . Azoospermia as a term means the absence of notable sperm during semen analysis for three months apart providing that patients should be on abstine for three days before each examination trail. The manifestation of azoospermia is failure in assesting conception in his mate although normal sexual life was evident. The spermatologic parameters were noted in table I . Means of their hermonal profile were listed in table II .

٢.١.٢ Oligospermia Patients Characteristics :

Eighty eight patients were elected for genetic analysis. Oligospermia as scientific entity means those subjects having sperm count less than ١٠ millions on three semen exams in three successive months apart providing that patients should be on abstine for three days before semen exam trial. The manifestation of oligospermia is either sub or hypofertility appeared in a failure in assisting conception in his mate although normal sexual life was indicated.A spermatologic parameters accompanied , hormonal states were mentioned in tables I, II.

٢.١.٣ Klinefilters syndrome:

A genetic disorder in human being concerning the chromosome constitution of ٤٧ ,XXY ,e.i. they have an extra X chromosome . A total of ١٢ Klineferters cyndrome patients were scored for genetic screening in this study . So far the reproductive fitness of the KF patients is concerned , they

where azoospermic subjects as spermatologic examination indicated. Mostly they showed gynecomastia ,small testis under developed sexual characteristics. Some were with mental retardation and delayed puberty. The sperm parameters were listed in table I. Hermonal profiles were also mentionened in table II .

Table (I): Characteristic Endocrine Profiles in Infertile Male Patients

Conditions	T	FSH	LH
Azoospermia	low	high	High
Oligospermia	low	high	High
Klinefelter's syndrom	low	high	High

T, testosteron: FSH , follicle-stimulating hormone; LH, luteinizing hormone

Table (II): Semen Evaluation of Azoo.,Oligo., and KF

Conditions	Azoo.	Oligo.	KF
Ejaculate rate	١-٢ ml	٠.٥-٥ ml	١-٣ ml
Sperm concentration	Zero (sperm/ml)	<١٠ millions (sperm/ml)	Zero (sperm/ml)
Motility	< ٥٠٪	< ٥٠٪	< ٥٠٪

٢.٢ Preparation of culture Media (Microculture technique):

Human microculture technique was carried out according to the method of Moorhead *et al.*, (١٩٦٠).

The culture media were prepared from XI stock of RPMI culture media (Gibco laboratory) and buffered by sodium bicarbonate.

All culture contained (٥٠) units per ml or (١٠٠Ml/ml)-benzyl penicillin and (١٠٠Ml/ml) streptomycin, ٢٪ phytohaematoagglutinin and ٢٠٪ foetal bovine serum.

Initiation of peripheral blood culture prerequisites adding of ٠.٥ml of blood within aseptic conditions to each ٥ml of complete media which

perceivable in tithium heparinized tube. The contents are mixed gently and incubated at ٣٧C for ٧٢ hours and shaked twice daily.

٢.٣ Chromosome Cytology:

Colchicine (sigma) was added to exponentially growing culture at a final concentration ٠.٠٠٤٪ (µg/ml) (W/V) and incubation continued for a further ٢ hours. At the end of the incubation period, the contents of culture tubes were transferred to conical ١٥ml centrifuges tube, and centrifuged at ١٥٠g for ٥ minutes at room temperature. The supernatant was discarded and the pelleted cells gently re – suspended in the remaining media. Approximately ١٠ml of prewarmed ٠.٠٧٥M KCL was added and the suspension was allowed to incubate in a water bath at ٣٧C for ٣٠ minutes with agitation every ١٠ minutes. After centrifugation the supernatant was removed and the cells were re – suspended as above. Two ml of freshly prepared chilled fixative (methanol: glacial acetic acid; ٣:١ V/V) was added dropwise with constant agitation and the tubes were then kept in refrigerator for at least ١٥ minutes. The fixative was changed twice and finally suspended in I ml of fixative.

The cells suspension was added dropwise using a Pasteur pipette to cold wetted slides from a height of about ٣٠ cm. The slides were dried in a stream of cold air and stored in dust – proof boxes at room temperature.

Slides were cleaned before use by boiling in tap water for ٣٠ minutes and rinsed in distilled water. They were then stored at ٤ C in distilled water.

٢.٤ Giemsa Staining Technique:

Air dried slides were placed flat on staining rack and flooded with ١٠.٠٪ (V/V) Giemsa in distilled water for ١٠ minute followed by a brief washing in running tap water. The slides were then mounted in depex after air-drying.

٢.٥ C – banding Technique:

C-banding was carried out by a modification of the method of Arrighi (١٩٧٤).

Freshly prepared slides, one to three days old, were placed in freshly prepared ٥٪ (W/V) saturated aqueous solution of barium hydroxide octahydrate [$Ba(OH)_{٢}.٨H_{٢}O$] for ٥ minutes at ٣٧C. This was followed by thorough rinsing with distilled water to remove all of the remaining barium hydroxides. The slides were then incubated for one hour in ٢X SSC (٠.٣M sodium chloride containing ٠.٠٣M trisodium citrate) at ٦٠-٦٥C followed by a quick rinse in distilled water and staining in ٢٪ (V/V) Giemsa in distilled water for ١ ½ hours. The slides were then washed briefly in running tap water and dried before mounting.

٢.٥.١ C – band heteromophism calculation:

To determine the lengths of C-bands the methods of linear measurement was used (Balicek *et al.*, ١٩٧٨; Podugolnikova *et al.*, ١٩٧٩). Measurements were performed directly from the positive photographs . Every measurement was repeated five times.

The absolute lengths of C-bands are presented in micrometers and the relative lengths as percentage of total length of C-bands in chromosomes ١,٩,١٦ and Y.

Three metaphases were photographed from each individual and reviewed. The length of the heterochromatic regions was classified to five levels (level ١,٢,٣,٤ and ٥) according the qh region to the short arm (p) of chromosome ١٦.

The localization variants were considered partial inversion when more than one-third of the c-segment located on the short arm and considered total inversions when the entire C-segment located on the short arm.

The heteromorphism among homologous chromosomes ١,٩ and ١٦ was defined as a difference of one or more c-segment length levels.

٢.٦ G – banding Technique:

This procedure was carried out by a modification of the method originally described by seabright ١٩٧٢.

Three days old slides, were incubated for ٢٠-٣٠ second in the ٠.٠٠٥٪ trypsin solution in acoplin jar. The slides were then rinsed thoroughly with cold (refrigerated) PBS, followed by staining with ٥٪ V/V Geimsa in distilled water for ١٠ minutes. These slides were then washed briefly in running tap water and dried before mounting.

٢.٦.١ Chromosome counting and satellite Association Scoring:

Well spread metaphases were selected under the low power of the microscope and transferred to oil immersion (X١٠٠ objective). Good spreads with clear morphology were counted by eye. Three separate counts were made for each spread. Whenever spreads were not so clearly arranged So as to allow direct counting by eye. A quick line drawing was made for each chromosome and the chromosomes then counted from the drawing as that suggested by Brievt. At least twenty spreads were counted for each individual cell type. Satellite association was scored if the satellited ends of two or more acrocentric chromosomes lay within ١.٢ μm (Ying Yip and fox, ١٩٨١) of each other.

٢.٧ Y chromosome measurments:

One hundred fertile males of different age group were included in the present investigation. Human Y chromosome was classified into five classes (very small, small, medium, large and very large) according to that published by other investigators (Verma *et al.,* ١٩٨٢). The classification was based on Y/F index. (F) the value of F was based on the average lengths of chromosomes ١٩ and ٢٠.

٢.٨ Photomicroscopy :

A Reichart microscope fitted with olympus camera was used for the photography of Giemsa stained, C-banded and G-banded chromosomes . Kodak (٣٢-ASA) film was found to be a suitable film for obtaining a clear photograph .

٢.٩. Statistics

The results of the investigation were statistically evaluated using the chi-square test (Yates correction factor was employed) and a NOVA test , Z- test, simple linear regression and Correlation coefficient were also used and the hypotheses were tested by the t-test. The evaluation of the genetic analysis in this study according to Daniel,١٩٨٤.

Chapter Three

3. The Results:

A total of two–hundred and seventy–seven male patients with clinical diagnosis of male infertility as have been assessed by urologist were subjected to the present study. A further one hundred normal controls were included in parallel during two–year period starting from July ١٩٩٨ till July ٢٠٠١. The lymphocyte of two hundred and twelve patients were successfully cultured and adequate metaphase spreads were obtained. The cultures of sixty-five patients failed to grow, thus they were excluded from the study. The patient's ages ranged from ١٩ – ٥٤ years. One or two slide were conventionally stained with Giemsa stain for chromosome counting and preliminarily analysis. The rest of the slides were subjected to C and G – banding techniques for further cytogenetic studies.

For each patient, fifty spreads were selected for chromosome counting and only ten C – banded metaphase spreads were photographed for the study of C – band polymorphism. All the spreads were randomly selected providing that the chromosome should be well separated from each other. In case of G – band, twenty-five G – banded spreads were studied for satellite association scoring. Due to the large number of photographs a representative plates are displayed when required.

3.1 Giemsa Stain and Chromosome Analysis:

Out of a total of two – hundred and twelve infertile men who were subjected to the present investigation only twelve patients showed an obvious chromosomal aberration which account to ٥.٧٪. Table (١) shows that out of one – hundred twenty four azoospermiac, only four patients had abnormal karyotypes, which account to ٣.٢٪. Only eight oligospermic

(٩.١٪) out of eighty-eight patients showed an obvious chromosomal aberration.

Table (٢) summarizes the type of chromosomal abnormalities, which have been detected in four azoospermia patients. It is clear that sex chromosomal abnormalities were the sole causes. No autosomal abnormalities were associated with azoospermia in our results. Autosomal abnormalities were found in only one out of eighty-eight oligospermia (table – ٣). Seven other oligospermic patients were found to have sex chromosome anomalies which account to (٧.٩٥٪).

The chromosomal constitutions of twelve infertile men who showed an obvious chromosomal aberration are grouped in table (٤).

Karyotype investigation revealed the following:

١-Four azoospermia patients showed ٤٦,XXY male mitotic karyotype, which was associated with klinefelter's syndrome. No other abnormalities were detected.

٢-Seven oligospermiac patients revealed a mosaic ٤٦,XY/٤٧, XXY male mitotic karyotypes, which is associated with klinefelter's syndrome, although the mosaic ratio seems to be not the same (table – ٤). The majority of these patients did not have a typical klinefelter's syndrome clinical feature (e.g. Small testes, gynecomastia).

٣-One patient showed ٤٦,XY/٤٥,XY, t(D;D) male mitotic karyotype with mosaic ratio of ٧٠:٣٠ respectively.

٤-The rest of patients showed normal human karyotype with chromosomal constitutes of ٤٦,XY male mitotic karyotype with no obvious chromosomal aberration seen.

Table (٥) summarizes the percentage of occurrence of chromosomal abnormalities among the infertile males.

Fig (١) is a representative metaphase of azoospermic patients with ٤٧,XXY which is associated with (klinefelter's syndrome).

Fig (٢) A representative C-banded metaphase spread of an oligospermic patients with ٤٥,XY,t(D;D). The translocation is arrowed

Table (١): Chromosome studies of infertile men (Azoospermia and oligospermia).

Type of infertility	Number of patients analyzed	Number of normal karyotype (%)	Number of abnormal karyotype (%)
Azoospermia	١٢٤	١٢٠(٩٦.٨)	٤(٣.٢)
Oligospermia	٨٨	٨٠(٩٠.٩)	٨(٩.١)
Total	٢١٢	٢٠٠(٩٤.٣)	١٢(٥.٧)

Table (٢): Type of chromosome abnormalities among azoospermia.

Type of chromosome abnormalities	Number of patients	(%) of anomaly detected (Total = ١٢٤)
Sex chromosomal abnormalities	٤	٣.٢
Autosomal abnormalities	٠	٠
Total	٤	٣.٢

Table (٣): Type of chromosome abnormalities among eight oligospermia.

Type of chromosome abnormalities	Number of patients	(%) of anomalies detected (Total =٨٨)
Sex – chromosome	٧	٧.٩
Autosomal	١	١.١
Total	٨	٩

Table (٤): Chromosomal constitution of twelve patients with chromosomal abnormalities.

Individual no.	Age	S.F.A.	Chromosome constitution	Mosaicism ratio
Y١ – ٤	٣٠ – ٤٠	Azoospermia	٤٧,XXY	Non
Y٥ - ٨	٣٥ – ٤٥	Oligospermia	٤٦,XY/٤٧,XXY	٨٠:٢٠
Y٩ – ١٠	٥٠	Oligospermia	٤٦,XY/٤٧,XXY	٣٠:٧٠
Y١١	٤٠	Oligospermia	٤٦,XY/٤٧,XXY	٧٠:٣٠
Y ١٢	٤٥	Oligospermia	٤٦,XY/٤٥,XY(D; D)	٧٠:٣٠
Total = ١٢				

Table (٥): The percentage of occurrence of chromosome abnormalities among infertile males (n = ٢١٢).

Group	Number of patients	Percentage of group relative to total (n = ٢١٢)
Classic klinefelter's Syndrome	٤	١.٩
Mosaicism klinefelter's syndrome	٧	٣.٣
Total	١١	٥.٢
Autosomal anomalies	١	٠.٥
Total	١٢	٥.٧

Fig. (١) G- banded metaphase spread of azoospermia patients associated with Klinfelter's syndromes (٤٧, XXY)

Fig. (٢) C- banded metaphase spread of oligospermia patients (٤٥, XY) t (D; D). The translocation is arrowed.

3. 2. C-band polymorphism Calculations

٣. ٢. ١ C- Band and Euchromatin Values:

Since the total length of the chromosome is affected by colchicine and cell cycle duration, which will consequently effect the size of C-band, thus the error correction must be determined to overcome the variation due to these reasons. To determine the error correction, a reference parameter is required. In our case, we considered the chromosome number ١ as it has a reference parameter as has been suggested by (Erdtmann et al., ١٩٨٢) Fig. (٣) shows the C- band metaphase of normal chromosome constitution, which is note a heterochromatic regions on the distal part of the Y chromosome. It is well known that if the total length of the chromosome is increased the C-band, will be increased too, accordingly the relationship between the reference parameter and C-band size for any of chromosome (١,٩ and ١٦) is evaluated by the study of regression coefficients.

As grouped in table (٦) the regression coefficient values are very low which means that, although the size of chromosome was increased, the C-band size did not increase proportionally. It showed a slight increase in comparison with euchromation length of chromosome number one.

To calculate this unproportional increase, the error correction is determined by dividing the slope/C-band means.

The results are grouped in table (٦):

١. it is obvious that the regression coefficient showed variability between the C-bands of chromosome number ١, ٩ and ١٦.

٢. The larger and smaller C-bands of chromosome number (١) had regression coefficients of (٠.١٩٢) and (٠.١٢٣) respectively.

٣. The larger and smaller C-bands of chromosome number (٩) had regression coefficients of (٠.١٣٥) and (٠.٠٩٥) respectively.

٤. The larger and smaller C-bands of chromosome number (١٦) had regression coefficients of (٠.٠٧٣) and (٠.٠٥٢) respectively.

Similarly slope/C-band means (the average error) were varied and the following result was obtained:-

١- The high value of average error was found in the larger and smaller C-band of chromosome number (١). It was (٠.٠٧٤) and (٠.٠٧١) respectively.

٢- The lower values were observed in the larger and smaller C-band of chromosome number (١٦). It was found to be (٠.٠٤٨) and (٠.٠٤٢) respectively.

٣- With regard to chromosome number (٩), the average error of the larger and smaller C-band found to be (٠.٠٦٩) and (٠.٠٥٤) respectively.

A similar study was performed among normal fertile group and the following results were obtained: the results of normal fertile group are presented in table (٧): it is clear that the regression coefficient shows variability between C- bands of chromosomes number ١.٩ and ١٦:

١. The larger and smaller C- bands of chromosome number ١ had a regression coefficient of (٠.١٨٨) and (٠.١٦٥) respectively.

٢. The larger and smaller C- bands of chromosome number ٩ had a regression coefficient of (٠.١٦٣) and (٠.١١٤) respectively.

٣. The larger and smaller C- band of chromosome number ١٦ had a regression coefficient of (٠.١١٥) and (٠.٠٨٤) respectively.

Similarly slope/C-band means (the average error) were varied and the following results were obtained:

١. The high value of average error was found in the larger and smaller C- band of chromosome number ١. It was (٠.٠٩٢) and (٠.٠٨٢) respectively.

٢. The lower value was observed in larger and smaller C- band of chromosome number ١٦. It was found to be (٠.٠٨٠) and (٠.٠٧٢) respectively.

٣. With regard to chromosome number ٩ the average error of the larger and smaller C- band was found to be (٠.٠٧٧) and (٠.٠٧٠) respectively.

جـــدول رقـــــــــــــــــــــــــــــــــــــــم ٦

جـــدول رقـــم ٧

Fig. (٣) C- band metaphase of normal chromosome constitution, note a heterochromatic regions on distal part of the Y chromosome (arrowed)

3.2.2 The means of C – band size:

3.2.2.1 The C – band absolute means:

Table (٨). Summarizes the results of the C – band size which are located on chromosomes number١, ٩ and ١٦ only. Fig. (٤) shows a represented C- banded metaphase of patient with normal constistution note, C- band heteromorhpism on chromosome number (١). It is clear that there is an overall significant difference between the infertile groups (oligo and azoospermia) compared with control groups. The ANOVA test which has been applied revealed an obvious difference in the absolute size mean among the infertile group in comparison to control group ($F=٤٣.٣٣$, $P < ٠.٠٥$). However, when C – band size has been measured for each individual chromosome separately and a comparison has been made between the infertile and fertile groups, the following pictures were obtained:

a- No significant difference in the C – band size of chromosome number ١ of the azoospermia patients compared to normal control group

($z = ٠.١٣$, $P > ٠.٠١$).

b- No significant increase in C – band size of chromosome number ١ of the oligospermia patients as compared to normal fertile group ($z=٢.٣$, $P < ٠.٠١$).

c- No significant increase in the C – band size of chromosome number ١ could be recognized among the oligospermia compared to azoospermia patient ($z=١.٩٥$, $P > ٠.٠١$).

d- With regard to chromosome number ٩ a significant increase in C – band size was recorded in both groups of infertile patients (oligo, and azoo) compared to the normal fertile group (z=٣.١٤, ٤.٢, P < ٠.٠١).

e- A significant increase in C – band size of chromosome number ٩ among the oligospermia compared to azoospermia patients (z=٦.٠٨, P < ٠.٠١).

f- As far as chromosome no. ١٦ is involved, a significant increase in the C– band size was noticed among both the oligo and azoospermia patients compared to control group (z=٧.٠٨, ٦.١٣, P < ٠.٠١). Indeed no significant differences were recorded when both the infertile groups were compared with each other (z=١.٠٤, P < ٠.٠١).

Fig. (٤) C- band metaphase of normal chromosome constitution, note the C- band heteromorphism on chromosome number (١) (arrowed)

Table (٨): Absolute lengths of C- band on chromosome ١,٩ and ١٦ in patients (Oligospermic, Azoospermic) and control group.

Length of C- band Absolute µ m	Groups	chromosomes					
		(١)		(٩)		(١٦)	
		Larger (M ± SD)	Smaller (M ± SD)	Larger (M ± SD)	Smaller (M ± SD)	Larger (M ± SD)	Smaller (M ± SD)
	Azoospermic n = ١٢٠	٣.٢ ± ٠.٥	٢.١ ± ٠.٨٦	٢.١ ± ٠.٧٤	١.٧ ± ٠.٧٤	١.٧٨ ± ٠.٢٩	١.٥٨ ± ٠.٣٦
		٢.٧ ± ٠.٦٨		١.٩ ± ٠.٦		١.٦٨ ± ٠.٦	
	Oligosperamic n = ٨٠	٢.٩٩ ± ٠.٦٩	٢.٧٦ ± ٠.٦٥	٢.٤ ± ٠.٣٧	٢.٢٣ ± ٠.٤٣	١.٨١ ± ٠.٢٧	١.٤ ± ٠.٢٣
		٢.٨٩ ± ٠.٦٧		٢.٣٣ ± ٠.٤		١.٦١ ± ٠.٢٥	

	Control **n = ١٠٠**	٢.٧٤ ± ٠.٤٥	٢.٦٤ ± ٠.٤	١.٩٩ ± ٠.٢٨	١.٥٩ ± ٠.٢٦	١.٤٨ ± ٠.٤٢	١.٠٨±٠.٢٩
		٢.٦٩ ± ٠.٤٢٥		١.٧٩ ± ٠.٢٧		١.٢٨ ± ٠.٣٥٥	

Comparison // A & O & C significant differences F – Test RCBD ANOVA

P < ٠.٠٥

3.2.2.2 The C – band relative length:

The results of the measurement of C – band size relative length among the infertile and control groups are grouped in table (١٠). Considered as a whole no significant difference was found in the C – band size relative length among the infertile patients compared to the normal control group (F=٢.٠٥, P < ٠.٠١) ANOVA test.

However, when the relative length of each individual chromosome (i.e chromosome no.١,٩ and ١٦) was compared with normal control group, some important observations were noticed.

a-A significant increase in the C – band size relative length of chromosome no.٩ of patients suffering from oligospermia compared with normal control group (z=٣.٨, P < o.o١).

b-No significant increase in the C – band size relative length of chromosome no.٩ of patients suffering from azoospermia compared with normal control group.

c-No significant differences in C – band size among the oligospermia compard to azoospermia patients (z=١.٢, P > ٠.٠١).

d-The C – band size relative length of chromosome no.١٦ showed a highly significant increase in both the azoospermia and oligospermia patients compared with control group (z=٧.٥, ٥.٠, P < ٠.٠١).

e-No significant increase in the C – band relative length of chromosome no.١٦ among the azoospermia compared with the oligospermia patients.

f-With regard to chromosome no.١ . No significant increase or decrease in C – band size relative length could be recorded compared to control (z=٢.٤٨, ٢.٧, P > ٠.٠١). Indeed, no significant difference was noticed

when both the infertile groups were comapred with each other (z=١.٣٥, P > ٠.٠١).

جـــــــــــــــــــــــــــــدول رقـــــــــــــــــــــــــــــم 9

3.2.3 C – band heteromorphisms:

Table (١٠): Shows the frequency of C – band heteromorphisms among the infertile and fertile groups, which were subjected to the present investigation. Figures. (٥) (٦) and (٧) represent a C- banded metaphase spread of patient with oligospermia. Note, C- segment asymmetry (heteromorphism) on chromosomes (١) and (٩) (arrowed). It is clear,as shown in fig.٥,٦ & ٧ that there is a clear increase in the frequency of the C – band heteromorphisms among the infertile groups in comparison to normal control group both in the overall frequency or when each individual chromosome was considered separately.

As far as the oligospermic patients are concerned, the percentages of the heteromorphisms in chromosomes number ١,٩ and ١٦ were ٤٥٪, ١٢٪ and ٢٢.٥٪ respectively.

Indeed, among the azoospermias, percentages of ٣٣.٣٪ ٤٠٪ and ٢٦.٧٪ has been recorded. The control group gave much less frequency than that. The percentage of C – band heteromorphism among the control group were ٩٪, ٣٪ and ٧٪.

Statistical analysis which has been conducted by applying chi-square test revealed that there was a significant increase in the frequency of C – band heteromorphism among the oligospermia compared to normal control group ($X^{٢}$=٩.٥٨, df=٢, P < ٠.٠١).

Again there was a significant increase in the frequency of C – band between the azoospermic patients compared to the control group ($X^{٢}$=٢١.١٥, df=٢, P < ٠.٠١). No significant difference was found in the C

– band heteromorphism between oligospermia and azoospermia patients ($X^{٢}$=٦.٧٥, P > ٠.٠١).

Table (١٠): C – band heteromorphisms in oligospermic & Azoospermic and controls.

Group	Chromosomes		
	١	٩	١٦
***Oligospermic n = ٨٠**	٣٦(٤٥ %)	٢٦(١٢.٥ %)	١٨(٢٢.٥ %)
**** Azoospermic n = ١٢٠**	٤٠(٣٣.٣ %)	٤٨(٤٠ %)	٣٢(٢٦.٧ %)
Control n = ١٠٠	٩(٩ %)	٣(٣ %)	٧(٧ %)

* Significant difference P < ٠.٠١ $X^{٢}$ = ٩.٥٨ between (O & C) df = ٢ $X^{٢}$ (٢ X ٣) $X^{٢}_{Tab}$ (٩.٢١٠)

** Significant difference P < ٠.٠١ $X^{٢}$ = ٢١.١٥ between (A & C) df = ٢ $X^{٢}$ (٢ X ٣) $X^{٢}_{Tab}$ (٩.٢١٠)

Fig. (٥) C- banded metaphase spread of patient with oligospermia note, C- segment asymmetry (heteromorphism) on chromosomes (١) and (٩) (arrowed)

Fig. (٦) C- banded metaphase spread of patient with oligospermia note, C- segment asymmetry (heteromorphism) on chromosomes (١) (arrowed)

Fig. (٧) C- banded metaphase spread of patient with oligospermia note, C- segment asymmetry (heteromorphism) on chromosome (١) (arrowed)

3.2.4 Autosomal Pericentric Inversion:

The results of the study for the occurrence of pericentric inversion on chromosome numbers ١, ٩ and ١٦ among the infertile patients in comparison to normal control group are summarized in table (١١) fig. (٨) and (٩) represent a C- banded metaphase spread of oligospermia patient note, heterochromatin and the partial inversion on chromosome number ١ (arrowed). Fig. (٩) represents C- banded metaphase of patient with azoospermia, note, the inversion C- segment on chromosome number ٩.

Considered as a whole, no significant difference for the occurrence of pericentric inversions could be recorded when the infertile group is compared with fertile group.

A pericentric inversion is considered to occur only if ٥٠٪ of the cells had identified C – band inversion as calculated by the size of this part of C-band that had been inverted to the short arm of the chromosome. All the inverted chromosomes which have been measured were well below the ٥٠٪ that had been suggested by (Mikelsaar *et al*, ١٩٧٨).

Table (١١): Frequencies (%) inversion levels partial inversion (PI) and non inversion (NI) of the C – bands of chromosomes ١, ٩ and ١٦ in ٢٠٠ patients, (Oligospermic, Azoospermic) and ١٠٠ controls.

Groups	Chromosomes	Inversion levels					Non inversion	
		Inv $(>\frac{1}{3}-\frac{1}{2})$	%	Inv $(<\frac{1}{3})$	%	Total	%	n
Oligospermic	١	٣	(٣.٧ %)	١٩	٢٣٪	٢٢	٢٧٪	٥٨
	٩	١	(١.٢ %)	٤	٥٪	٥	٦.٢٪	٧٥
	١٦	-	-	-	-	-	-	٨٠
Azoospermic	١	٦	(٦.٦ %)	٢٢	٢٤٪	٢٨	٣١.١٪	٦٢
	٩	٣	(٣.٣ %)	١١	١٢.٢٪	١٤	١٥.٥٪	٧٦
	١٦	-	-	-	-	-	-	٩٠
Control	١	٣	(٣٪)	١٦	١٦٪	١٩	١٩٪	٨١
	٩	٦	(٦ %)	١٠	١٠٪	١٦	١٦٪	٨٤
	١٦	-	-	-	-	-	-	١٠٠

Comparison // A & O & C for IN and non IN to chromosome (١) $x^{٢}$ (٢ X ٣) contingency table .

Non significant P < ٠.٠٥

Comparison // A & O & C for IN and non IN to chromosome (٩) $x^{٢}$ (٢ X ٣) contingency table .

Non significant P < ٠.٠٥

Fig. (٨) C- banded metaphase spread of oligospermia patient. Note, heteromorphism on chromosomes number ٩ and the partial inversion on chromosome number ١ (arrowed)

Fig. (٩) C- banded metaphase spread of azoospermia patient. Note, the inversion C- segment on chromosome number (٩) (arrowed)

3.2.5 Autosomal C – band Distribution Level:

The C – band is classified into five levels according to their size (level ١,٢,٣,٤,٥) as has been suggested by (Patil and Lub, ١٩٧٧). It is clear from table (١٢) that there was a significant increase in level ٣ C – band (large size) among both the oligo and azoospermic patients compared to control group ($X^{٢}$=٦٠,٧٨, df=١, P < ٠.٠٥). Among the oligospermia the precentage of cells which showed level ٣ C – band in chromosome number ١ reached a percentage of ٤٧٪ and among the azoospermia reached a percentage of ٤٣٪ while only ٢٢٪ of the normal control group had a large C – band.

Furthermore, no significant increase was noticed in the small C – band (level ١) or (level ٢) in either the oligospermia or azoospermia compared to the control.

On the other hand, a significant increase has been noticed in the C – band level ١ (small size) of chromosome number ١٦ among both the oligospermia and azoospermia patients compared to control group, ($X^{٢}$=٦.٨, df=٢, P < ٠.٠٥). The percentage of the cells which showed a small C – band size among the oligospermia, azoospermia and control group were ٧٥٪, ٨٦٪ and ٢٤٪ respectively. No other significant results could be detected in other C – band size in the examined cells.

Table (١٢): Distribution of homologous chromosome ١,٩ and ١٦ according to C-band size in Oligo , Azoo & control group.

			chromosomes		
Levels	**Groups**	**No. of cells examined**	١	٩	١٦
١	**Oligo**	٨٠	٥ (٦.٢ %)	٨ (١٠ %)	٦٠ (٧٥٪)
١	**Azoo**	١٢٠	-	٢٢ (١٨.٣ %)	١٠٤ (٨٦٪)
١	**Control**	١٠٠	-	٤ (٤ %)	٢٤ (٢٤٪)*
٢	**Oligo**	٨٠	٤٠ (٥٠ %)	٤٠ (٥٠ %)**	٢٠ (٢٥٪)
٢	**Azoo**	١٢٠	٦٢ (٥١.٦ %)	١١١ (٩٢ %)	٦٠ (٤١٪)
٢	**Control**	١٠٠	٧٨ (٧٨ %)	٩٦ (٩٦ %)	٧٦ (٧٦٪)
٣	**Oligo**	٨٠	٣٨ (٤٧.٩ %)	٣٠ (٣٧.٥ %)	-
٣	**Azoo**	١٢٠	٥٢ (٤٣.٣ %)*	٨ (٦.٦ %)	-
٣	**Control**	١٠٠	٢٢ (٢٢ %)	-	-
٤	**Oligo**	٨٠	-	-	-
٤	**Azoo**	١٢٠	-	-	-
٤	**Control**	١٠٠	-	-	-
٥	**Oligo**	٨٠	-	-	-
٥	**Azoo**	١٢٠	-	-	-
٥	**Control**	١٠٠	-	-	-

* Significant difference . $P < ٠.٠٥$ $X^{٢} = ٦٠.٧٨$ df = ١ , In level (٣) to chromosome NO. (١) for comparison (A & C) $X^{٢}$ tab = ٥.٠٢٤

** Significant difference . $P < ٠.٠٥$ $X^{٢} = ٦.٨$ df = ١ , In level (٢) to chromosome NO. (٩) for (O & C)

*** Significant difference . $P < ٠.٠٥$ $X^{٢} = ١٢.٣$ df = ١ , In level (١) to chromosome NO. (١٦) for (O & C)

3.2.6 Y Chromosome measurements:

Before we proceed any further, it is beneficial to measure the normal and standard size of Y – chromosome among Iraqi male population. This study will represent the base line for any change in the size of this chromosome in future. The results are compared with that published elsewhere.

3.2.6.1 Y Chromosome total length distribution:

One hundred fertile males of different age group were included in the present investigation. Human Y chromosome was classified into five classes (very small, small, medium, large and very large) according to that published by other investigators (Verma *et al.,* ١٩٨٢). Fig. (١٠) showed a representative C- banded metaphase spread of large Y chromosome. The arrow indicates the large Y chromosome. The classification was based on Y/F index. It is clear from table (١٣) that the Y – chromosome of about ٦٣٪ of the Iraqi fertile males were of medium size. The Y/F index was ٠.٩٤ – ١.٠٩. table (١٣).

The Iraqi large size Y chromosome and very large size showed a percentage of ٢٢٪ and ٨٪ respectively. The Y/F index for both the large and very large were ١.١٦ – ١.٢٢ and > ١.٢٣. The small size Y chromosome showed a percentage of ٧٪ with ٠.٨١ – ٠.٩٤ index. The standard size of the Y-chromosome of other population is grouped in table (١٣) for comparison.

Table (١٣) Classification and comparison of Y / F indices from ١٠٠ normal Iraqis Population. ٦٠ normal Caucasian and ٦٠ normal American Blacks.

Criteria Y / F index	Size	Caucasian		American Black		Iraqi Pop.	
		No.	%	No.	%	No.	%
< ٠.٨٠	Very small	٠	٠	٠	٠	٠	٠
٠.٨١ – ٠.٩٤	Small	٩	١٥	٢	٣.٣٣	٧	٧
٠.٩٤ – ١.٠٩	Medium	٤٠	٦٦.٧	٣٤	٥٦.٦٧	٦٣	٦٣
١.١٠ – ١.٢٣	Large	٨	١٣.٣	١٨	٣٠.٠٠	٢٢	٢٢
> ١.٢٣	Very large	٣	٥.٠	٦	١٠.٠٠	٨	٨

Data from Verma et al. ١٩٧٨

Iraqi & Caucasian $X^{٢}$ = ٤.٤٥ df = ٤ P > ٠.٠٥ $X^{٢}$ Tab = ٩.٤٨٨

Iraqi &

American Bl. $X^{٢}$ = ٢.٢٦ df = ٤ P > ٠.٠٥ $X^{٢}$ Tab = ٩.٤٨٨

There is No Significant difference between them.

٣.٢.٦.٢ Y chromosome length among Iraqi infertile male:

١. The frequency of Y-chromosomes size of ٢٠٠ infertile and ١٠٠ normal fertile males are grouped in table (١٤). The table shows that the distribution of total length of Y chromosome in infertile group was significantly different from that of control group ($P < ٠.٠٥$).

Furthermore, our results also showed that about ٣٨٪ of our infertile patients had small and very small size of Y chromosome while the normal fertile group showed a percentage of ٧٪ of the small type.

٢. The frequency of Y chromosome size of ٨٠ oligospermia, ١٢٠ azoospermia and ١٠٠ normal fertile males are grouped in table (١٥). The table shows that the distribution of total length of Y chromosome in both infertile group (oligo and azoospermia) are significantly different from that of control groups ($X^{٢}=٣٤$, df=٨, $P < ٠.٠٥$).

Furthermore, our result also showed that about ٨.٣٪ and ٢٥٪ of our azoospermia patients and about ٧.٥٪ and ١٧.٥٪ of our oligospermic had small and very small size Y chromosome respectively while the normal fertile group showed a percentage of ٧٪ of the small type only.

Chi – test analysis which has been carried out revealed the following:-

A-There is a highly significant difference in the Y – chromosome length distribution between the azoospermia patient, and normal control group. The difference is calculated to be ($X^{٢}=٣٣.٦٨$, df=٤, $P < ٠.٠٥$).

B-There is a significant difference in total length distribution of the oligospermia patients Y chromosome compared to that of normal control males ($X^{٢}=١٤.٢$, df=٤, $P < ٠.٠٥$).

Fig (١٠) is C- banded metaphase spread of oligospermia patient. With normal chromosomal constitution. The arrow indicates the total length of Y chromosome.

Fig. (١١) shows a C- banded metaphase spread of normal in which the heterochromatic in the distal part of Y chromosome is seeing as dark stained region (the arrowed indicates small Y chromosome.

Fig (١٢) (١٣) (١٤) are C- banded metaphase spread of infertile male in which the heterochromatic distal part showed a heteromorphism.

Table (١٤): Classification of Y/F indices from ٢٠٠ infertile patients and ١٠٠ Iraqi normal pop. Control.

Criteria Y / F index	Size	Normal Iraqi pop.		* Infertile group	
		No.	%	No.	%
< ٠ – ٨٠	Very small	٠	٠	١٦	١٦
٠.٨١ – ٠.٩٤	Small	٧	٧	٤٤	٢٢
٠.٩٤– ١.٠٩	Medium	٦٣	٦٣	٩٤	٤٧
١.١٠ – ١.٢٣	Large	٢٢	٢٢	٤٣	٢١.٥
> ١.٢٣	Very large	٨	٨	٣	١.٥

The length distribution is significantly different between infertile group and normal control.

X^2 = ٣٤.٣٤ df=٨ X^2 tab = ١٥.٥٠٧ P < ٠.٠٥

Table (١٥): Classification of Y/F indices from ٨٠ Oligospermic and ١٢٠ Azoospermic Patient and ١٠٠ Iraqi normal Control.

Criteria Y / F index	Size	Normal Iraqi PoP.***		Azoospermic **		Oligospermic *	
		No.	%	No.	%	No.	%
< ٠.٨٠	Very small	٠	٠	١٠	٨.٣	٦	٧.٥
٠.٨١ – ٠.٩٤	Small	٧	٧	٣٠	٢٥	١٤	١٧.٥
٠.٩٤ – ١.٠٩	Medium	٦٣	٦٣	٥٠	٤١.٧	٤٤	٥٥
١.١٠ – ١.٢٣	Large	٢٢	٢٢	٣٠	٢٥	١٣	١٦.٢٥
> ١.٢٣	Very large	٨	٨	٠	٠	٣	٣.٧٥

*** P < ٠.٠٥ The length distribution is significantly different between A & O & C

$X^{٢}$ = ٣٤.٣٤ df = ٨ $X^{٢}_{Tab}$ = ١٥.٥٠٧

** A & C $X^{٢}$ = ٣٣.٦٨ df = ٤ P < ٠.٠٥ $X^{٢}_{Tab}$ = ٩.٤٨٨

* O & C $X^{٢}$ = ١٤.٢ df = ٤ P < ٠.٠٥ $X^{٢}_{Tab}$ = ٩.٤٨٨

Fig (١٠) C- banded metaphase spread of normal fertile. With normal chromosomal constitution. The arrow indicates the total length of the Y chromosome.

Fig.(١١) C- banded metaphase spread of normal in which the heterochromatic in the distal part of the Y chromosome is seen as dark stained region (arrowed) small Y chromosome.

Fig (١٢) C- banded metaphase spread of infertile male in which the heterochromatic distal part showed a heteromorphism.

Fig (١٣) C- banded metaphase spread of infertile male in which the proximal Euterochromatic part showed a heteromorphism.

Fig (١٤) C- banded metaphase spread of infertile male in which the heterochromatic distal part of Y chromosome showed a heteromorphism.

٣.٢.٦.٣ C – band Y chromosome size:

As grouped in table (١٦) the size of C – band are classified in to five levels (١ – ٥) according to that suggested by (Patil and Lubs ١٩٧٧). The parameters, which have been used, depend on the size of the heterochromatic region, which is located on the distal portion of the Y chromosome.

In our result, ١٨ patients out of ٨٠ who were suffering from oligospermia had a level ١ Y chromosome C – band size which accounted to ٢٢.٥٪. Again ٢٠ azoospermia patients out of ١٢٠ showed the same level size, which accounted to ١٦.٧٩. Only ten normal out of one hundred showed the level ١ C– band size, which accounted to ١٠٪.

As far as level ٢ is concerned, only ٢٨ patients out of a total of ٨٠ oligospermia patients showed level ٢ C – band which account to ٣٥٪ while ٥٧ azoospermia patients out of ١٢٠ showed this level which accounts to ٤٧.٥٪. The control group showed ٣٥٪ of level ٢ C – band of Y chromosome.

Furthermore, ٣٤ oligospermia patients out of ٨٠ showed level ٣ C – band which accounted to ٤٧.٥٪. Indeed ٤٣ patients and ٥١ normal male showed level ٣ C – band which accounted to ٣٥٪ and ٥١٪ respectively. No other C–band level of Y – chromosome has been recorded in our result table (١٦).

No statistical difference control to detect in the distribution of C-band size oligo and azoo as compared to normal fertile group.

Table (١٦): Distribution of C- bands Y chromosome according to their size.

Y chromosome Heterochromatin			
Level	**Group**	**No. of cell measured**	**C-band size**
١	Oligo n = ٨٠	٢٤٠	١٨ (٢٢.٥ %)
١	Azoo n = ١٢٠	٣٦٠	٢٠ (١٦.٧ %)
١	Control n = ١٠٠	٣٦٠	١٠ (١٠ %)*
٢	Oligo		٢٨ (٣٥ %)
٢	Azoo		٥٧ (٤٧.٥ %)
٢	Control		٣٥ (٣٥ %)*
٣	Oligo		٣٤ (٤٧.٥ %)
٣	Azoo		٤٣ (٣٥ %)
٣	Control		٥١ (٥١ %)*
٤	Oligo		----------
٤	Azoo		----------
٤	Control		----------
٥	Oligo		٤
٥	Azoo		----------
٥	Control		----------

In level (١) X^2 for O & C = ٠.٩ , X^2 for A & C = ٠.٦٥ df = ١ , In level (٢) X^2 for O & C = ١.٤ , X^2 for A & C = ١.٩ df = ١ , In level (٣) X^2 for O & C = ١.٧ , X^2 for A & C = ١.٨٣ df = ١ All of them non significant. P > ٠.٠٥ .

٣. ٢. ٦. ٤ C- band, Euchromatin and Y chromosome lengths

The total length of the C-banded Y chromosome was calculated by measuring the length of the C-band region and the length of the Euchromatin.

Accordingly, for human Y chromosome, C/F, E/F ratios were calculated.

Table (١٨) summarize the results of the measurements of the Y/F, C/F and E/F ratios among infertile groups and control group.

The mean of Y/F, C/F and E/F ratios among the azoospermia patients was about (١.٠ ± ٠.٦), (٠.٦٢ ± ٠.٠٢) and (٠.٥ ± ٠.٠١) respectively. and the oligospermia patients the indices were (١.٠٥ ± ٠.٠٢), (٠.٦ ± ٠.٠٢) and (٠.٦٨ ± ٠.٢٢) and whereas the control group Y/F index was (١.١٠ ± ٠.١٨) and C/F, E/F were (٠.٥ ± ٠.٢) and (٠.٤٦ ± ٠.٠٦) respectively.

Accordingly, the functional relationship between the two variables for example (C/F Vs Y/F) and (E/F vs Y/F) in azoosperima, oligospermia patients and normal fertile group, was calculated using the correlation coefficients. The correlation was tested by the t-test. It is clear from table (١٧) that a highly significant correlation has been found between C/F vs Y/F among the azoospermia patients ($t=١٢.١٦$, $p < ٠.٠٥$) and among the oligospermia as well ($t=٢٠.٢$, $p < ٠.٠٥$). The normal control group showed likwise a highly significant correlation coefficients ($t=٢٤.٢٤$, $p < ٠.٠٥$).

When the E/F values were correlated to Y/F indices, the result showed a significant correlation among the azoosperima, oligospermia and control groups (t=١٣.١, p < ٠.٠٥), (t=١٢.٤, p < ٠.٠٥) and (t=٤٠.٣, p < ٠.٠٥) respectively.

Finally, C/F values were correlated to E/F for all groups, which were subjected to the present study. The results of correlation coefficients showed no significant correlation was found with regard to C/F vs E/F value (t=٠.٧٥, p < ٠.٠٥), (t=٠.٧١٢, p < ٠.٠٥) and (t=٠.٥٩, p < ٠.٠٥) respectively.

Table (١٧): Comparison statistical parameters between oligospermia, azoosperima patients and normal fertile group.

Component	Oligospermia	Azoosperima	Control
A-mean			
Y/F	١.١ ± ٠.٠٦	١.٠٥ ± ٠.٠٢	١ ± ٠.١٨
C/F	٠.٦ ± ٠.٠٢	٠.٦ ± ٠.٠٢	٠.٥ ± ٠.٢
E/F	٠.٥ ± ٠.٠١	٠.٦٨ ± ٠.٠٢٢	٠.٤٦ ± ٠.٠٦
B-correlation (r)			
C/F vs Y/F	(٠.٩٥)*	(٠.٩٩)*	(٠.٩٤)*
E/F vs Y/F	(٠.٤)*	(٠.٦)**	(٠.٨١)*
C/F vs E/F	(٠.٣٢)	(٠.٠٨)	(٠.٣٨)

* Significant P<٠.٠١

٣.٣ Satellite Association :

The degree of satellite association (SA) was studied in phytohaemagglutinin – stimulated lymphocytes in ٢١٤ infertile male who were subjected to cytogenetic studies. Peripheral blood for one hundred normal fertile males was cultured in parallel as a control. fig. (١٥) is a representative metaphase spread of D/D association. The arrow indicates the associated chromosomes. To avoid any differences in the frequency of satellite association (SA) due to culture time (Mattie and Salzano, ١٩٧٥), all cultures were harvested at ٧٢ hours. Fig. (١٦) showed a represented metaphase spread of partial acrocentric association.

Three groups of satellite association parameters were scored for each subject: -

A-The mean number of cells containing association per scored cells (n=٥٠). See table (٢٠).

B-The mean number of small association (when two chromosomes are involved) and the mean number of large association (when more than two chromosomes involved).

C-The mean number of cells with different number of associations. In this instance, the cells were classified into three categories:

١-cells with one association.

٢-cells with two associations.

٣-cells with more than two associations.

٣.٣.١ Relationship between SA and infertile patients :

Table (١٨) shows a higher percentage of mitotic cells containing association among the infertile groups (oligo, azoo and KF). The mean numbers of cells containing association among the oligo, azoo and

klinefelter syndrome were (٣٧.٠) (٣٥.٠) (٢٩.٠) respectively while the mean number among normal control group were (١٠.٠) only.

The Z – test analysis shows that the differences are highly significant (z=٣٩.٧٠, ٣٩.٠٦ and ٣٥.١٩, respectively, P < ٠.٠١).

Table (١٨): The mean number of cells containing association for infertile groups as compared to normal control.

Individuals	No. of cells scored per subject	No. of cells containing asso. for scored cells. Mean ± SD	Z – test cal.
Oligo (n=٨٠)	٥٠	* ٣٧.٠ ± ٤.٦٢٥	٥٠.٧٥
Azoo (n = ١٢٠)	٥٠	* ٣٥.٠ ± ٤.٣٧٥	٥٩.٧٣
Klinfelter (n = ١٢)	٥٠	* ٢٩.٠ ± ٣.٩٢٥	١٧.٩٨
Control (n=١٠٠)	٥٠	* ١٠.٠ ± ١.٢٥	

* P < ٠.٠١

There was astonishing increase in the number of satellite association, among the infertile patients in comparison to normal fertile control.

Fig. (١٥) G- banded metaphase spread of oligospoermic patient. acrocentric association D/D chromosome (arrowed).

Fig (١٦) C- banded metaphase spread. An acrocentric partial association D/D chromosomes (arrowed).

٣.٣.١.١ The Relationship Between Satellite Association and Azoospermia :

As grouped in table (١٩) the following observations can be noticed:-

١-The mean number of association per cell among the azoospermic is (٠.٩٢٦ ± ٠.١١٥) compared with (٠.٤٧٤ ± ٠.٠٥٩) for the normal control group. The z – test analysis revealed that the difference is highly significant (z=٣٧.٥, P < ٠.٠١).

٢-The mean number of associated chromosome per cell is (١.٨٧١ ± ٠.٢٣٤) among the azoospermic and (٠.٤٧ ± ٠.٠٥٤) among the fertile group with a significant z-test analysis in favor of azoospermiac as (z=٦٣.٧, P < ٠.٠١).

٣-The mean number of small association per cell among the azoospermiac is (٤١.٥٠ ± ٥.١٩) which is about four time higher than that of normal control group which showed about (١١.٨٣ ± ١.٤٧٤) small association. Again the difference is highly significant (z=٥٩.٧٢, P < ٠.٠١).

٤-The mean number of large associations per cell among the azoospermiac is (٤.٥٦ ± ٩.٨٧) which is much higher than that of normal group which gave only (١.١٩٧ ± ٠.٥) number of large association. As expected the difference is highly significant (z=٥٧.٤٧, P < ٠.٠١).fig. (١٧) showed a C- band metaphase spread for patients with azoospermia. The arrow indicates the large association of acrocentric chromosome.

٥-The mean number of cell, with one association is much higher among this group of patients (٢٦.٩٢ ± ٦.٧١٥) compared to normal group

which showed only (١٨.١ ± ١.٨٨٨) number of association (z=٢١.٤, P < ٠.٠١).

٦-The mean number of cell with two associations and more than two associations are also higher among our azoospermia patients. The mean number of two associations is (٦.٧٩ ± ٣.١٦٥) and for more than two associations is (٢.٠٧ ± ٠.١٤٦). The same parameters show (٣.٦ ± ٠.٨٢٥) and (١.٤٢ ± ٠.١٧٨) among control groups respectively. Z – test analysis revealed the differences in both parameters are highly significant (z=١٠.٦٧, P < ٠.٠١) and (z=٢٩.٢٣, P < ٠.٠١).

Fig (١٧) C- banded metaphase spread, large association of acrocentric chromosome (arrowed).

Table (١٩): The satellite association for ١٢٠ azoospermic patients as compared to normal control.

	Asso./cell mean ± SD	Asso. Chro. Per cell Mean ± SD	No. of small asso. Mean ± SD	No. of large asso. Mean ± SD	No. of cells with one asso. Mean ± SD	No. of cells with two asso. Mean ± SD	No. of cells with more than two asso. Mean ± SD
Azoo. n = ١٢٠	* ٠.٩٢٦ ± ٠.١١٥	* ١.٨٧١ ± ٠.٢٣٤	* ٤١.٥٠ ± ٥.١٩	* ٤.٥٦ ± ٠.٨٧	* ٢٦.٩٢ ± ٦.٧١٥	* ٦.٧٤ ± ٣.١٠٥	* ٢.٠٧ ± ٠.١٤٦
Control n = ١٠٠	٠.٤٧٤ ± ٠.٠٥٤	٠.٤٧± ٠.٠٥٩	١١.٨٣ ± ١.٤٧٩	٠.٠	١٨.١ ± ١.٨٨٨	٣.٦ ± ٠.٨٢٥	١.٤٢ ± ٠.١٧٨
Z – test cal.	٣٧.٥	٦٣.٢	٥٩.٧٨	٥٧.٤٢	٢١.٤٤	١٠.٦٤	٢٩.٢٣

* P < ٠.٠١

٣.٣.١.٢ The Relatiomship Between satellite association and oligospermia:

The relationship between eighty patients suffering from a decrease in sperm count and one – hundred normal fertile control are grouped in table (٢١). It is clear, that there is a significant increase in the frequency of satellite association among the patients compared to normal control. The detail of the study can be summarized as follow:-

١-The mean number of association per cell among the oligospermic is twice that of normal control. The first group shows a mean association number of (٠.٩٣٦ ± ٠.١١٧) while the second group shows (٠.٤٧٤ ± ٠.٠٥٩). Z – test analysis is highly significant in favour of oligospermic (z=٣٢.١٩, P < ٠.٠١).

٢-The mean number of associated chromosomes per cell is (١.٩٦٤ ± ٠.٢٠٦) among the oligospermic patients and (٠.٤٧٤ ± ٠.٠٥٩) among the normal control. The increase is about four times (z=٥٣.١١, P < ٠.٠١).

٣-The mean number of cells with small association among the oligospermic is (٤٢.١٦ ± ٥.٢٧) while the same parameter among the normal fertile groups is (١١.٨٣ ± ١.٤٧٤). The results shows a significant z– test analysis (z=٤٩.٩, P < ٠.٠١).

٤-The mean number of cells with large association per cells is (٤.٦٦ ١٠.٤٤٥) among the oligospermia. No large association has been recorded among the normal control group.

٥-The mean number of cells with two associations and more than two associations are obviously high among the oligospermia compared to normal control group. The mean number of two association is (٦.٨٨ ± ٣.٢٦) and more than two is (٢.١٤ ±

٠.٦٦٨) among the oligospermia, the same parameters showed about (٣.٦ ± ٠.٨٢٥) and (١.٤٢ ± ٠.١٧٨) among the normal group. Z – test analysis confirmed the difference is significant in both parameters (z=٨.٧٨, P < ٠.٠١) and (z=٩.٣٧, P < ٠.٠١).

Table (٢٠): Relationship between oligospermia and satellite association compared to normal control group.

	Asso./cell mean ± SD	Asso. Chro. Per cell	No. of cells with small asso. Mean ± SD	No. of cells with large asso. Mean ± SD	No. of cells with one asso. Mean ± SD	No. of cells with two asso. Mean ± SD	No. of cells with more than two asso. Mean ± SD
Oligo N = ٨٠	* ٠.٩٣٦ ± ٠.١١٧	* ١.٩٦٤ ± ٠.٢٤٦	* ٤٢.١٦ ± ٥.٢	* ١٠.٣٩ ± ٤.٦٦	* ٢٧.٧٠ ± ١٠.٤٦	* ٦.٨٨ ± ٣.٢٦	* ٢.١٤ ± ٠.٦٦٨
Control N = ١٠٠	٠.٤٧٤ ± ٠.٠٥٤	٠.٤٧ ± ٠.٠٥٩	١١.٨٣ ± ١.٤٧٩	٠.٠	١٨.١ ± ١.٨٨٨	٣.٦ ± ٠.٨٢٥	١.٤٢ ± ٠.١٧٨
z-test cal.	٣٢.١٩	٥٣.١١	٤٩.٩	٤.٠	٨.١	٨.٧٨	٩.٣٧

* P < ٠.٠١

٣.٣.١.٣ The Relationship of satellite association and klinefelter's syndrome:

Due to the fact that the klinefelter's syndrome has a distinctive clinical feature (i.e. small test which are unable to produce spermatozon) thus the frequency of satellite association is studied separately. Table (٢١) gives a clear picture on the relationship between the klinefelter syndrome and satellite association compared to normal control. In this study only ١٢ a clear distinct klinefelters syndrome were studied and the results are grouped in table (٢١). Fig. (١٨) is a G- banded metaphase spread of kline felter's syndromes and the acrocentric association D/D chromosome is (arrowed)

١-The mean number of association per cell among klinefelter's syndrome is (٠.٨٠٥ ± ٠.١٠١) compared to (٠.٤٧٤ ± ٠.٠٥٩) association per cell among the normal control. The z – test analysis is significant (z=١١.٩٧, P < ٠.٠١).

٢-The mean number of association chromosome per cell is (١.٦ ± ٠.٢) among the klinefelter's syndrome which is three times higher than that of normal control group which give a frequency of (٠.٤٧٤ ± ٠.٠٥). Indeed, the difference is significant as proved by z – test analysis (z=١٢.٢٦, P < ٠.٠١).

٣-The mean number of small association per cell among the KF is (٣٨.٤٨ ± ٦.٨٣٨) while the same parameter is about (١١.٨٣ ± ١.٤٧٤) among the normal control. The increase is about four times and the z- test analysis highly significant (z=٨.٠١, P < ٠.٠١).

٤-The mean number of large association per cells among KF is (١.١٧٨ ± ٠.٥). No large association has been recorded among the fertile control (z=٤.٨٧, P < ٠.٠١).

٥-The mean number of cells with small association is about (٣.١٢٥ ٢٤.٠) among the KF while the same parameter is about double than that of normal control (١٨.١ ١.٨٨٨). Again the increase in the association is significant (z=٦.٤٩, P < ٠.٠١).

٦-The mean number of cells with two or more than two associations is (٥.٧ ± ٠.٧١٣) and (١.٩ ± ٠.١١٢٥) among the KF compared to (٣.٦ ± ٠.٨٢٥) and (١.٤٢ ± ٠.١٧٨) in the normal control. The difference is highly significant (z=١٠.١١, P < ٠.٠١), (z=١٣.٧, P < ٠.٠١) respectively.

Fig (١٨) G- banded metaphase spread and the acrocentric association D/D chromosome is arrowed.

Table (٢١): The satellite association for klinefelter patients as compared to normal control group.

	Asso./cell mean ± SD	Asso. Chro. Per cell Mean ± SD	No. of cells with small Asso. Mean ± SD	No. of cells with large Asso. Mean ± SD	No. of cells with one Asso. Mean ± SD	No. of cells with two Asso. Mean ± SD	No. of cells with more than two Asso. Mean ± SD
Klinefelter N = ١٢	* ٠.٨٠٥ ± ٠.١٠١	* ١.٦ ± ٠.٢٠٨	* ٣٨.٧٨ ± ٦.٨٣٨	* ١.١٧ ± ١.١٩٨	* ٢٤.٠ ± ٣.١٢٥	* ٥.٧ ± ٠.٧١٣	* ١.٩ ± ٠.١١٢٥
Control N = ١٠٠	٠.٤٧٤ ± ٠.٠٥٩	٠.٤٧ ± ٠.٠٥٩	١١.٨٣ ± ١.٤٧٤	٠.٠	١٨.١ ± ١.٨٨٨	٣.٦ ± ٠.٨٢٥	١.٤٢ ± ٠.١٧٨
Z –test cal.	١١.٩٧	١٢.٢٦	٨.٠١	٤.٨٧	٦.٨٩	١٠.١١	١٣.٧

* P < ٠.٠١

٣.٣.١.٤ A comparative view to the satellite associations of the oligospermia and azoospermia patients:

In order to shed the light on whether the number of sperm count influences the frequency of SA. A comparative study has been made to evaluate the frequency of SA among the oligospermic (patients with low sperm count) and azoospermic (patients).

The same previous parameters have been used in this investigation, considered as whole, no obvious differences could be detected. The z-test analysis, which has been used, gave no difference. The frequency of SA was the same. The result of the investigation is grouped in table (٢٢).

Table (٢٢): The satellite association frequency among oligospermic patients as compared to azoospermic patients.

	Asso./cell mean ± SD	Asso. Chro. Per cell Mean ± SD	No. of cells with small asso. Mean ± SD	No. of cells with large asso. Mean ± SD	No. of cells with one asso. Mean ± SD	No. of cells with two asso. Mean ± SD	No. of cells with more than two asso. Mean ± SD
Oligo N = ٨٠	* ٠.٩٣٦ ± ٠.١١٧	* ١.٩٦٤ ± ٠.٢٤٦	* ٤٢.١٦ ± ٥.٢٧	* ٤.٦٦ ± ١٠.٣٩٥	* ٢٧.٧٠ ± ١٠.٤٦	* ٦.٨٨ ± ٣.٢٦	* ٢.١٤ ± ٠.٦٦٨
Azoo N = ١٢٠	٠.٩٢١ ± ٠.١١٥	١.٨٧١ ± ٠.٢٣٤	٤١.٥٠ ± ٥.٧٩	٤.٥٦ ± ٠.٨٧	٢٦.٩٢ ± ٦.٧١٥	٦.٧٤ ± ٣.١٠٥	٢.٠٧ ± ٠.١٤٦
z-test cal.	٠.٨٩	٢.٦٧	٠.٨٣	٠.٠٩	٠.٥٩	٠.٣٠	٠.٩٢

* P> ٠.٠١

٣.٣.١.٥ A comparative view to the satellite association of the klinefelter's syndrome and azoospermia patients:

To study whether there is any significant difference in the frequency of SA between the KF patients, and azoospermic patients standard parameters have been used for this purpose.

As summarized in table (٢٣) there is a significant difference in the frequency of SA between these two groups of infertile patients for most of the parameters used although there are many exceptions:

١-The mean number of association per cell among the azoospermia patients is (٠.٩٢١ ± ٠.١١٥) compared to (٠.٨٠٥ ± ٠.١٠١) among the klinefelter syndrome.The z-test analysis shows a significant increase in azoospermia compared to klinefelter (z=٣.٦, P < ٠.٠١).

٢-The mean number of associated chromosome per cell is (١.٨٧١ ± ٠.٣٣٤) among azoospermia patients which shows a slight increase compared to KF which shows a frequency rate of (١.٦ ± ٠.٢). The difference is significant as tested by z-test analysis (z=١.٤٣, P < ٠.٠١).

٣-The mean number of small association per cell among the azoospermia is (٤١.٥٠ ± ٥.٧٩) while the same parameter is (٣٨.٧٨ ± ٦.٨٣٨) among the KF patients. The increase in azoospermia patients is slight but did not reach the significant level as proved by analysis (z=١.٤٣, P < ٠.٠١).

٤-The mean number of large association per cell among the azoospermia patients is (٤.٥٦ ± ٩.٨٧), while the same parameter among KF patient is (٠.٥ ± ١.١٩٨).

The increase is about four times and the z-test analysis shows a significant increase in azoospermia patients (z=٤.٢, P < ٠.٠١).

٥-The mean number of cells with one association among the azoospermia patient, is (٢٦.٩٢ ± ٦.٧١٥) while the same parameter is about (٢٤.٠ ± ٣.١٢٥) among the klinefeleter patients. No significant difference is recorded for this parameter.

٦-The mean number of cells with two and more than two associations are (٦.٧٤ ± ٣.١٠٥) and (٢.٠٧ ± ٠.٤٤٦) among the azoospermia patients compared to (٥.٧ ٠.٧١٣) and (١.٩ ٠.١١٢٥) among the KF patients. The increase in the means number among the azoospermia patients appear to be slight. No significant result could be reached as proved by z-test analysis(z=١.٣٤,١.١٨٩,P<٠.٠١).

Table (٢٣): The satellite association for azoospermic patients as compared to klinefelter's syndrome.

	Asso./cell mean ± SD	Asso. Chro. Per cell Mean ± SD	No. of cells with small Asso. Mean ± SD	No. of cells with large Asso. Mean ± SD	No. of cells with one Asso. Mean ± SD	No. of cells with two Asso. Mean ± SD	No. of cells with more than two Asso. Mean ± SD
Azoo./ n = ١٢٠	٠.٩٢١ ± ٠.١١٥	١.٨٧١ ± ٠.٣٣٤	٤١.٥٠ ± ٥٧٩	٩.٨٧ ± ٤.٥٦	٢٦.٩٢ ± ٦.٧١٥	٦.٧٤ ± ٣.١٠٥	٢.٠٧ ± ٠.٤٤٦
K.F. n = ١٢	٠.٨٠٥ ± ٠.١٠١	١.٦ ± ٠.٢	٣٨.٧٨ ± ٦.٨٣٨	٠.٥ ± ١٠١٩٨	٢٤.٠ ± ٣.١٢٥	٥.٧ ± ٠.٧١٣	١.٩ ± ٠.١١٢٥
Z – test	٢.١٢	* ١.٤٣	١.٤٣	* ٤.٢	٢.٨	١.٣٤	١.١٨

* P < ٠.٠١

٣.٣.٢ Specific Satellite Association Frequency:

٣.٣.٢.١ Acrocentric-associated chromosomes per cell:

After we have shown that there were obvious and significant increase in the frequency of satellite association among the infertile patients (Oligo and Azoo and KF), compared to normal control group, the attention has been turned to study whether these high frequencies of association is random or non – random. G – banding technique is applied for this purpose.

Table (٢٤) shows a comparison in the mean number of associated chromosome per cell among the three groups of infertile patients compared to normal fertile group. The acrocentric chromosomes (١٣,١٤,١٥,٢١,٢٢) which are the satellited chromosomes are involved in the association. It is clear from this table that the three medium sized acrocentric chromosome (١٣,١٤,١٥) are the most frequent associated chromosomes than the smaller one (٢١ and ٢٢). Among the oligospermic, chromosome no.١٣ had an association ability reached to ٤٦٢ association per total sample (n=٣٥) while chromosome no.١٤ and ١٥ had as association ability of ٣٨٥ and ٣٠٨ respectively per total sample. This ability is decreased to ٢٨٨ and ٢٩٠ in chromosome no.٢١ and ٢٢ respectively. Among the azoospermic patients the association ability of chromosome no.١٣ and ١٤ is ٤٩٢ and ٤٤٠ respectively.

In the other acrocentric chromosome (١٥,٢١,٢٢) the specific association ability start to decrease to ٢٩٩ in chromosome no.١٥, ٣٤٠ in chromosome no.٢١ and ٣٣٠ in chromosome no.٢٢.

With respect to klinefelter's syndrome, the specific satellite association ability for each five acrocentric chromosomes are almost the same, no clear difference could be noticed expect for the acrocentric chromosome no.١٤ and ١٥ in which the specific satellite association ability started to decrease to ١٠١ and ٩١ respectively.

The specific satellite ability for the same five chromosomes among the normal control decreased and there is no sharp difference between them (Table (٢٤)).

Table (٢٤): Specific Satellite Association frequency among infertile and fertile groups.

Individuals	No. of scored cells	means	Acrocentric chromosomes					
			١٣	١٤	١٥	٢١	٢٢	Total
Oligospermia N=٣٥	٨٧٥	١.٩٨٤	٤٦٢	٣٨٥	٣٠٨	٢٨٨	٢٩٠	١٧٣٣
Azoospermia N=٤٠	١٠٠٠	١.٨٧١	٤٩٢	٤٤٠	٢٩٩	٣٤٠	٣٢٠	١٨٧١
Klinefelter N=١٢	٣٥٠	١.٦	١٢٤	١٠١	٩١	١٢١	١٢٣	٥٦٠
Control N=٣٠	٧٥٠	٠.٤٧	٧١	٧٨	٦٩	٦٤	٧٠	٣٥٢

٣.٣.٢-٢ Specific Acrocentric Association Per Cell among Oligospermia Patients:

The number of specific acrocentric association which were detected among thirty-five oligospermia patients are grouped in table (٢٥). The mean number of chromosomal association per cells for all the patients was around (١.٩٩٦).

The most frequent association chromosomes were number ١٣ with association ability of (٤٣٦) per total sample. The second most frequent associated chromosomes were chromosomes number ١٥ and ١٤ with association ability of (٣٦٧) and (٣٦٢) per total sample respectively. The third most frequent association chromosomes were chromosomes number ٢١ and ٢٢ with association ability of (٢٦٨) and (٢٥٨) per total sample respectively table (٢٥).

Furthermore, the chi-square analysis revealed a significant difference in the association ability between chromosomes number ١٣ and chromosomes number ١٤ ($x^{٢}$=٦٤.٦٦, P<٠.٠٠٥), again the association ability between chromosomes number ١٣ and chromosomes number ١٥ ($x^{٢}$=٥٩.٩٧, P<٠.٠٠٥). Finally, the association ability of chromosomes ١٣ with chromosomes number ٢١ and ٢٢ ($x^{٢}$=٦٠.٤٤, ٥٣.٧٠, P<٠.٠٠٥).

Fig. (١٩) is a representative G- banded metaphase spread of oligospermia patient with ١٣/٢٢ association. The arrow indicates the associated chromosomes.

Fig. (٢٠) showed representative G- banded metaphase spread of oligospermia patient showed with ١٣/١٤ association. The arrow indicates the associated chromosomes.

Table (٢٥): Number of association for each acrocentric chromosome in metaphase among the oligospermia patients.

Patients No.	Association scored for each pair acrocentric Per ٢٥ cells					Asso./cell
	١٣	١٥	١٤	٢١	٢٢	
١	١٠	٩	٧	١٠	٥	١.٦٤
٢	١٢	١٠	٨	٧	٨	١.٨٠
٣	١٠	٩	١٠	٥	٥	١.٥٦
٤	١١	٨	٧	٠	٧	١.٣٢
٥	٨	٧	٦	١٠	٤	١.٤
٦	٨	٦	٢	٠	٣	٠.٧٦
٧	٩	٧	١٠	٦	٠	١.٢٨
٨	٤	٤	٣	٢	٢	٢.١٢
٩	٩	٧	١٠	٧	٦	١.٥٦
٠	١٣	١١	٥	١٠	١٧	٢.٢٤
١	٥	٤	١١	٣	٣	١.٠٤
٢	١٤	١٠	٥	١٣	٥	١.٨٨
٣	١٥	١٤	١٤	٣	١٦	٢.٤٨
٤	١٦	١٤	٤	١٢	٠	١.٨٤
٥	١٨	١٤	٧	٨	٦	٢.١٢
٦	١٣	١٦	٠	٨	٤	١.٦٤
٧	١٨	١٤	١٢	١١	٨	٢.٥٢
٨	٢٠	١٥	١٤	١٣	٩	٢.٨٤
٩	١٢	١٢	٦	٦	١٣	١.٩٦
٠	١٦	١٠	٦	٩	٧	١.٩٦
١	١١	٦	١١	١٢	١٣	٢.١٢
٢	١٦	٢٠	١٣	١٢	١١	٢.٨٤
٣	١٤	١٢	١٠	٣	٥	١.٧٦
٤	٩	٨	١١	١٠	١٠	١.٧٦
٥	١٥	١٢	١٠	٦	٧	١.٨٨
٦	١٥	١٤	١٢	٠	١٢	٢.١٢
٧	١٢	١١	١٠	٨	٩	٢
٨	١٠	٩	١٢	١٣	١٢	٢.٢٤
٩	٩	٤	٦	٤	٧	١.٢
٠	١٨	٩	١٢	٨	٧	٢.١٦
١	١١	١٠	١٨	١١	٨	٢.٣٢
٢	١٩	١٣	١٠	١٩	٠	١.٨٨
٣	١٣	١١	١	١٠	١٥	٢
٤	١٥	١٤	١٠	٨	٧	٢.١٦
٥	١٦	١٣	٩	٨	٧	١.٨٨
	٤٣٦	٣٦٧	٣٠٢	٢٦٨	٢٥٨	١.٨٩٤

$X^{٢}$ = ٢٦٨.٨٤

Fig. (١٩) G- banded metaphase spread of oligospermia patient showed with ١٣/٢٢ association. The arrow indicates the associated chromosomes.

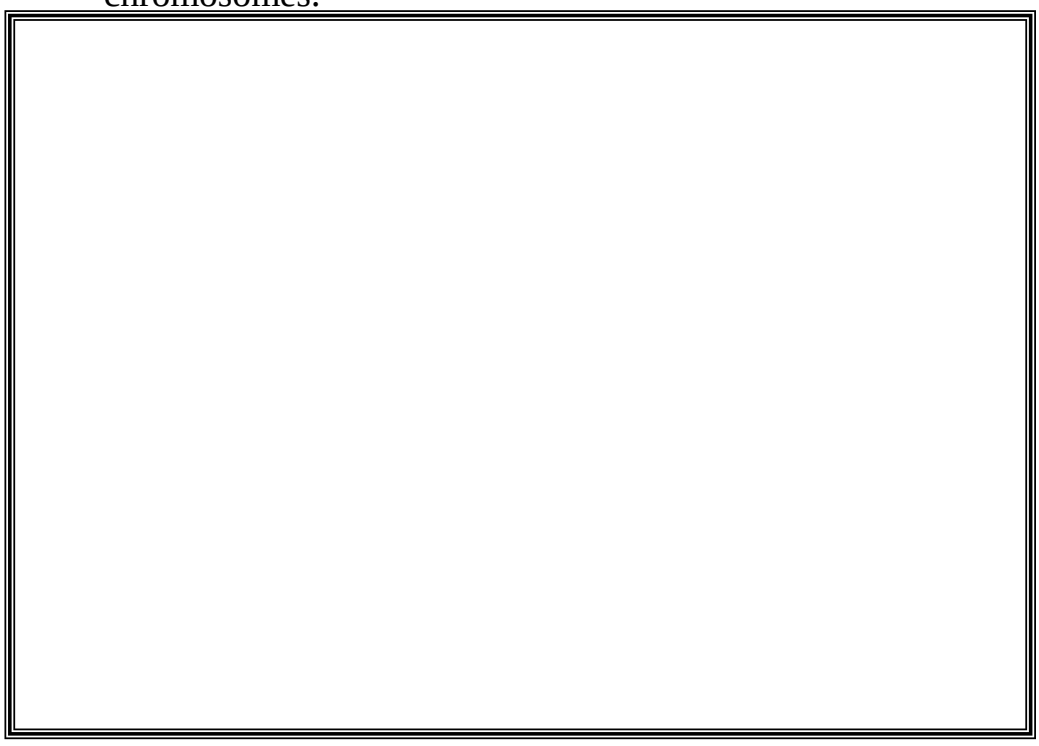

Fig. (٢٠) G- banded metaphase spread of oligospermia patient showed with ١٣/١٤ association. The arrow indicates the associated chromosomes.

٣.٣.٢-٣ Specific Two-By-Two Acrocentric Association among Oligospermia Patients:

Table (٢٦). Shows the distribution of two-by-two associated chromosomes among the thirty-five oligospermia patients. The highest association was occurred between chromosome number ١٣ and ١٤ with an association frequency of (٢١٦) per total sample. The second most frequent associated chromosome between chromosomes number (١٣-١٥) with association frequency of (١٤٢) per total sample. Then followed by chromosomes number (١٣-١٣) (١٢٦), chromosome number ١٣ and chromosome number ٢٢ (١١٠), chromosome number ١٤ and chromosome number ٢٢ (١١٠), (٢١-٢٢) (١٠٢), (١٣-٢١) (٩٦), (١٥-٢٢) (٩٥), (١٤-١٤)(٨٥), (٢٢-٢٢) (٨٤), (٢١-٢١) (٨٣), (١٥-٢١) (٨٠), (١٥-١٥) (٧٤) per total sample.

The chi-square test analysis which have been applied revealed that the association frequency is not random:-

a- The association frequency of (١٣-١٤) and (١٣-١٥) is significant ($x^{٢}$=٥٦.٣, P<٠.٠٠٥).

b- The association frequency of (١٣-١٤) and (١٣-٢١) is significant ($x^{٢}$=٥٥.١٠, P<٠.٠٠٥).

c- The association frequency of (١٣-١٤) and (١٣-٢١) is also significant ($x^{٢}$=٥٥.٩٨, P<٠.٠٠٥).

d- The association frequency of (١٣-١٤) and (١٣-٢٢) is significant ($x^{٢}$=٥٣.٦٢, P<٠.٠٠٥).

جـــــــــــــــــــــــــــــدول رقـــــــــــــــــــــــــــم ٢٦

٣.٣.٢-٤ Specific Acrocentric Chromosomal Association Per Cell among Azoospermiac Patients:

The numbers of specific acrocentric association, which have been detected among fourty azoospermia patients, are grouped in table (٢٧). It is clear from the table that the mean number of association ability per cell for all the patients was (١.٨٨). Again, it is obvious that the chromosome number ١٣ showed a higher association ability per cells which was (٥٠١) followed by chromosome number ١٤ (٤٤٦), chromosome number ٢١ (٣٢٧), chromosome number ٢٢ (٣٠٤) and finally chromosome number ١٥ (٣١٢). The chi-square test which have been applied revealed the following:-

a- Although chromosome number ١٣ posseses the higher association ability compared to the other four acrocentric chromosomes. No significant differences were detected.

Table (٢٧): Number of association for each acrocentric chromosome in metaphase among the Azoospermia patients.

Patients No.	Association scored for each pair of acrocentric shr (s) per ٢٥ cells					Association /cell
	١٣	١٤	١٥	٢١	٢٢	
١	٧	١٢	٩	١٠	٨	١.٨٤
٢	١١	١١	٧	١١	٨	١.٩٢
٣	١٣	١٢	٦	١٢	٩	٢.٠٨
٤	١٠	١١	١٠	٩	٧	١.٨٨
٥	١٣	١١	٧	٩	٧	١.٨٨
٦	١٥	١٣	١٠	١٠	١٢	٢.٤
٧	١٧	١٣	١٠	١٢	١١	٢.٥٢
٨	١١	١٠	٥	٦	٦	١.٥٢
٩	١٢	١١	٩	٨	٨	١.٩٢
٠	١٤	١٢	٦	٨	٨	١.٩٢
١	١٢	١١	٦	٨	٨	١.٨
٢	١٤	١٣	٩	٨	٩	٢.١٢
٣	١٢	١١	١٠	١١	١٠	٢.١٦
٤	١٣	١١	٦	٦	٥	١.٦٤
٥	١٢	٩	٦	٧	٦	٢.٢
٦	١٤	١٠	٧	١٠	٨	١.٩٦
٧	١١	٩	٧	٦	٧	١.٦
٨	١٢	١٠	٦	٦	٧	١.٦٤
٩	١٣	١٥	١١	١١	١٢	٢.٤٨
٠	١٥	١٢	٩	١١	١٠	٢.٢
	٢٥٥	٢٣٠	١٥٩	١٨١	١٦٨	١.٨٨

Table (٢٧): Number of association for each acrocentric chromosome in metaphase among the Azoospermia patients.

Patients No.	Association scored for each pair of acrocentric chr (s) per ٢٥ cells					Association /cell
	١٣	١٤	١٥	٢١	٢٢	
١	٩	١١	٩	٤	٧	١.٦
٢	١٠	٩	٥	٤	٤	١.٢٨
٣	١٠	٩	٦	٥	٥	١.٤
٤	١٣	١٢	٨	٧	٧	١.٨٨
٥	١٢	١١	٩	٦	٦	١.٨
٦	١١	١٠	٧	٨	٧	١.٧٢
٧	٢٠	١٨	١٤	١٥	١٣	٢.٨
٨	١٢	١٠	٧	٩	٧	١.٨
٩	١٤	١١	٧	٨	٧	١.٨٨
٠	١٦	١٣	١٠	٩	٨	٢.٢٤
١	١٥	١٤	١٤	٧	٧	٢.٢٨
٢	٨	٨	٦	٦	٥	١.٣٢
٣	١٧	١١	٧	٨	٧	٢
٤	١٧	١٦	٧	١٠	١٠	٢.٤
٥	١٠	١٠	٦	٧	٨	١.٧٦
٦	٩	٨	٦	٦	٤	١.٣٢
٧	٩	٧	٦	٨	٧	١.٤٨
٨	١١	٩	٦	٧	٧	١.٦
٩	١٢	١٠	٧	٧	٥	١.٦٤
٠	٨	٩	٦	٥	٥	١.٤٢
Pooled	٢٤٦	٢١٦	١٥٣	١٤٦	١٣٦	١.٧٧٦
	٥٠١	٤٤٦	٣١٢	٣٢٧	٣٠٤	١.٩٨٤

$P < ٠.٠٠٥$ $df=٣٩$

b- The chi-square analysis revealed that the association ability of chromosome number ١٣ with chromosome number ١٤ showed

($x^{٢}$=٦.٩, P>٠.٠٠٥), and with chromosome number ١٥ revealed ($x^{٢}$=٢٨.٧٤, P>٠.٠٠٥), and with chromosome number ٢١ and ٢٢ was ($x^{٢}$=٩.٩٧ and ٨.٨٥, P>٠.٠٠٥). All of them are not significant.

٣.٣.٢-٥ Specific Two-By-Two Acrocentric Association among Azoospermiac Patients:

Table (٢٨). Displays the specific two-by-two acrocentric association, which has been recorded among fourty azoospermia patients. The table shows that the association between chromosome number ١٣ and ١٤ (١٣-١٤) were most frequent with a total of ١٨١ association per sample. The second most frequent association was that between chromosome number ١٣ and ١٣ (١٣-١٣) with a total of ١٤٧ association per sample, followed by the association between chromosome number ١٥ and ٢٢ (١٥-٢٢) and ١٣ and ١٥ (١٣-١٥) which showed a total association of ١٤٦ and ١٤٣ per sample. The rest of acrocentric chromosomes showed the following association per sample.

a- Chromosomes (١٤-٢٢) were with association number of ١٣٥ per sample.
b- Chromosomes (١٣-٢٢) were with association number of ١٣٢ per sample.
c- Chromosomes (٢١-٢١) were with association number of ١٢٦ per sample.
d- Chromosomes (١٤-١٥) were with association number of ١٢٢ per sample.
e- Chromosomes (١٤-٢٢) were with association number of ١١٦ per sample.

f- Chromosomes (١٥-١٥) with association number of (١١٣) per sample.

g- Chromosomes (٢١-٢٢) with association number of (١١٢) per sample.

h- Chromosomes (١٤-١٤) with association number of (١١٢) per sample.

i- Chromosomes (١٥-٢١) with association number of (١٠٨) per sample.

j- Chromosomes (٢٢-٢٢) with association number of (٩٠) per sample.

k- Chromosomes (١٣-٢١) with association number of (١٢٦) per sample.

The chi-square test analysis which have been applied showed that the association frequency which are involving chromosome number (١٣-١٤) and chromosome ١٣ with the rest of acrocentric is random according to the following results:

a- The (١٣-١٤) and (١٣-١٣) is not significant ($x^{٢}$=٥٤.٤٤, df=٢,P>٠.٠٠٥).

b- The (١٣-١٤) and (١٣-١٥) is not significant ($x^{٢}$=٤٣.٣٣,df=٢, P>٠.٠٠٥).

c- The (١٣-١٤) and (١٣-٢١) is not significant ($x^{٢}$=٤٠.٤٠,df=٢, P>٠.٠٠٥).

d- The (١٣-١٤) and (١٣-٢٢) is not significant ($x^{٢}$=٤٤.٧٠, df=٢,P>٠.٠٠٥).

Fig. (٢١) is represented G- banded metaphase spread of normal fertile group as a control for comparison.

Fig. (٢٢) showed G- banded metaphase of patient with azoospermia, the arrow indicates association between ١٣/٢٢ (metaphase with one association).

Fig. (٢٣) is a G- banded metaphase of patient with azoospermia the arrow indicates the association between ١٥/٢١, ١٣/٢١, ١٣, ١٤ (metaphase with more than two associations)

جدول ٢٨ ص ١٢٣

تكملة جدول ٢٨

Fig. (٢١) G- banded metaphase spread of normal fertile group as a control for comparison.

Fig. (٢٢) G- banded metaphase of patient with azoospermia, the arrow indicates association between ١٣/٢٢ (metaphase with one association).

Fig. (٢٣) G- banded metaphase of patient with azoospermia the arrow indicates the association between ١٥/٢١, ١٣/٢١, ١٣/١٤ (metaphase with more than two associations)

٣.٣.٢.٦ Number of Specific Acrocentric Chromosomal Association Per Cell among Klinefelter's Syndrome Patients:

The number of specific acrocentric association, which was detected among twelve Klinefelter's Syndrome, are grouped in table (٣٠). The mean number of chromosomal association per cell for all the patients is around (١.٥٣٧). It is clear that the distribution of association frequency is different from other infertile group which have been used in this study. The higher association frequency was recorded in chromosome number ١٣ and chromosome number ٢٢ with association ability of (١٠٩) and (٩٨) per total sample. The next most frequent associated chromosomes are chromosome number ١٤ and chromosome number ٢١ with association ability of (١٩٤) and (٩٦) per total sample and finally chromosome number ١٥ with association ability of (٧٣) per total sample.

The chi-square test which have been applied showed the following:-

١- The association ability chromosome number ١٣ with chromosome number ١٤ is not significant ($x^{٢}$=١.٤٢, P>٠.٠٠٥).

٢- The association ability chromosome number ١٣ with chromosome number ١٥ is not significant ($x^{٢}$=١.٨٧, P>٠.٠٠٥).

٣- The association ability chromosome number ١٣ with chromosome number ٢١ and ٢٢ are not significant ($x^{٢}$=٥.٦٦. ١.٤, P>٠.٠٠٥).

Fig. (٢٤) is a G- banded metaphase of azoospermia patient with ٤٧, XXY, klinfelter's syndromes, the arrow indicate the association including D/G.

Table (٢٩): Number of association for each acrocentric chromosome in metaphase among the Klinefelter patients.

Patients No.	Association scored for each pair of acrocentric shr (s) per ٢٥ cell					Association /cell
	١٣	١٤	١٥	٢١	٢٢	
١	١٠	٨	٥	١١	١٠	١.٧٦
٢	٩	٨	٥	٨	٩	١.٥٦
٣	٨	٧	٦	٤	٨	١.٣٢
٤	٩	٦	٧	٥	٨	١.٤
٥	٨	٦	٧	٨	٨	١.٤٨
٦	١٠	٦	٧	٨	٧	١.٦
٧	١٠	٨	٧	٥	٥	١.٤
٨	٩	٩	٧	١٠	٩	١.٧٢
٩	٩	٩	٦	٩	٨	١.٦٤
١٠	٨	٧	٦	٨	٧	١.٤٤
١١	٨	٧	٥	٩	٩	١.٥٢
١٢	١١	٨	٥	٩	٩	١.٦٨
	١٠٩	٩١	٧٣	٩٤	٩٨	١.٥٣٧

١٣&١٤ ١٣&١٥ ١٣&٢١ ١٣&٢٢

x^2=١.٤٢ x^2=١.٨٧ x^2=٥.٦٦ x^2=١.٤

df=١٣ x^2Tab.=٢٩.٨١٩ p< ٠.٠٠٥

x^2 total = ١٠.٠٧ non significant

٣.٣.٢-٧ Specific Two-By-Two Acrocentric Association among Klinefelter's Syndrome Patients:

Table (٣٠). Demonstrates the specific two-by-two acrocentric association, which has been, recorded among twelve Klinefelter Syndrome patients. As grouped in the table it is clear that the association frequency distribution is entirely different from that which has been found among other infertile groups. The higher number of association ability was found between the small acrocentric chromosomes mostly chromosome numbers (٢١-٢١), (٢١-٢٢) with an association ability of (٤٤)(٤٢) per sample, while the next most frequent was found in large acrocentric associated pairs (١٣-١٤), (١٣-١٣) and (١٣-١٥) with association ability of (٣٧)(٣٦) and (٣٦) per samples. The association between the small and the large acrocentric chromosomes e.g. (١٥-٢٢) (١٤-٢٢) (١٣-٢٢) and (١٣-٢١) showed a mild decrease in the association frequency (٣٤) (٣٣) (٣١) and (٣١) per samples respectively.

The rest of acrocentric chromosomes showed the following association per sample.

a- Chromosome numbers (١٤-١٤) were with association frequency of ٢٦ per sample.

b- Chromosome numbers (١٤-١٥) were with association frequency of ١٩ per sample.

c- Chromosome numbers (١٤-٢١) were with association frequency of ٢٣ per sample.

d- Chromosome numbers (١٥-١٥) were with association frequency of ٢٣ per sample.

e- Chromosome numbers (١٥-٢١) were with association frequency of ٢٤ per sample.

f- Chromosome numbers (٢٢-٢٢) were with association frequency of ٢٨ per sample.

The chi-square test analysis which have been applied revealed that the association frequency which are involving chromosome number ١٣ with the rest of acrocentric chromosome is not significant which a mean that the association between the acrocentric chromosome were entirely random due to the following statistical results:-

a- The (١٣-١٤) and (١٣-١٥) associations are not significant ($x^{٢}$=٩.٩٨, P<٠.٠٠٥).

b- The association frequency of (١٣-١٤) pair and the association frequency of (١٣-١٣) pair is not significant ($x^{٢}$=١٧.٩٤, P<٠.٠٠٥).

c- Finaly the (١٣-١٤) and (١٣-٢٢) is not significant ($x^{٢}$=١٠.٥٤, P<٠.٠٠٥).

جـــــــــــــــــــــــــــــــــدول رقـــــــــــــــــــــــــــــم ٣٠

Fig. (٢٤) A G- banded metaphase of azoospermia patient with ٤٧, XXY, klinfelter's syndromes, the arrow indicate the association including D/G.

Chapter Four

Discussion

٤.١ Chromosomal Anomalies

Chromosomal anomalies are common among men with infertility. It is estimated as being between ٢٪ to ٢٠٪ (Dutrillaux *et al.*, ١٩٨٢). Obviously the chromosomal abnormality is higher in the infertile men than in general population. Two main ways by which chromosomal disorders can influence fertility in man. Firstly they can disturb the development of testis leading to a serious impairment of its normal function. Such disorder, are usually caused by abnormality of sex chromosome (Chandly *et al.*, ١٩٧٥). Secondly, autosomal anomalies may be resulting in disruption of normal process of cell division that occurs during the development of the gametes. Furthermore, numerous factors can contribute to male infertility, including Chromosomal make up, single gene defect, hormonal melieu, infections, chemical and physical agents (Lange *et al.*, ١٩٩٧). However, despite a large body of work, which has been carried out to elucidate the relationship between chromosome anomalies and reduced fertility, no clear cut picture could be reached. The present investigation dealt with ٢١٢ patients in an attempt to study the relationship between chromosome defect and infertility in Iraq by applying cytogenetics techniques (C and G – banding techniques) .

٤.١.١ Karyotype:

Infertile males patients with numerically and structurally abnormal chromosome have been reported elsewhere (Retief *et al.*, ١٩٨٤) (Chandly,

١٩٧٩). Both the sex chromosomes and the autosomes were involved. Needless to say that many patients who exhibit sub-fertility showed normal human karyotype. Nevertheless, chromosome aberrations are an important cause of male infertility as has been suggested by many workers. (De brackleer and Dao, ١٩٩١; Meschede *et al.,* ١٩٩٥).

Accordingly, groups of men attending infertility clinics have been screened by cytogeneticists to determine the frequency of abnormalities among randomly chosen patient. In fact some authors reported high incidence of chromosomal abnormalities among infertile men. Koudah *et al.,* (١٩٩٢) stated an incidence of chromosome abnormality of about ١٣٪, where Croquatte and Fourline, (١٩٨٠) reported an incidence of ١٢.٦٦٪. More recently, Yasseen and his workers reported an incidence of ١٢.٥٪ among Iraqi subfertile patients (Yasseen *et el.,* ٢٠٠١ a,b).

On the other hand, other investigations registered a low incidence of abnormality that may be associated with infertility Chandly et al. (١٩٧٥) in their cytogenetic screening of (٢٣٧٢) reported an incidence of ٢.٢٪ and kjessler (١٩٧٢) registered an over all incidence of ٦.٦٪, similarly, Dutrilluax *et al.,* (١٩٨٢) stated an incidence of ٦.٦٥٪.

The present investigation registered an incidence of ٥.٧٪ Table (١) which is less than ١٢.٥٪ which has been reported by Yasseen and his co-workers (٢٠٠١) . The reason here is most probably due to the large number of patient who were subjected to the present investigation.

The number of patient, which has been used by Yasseen *et al.,* (٢٠٠١) were ٦٤ patients while the number of patients in present study were ٢١٢ patients. However our result tend to be nearly similar to that

reported by Kjessler (١٩٧٢) and Dutrilluax *et al.*, (١٩٨٢) Who obviously investigated a considerable number of infertile patients.

Furthermore, Table (١) shows that about ٣.٢٪ of our azoospermic patients who were subjected to this investigation showed chromosomal abnormal. This percentage was in agreement with that reported by Faed *et al.*, (١٩٧٩), who reported a percentage of (٢.٩٪), though Yasseen and his workers, (٢٠٠١a,b) recorded an incidence of ١٠.٨٪. Indeed, the difference between the two studies could be related to the large number of azoospermic patients who were subjected to cyrogentic screening in the present study. Surely the large number of patients will affect the final percentage of abnormality. The number of azoospermic patients in the present study were ١٢٠ patients while Yasseen *et al.*, (٢٠٠١) number were ٣٧ patients only.

On the other hand, The present investigation revealed that the percentage of chromosomal abnormality among the oligospermic patients was ٩.١٪. Again, this percentage seems to be higher than that reported by Retief *et al.*, (١٩٨٤) and Chandly *et al.*, (١٩٧٩) who reported a percentage of ٥.١٪ and ١.٧٪ respectively.

Obviously, the present study dealt with infertile patients who were severely oligospermic. Indeed, the previous studies has proposed a positive relationship between chromosomal abnormality and sperm counts. Chandly et al.,(١٩٧٩) reported a decline in frequency of chromosomal abnormalities with the increasing of sperm count. Accordingly, the above statement may explain to some extent the higher frequency although other reasons (e.g. race, environmental factors), can not be excluded.

However, Yasseen *et al.*, (٢٠٠١) a,b in their recent work registered an incidence of ١٥٪ though their work has been conducted under the same conditions. The small number of patients who were used may contribute to in their high frequency.

On the other hand, the ratio of autosomal to sex chromosomal abnormality is estimated in the present investigation to be ١:١١ table (٤). The Robertsonian's translocation is only abnormality observed and occurred at a frequency of ١.١٪. This percentage is in full agreement with that reported by Retief *et al.*, (١٩٨٤). Indeed Refief *et al.*, (١٩٨٤) reported a ratio of autosomal to sex chromosome abnormality ١:١ and the frequency of Robertsonian's translocation was reported to be ١٪. Yasseen and his workers reported a frequency of Robertsonian's translocation to be ٤٪, likewise the reason behind that probably owing to previously mentioned reasons.

Sex chromosomal abnormality seems to be the sole cause of azoospermia. No autosomal abnormalities that were associated with azoospermia were observed. This observation is in complete agreement with that reported by Yasseen *et al.*, (٢٠٠١a,b) and Retief *et al.*, (١٩٨٤).

Furthermore, the present investigation showed that about ٧.٩٪ of oligospermia patients had abnormal sex chromosome although the abnormality was in mosaic form.

Furthermore, autosomal abnormalities were found among the oligospermia patients only and the percentage was ١.١٪. One patient with DqDq translocation was identified which was associated with Oligospermia. Robertsonian's translocation was reported to reduce men

fertility (Centerwell and Merrell, ١٩٧٥). The D/D translocation is the most common type (Count Brown, ١٩٦٧). Furthermore, varying degrees of spermatogenesis impairment have been reported among carriers of both DqDq and DqGq translocation and the spermatognic arrest was observed in spermatocyte or spermatid (Chandly *et al.*, ١٩٧٥). No D/G or G/G translocation were observed in the present investigation, although its difficult to determine whether the rare G/G translocation has a greater effect up on male reproductive fitness as has been suggested by others (Gosden *et al.*, ١٩٧٨).

Several studies reported that sub-fertility in the translocation carriers can be brought about in two ways (Miroslave *et al.*, ١٩٨٠).

Firstly, it can result from the production of genetically unbalanced gametes, which lead to spontaneous abortion of unbalanced zygote.

Secondly, sub-fertility can be the consequence of the spermatogenic disturbances, which result in oligospermia and azoospermia.

٤.١.٢ Klinefelter's Syndrome and Chromosomal abnormality:

It is a well established fact that KF syndrome is associated with the sex chromosomal abnormality. Peripheral blood culture for the classic form of Klinefelter's Syndrome revealed ٤٧,XXY male mitotic karyotype with no other obvious autosomal abnormality could be seen. The KF normally is associated with azoopermia. As grouped in table (٥) the frequency of KF is ١.٩٪.

Indeed, our results seems to be less than that reported by Kouslisher and Schoysman in (١٩٧٤) who reported a percentage of ٦.٥٪ and Yasseen *et al.*, (٢٠٠١) who reported a percentage of ٦.٢٥٪. The small number of KF which was subjected to the present investigation may be behind this low frequency. Indeed, most urologist did not refer the classic KF for the cytogenetic screening and they relay on the clinical feature in stead.

In the present study the mosaicism form showed a ٤٦,XY/٤٧,XXY male mitotic karyotype, and it constituted about (٣.٣٪). It should be mentioned here, that the mosaicim ratio was not the same neither in the present work nor in that published previously (Koushiher and Schydom, ١٩٧٤) (Pulsen *et al.*, ١٩٦٨) (Yasseen *et al.*, ٢٠٠١a,b).

The difference in the mosaicism ratio will effect the phenotypic traits of patients. All the mosaic from of Klinefelter's syndrome were associated with oligospermia rather than azoospermia as might be expected. Thus one should believe that more ٤٦,XY chromosomal constitution in the mosaic

complement might decrease sterility and cover much of the clinical features of the patients. Oligospermia has seldom been reported in subjects affected by Klinefelter's Syndrome, and those associated with various degree of tubular alteration, arrest of maturation process at primary spermatocyte or spermatic level and the presence of spermatogenesis only in few seminiferous tubules (Kaplan *et al.*, ١٩٦٣; Regendra *et al.*, ١٩٨٤). It has been suggested in Klinefelter's Syndrome that the spermatogenesis was related to the presence of normal ٤٦,XY germ cells (Kjessler *et al.*, ١٩٦٦), and the different degrees of testicular alteration depend on the

proportion of normal ٤٦,XY tubular cells, including germ cells and cell surrounding them (Foreste *et al.,* ١٩٩٨).

٤.٢ C – band polymorphism and males infertility:

Due to the fact that the majority of the patients shows normal human karyotype with no any sign of chromosomal aberration, thus a C – band studies have been conducted in an attempt to shed the light if C – band polymorphism resolves the clue.

٤.٢.١ C – band Error correction calculation method:

The error correction on C – band size of both infertile and control group has been carried out. The most notable C – band are present in autosomes chromosomes number ١,٩ and ١٦ and in the sex chromosome (Y chromosome). The C – bands of autosomes usually show a continuos normal distribution (Balicek *et al.,* ١٩٧٨) (Podugolnihave *et al.,* ١٩٧٩) (Brown *et al.,* ١٩٨٠), while the terminal C – band of Y chromosome show a bimodal distribution. In order to evaluate a continuous variability of C – band, two ways of analysis were present; qualitative and quantitative analysis methods. The most efficient method is the quantitative one.

It is well known that the total length of the chromosomes is affected by cell cycle duration and colchicine treatment, thus the length of C – band size is also affected by the same factors too. The question which is often raised by the specialist in this field, is that which part of the chromosome is the most affected the euchromatin or the heterochromatin.

To answer such question it should first determine the correction error for different C – bands contractions by the use of a reference parameters. The reference parameter used in the present study was ١q – h region

(chromosome no.١, the long arm minus the heterochromatin region). To minimize the error due to different contraction stage, a linear regression method has been applied.

The present investigation is in complete agreement with that published elsewhere in evaluating this method to be the best to correct the variation in C – band size due to different contraction.

It must be mentioned here that some other investigators prefer the use of total chromosome length for all the karyotype as a reference parameters rather than confine to only one chromosome (Lelikova *et al.*, ١٩٧٧) (Brown *et al.*, ١٩٨٠) as in this study. Indeed such statement seems to be more logic but it is surrounded by some unpreferable difficulties that led us to exclude it specially when a large sample is needed to be measured. Beside all that previous work did not find any significant differences between them . Accordingly, it is obvious as grouped in table (١) that the regression coefficient showed a clear variability in the C – band size of chromosomes no. ١,٩ and ١٦. While the larger and smaller C – band of chromosome no. ١ among the infertility group had a regression coefficient of (٠.١٩٢) and (٠.١٢٣) respectively. they had a regression coefficient of (٠.١٨٨) and (٠.١٦٥) among the normal control group.

Likewise, the larger and smaller C – band of chromosome number ٩ had a regression coefficient of (٠.١٣٥) and (٠.٠٩٥) among the infertile group it had a regression coefficient of (٠.١٦٣) and (٠.١١٤) among the fertile group.

Indeed, it is expected to find that the slope/C – band mean (the average error) varied in both the infertile and fertile group. Thus, a

comparison study showed that in each group the high value of average error was found in the larger and smaller C – band of chromosome number ١. This is expected simply because of the large size of both chromosome no. ١ and C – band size.

The smaller or the lower value as expected was observed in the larger and smaller C – band of chromosome number ١٦ in both groups. The average error of larger and smaller C – band of chromosome no.٩ lied between the two and found to be (٠.٠٦٩) and (٠.٠٥٤) among the infertile group and (٠.٠٧٧) to (٠.٠٧٠) and the fertile group respectively.

Based on the above data it is obvious that there is no significant differences between the two groups could be recognized. The regression coefficient in the both groups was very low, thus, the relationship between reference parameter and C – band size for any of chromosome number (١,٩ and ١٦) was very weak.

Indeed, both region of the chromosome (the euchromatin and the heterochromatin) were affected by colchicine and cell cycle duration but the heterochromatin region (C – band region) was less affected than the euchromatine region (non C – band region). This finding is consistent with previous work of (Erdtman *et al.*, ١٩٨٢), (Brown *et al.*, ١٩٨٠) who reached the same conclusion. The sequences of DNA in the heterochromatine region which is known to be highly repetitive and short stranded DNA may play a role when a colchicine is applied.

Again, the large C – band which is usually located in the centromeric region of chromosome no.١ had both the largest regression coefficient and the highest average of error while the smallest C – band which is usually

located in the centromeric region of chromosome no.١٦ had the lower value of regression coefficient and the lowest value of average error in both the infertile and fertile groups. On the other hand, many previous investigations tended to correlate the C – band region with the euchromatin region (Belicek *et al.,* ١٩٧٨) rather than chose a reference parameter which is used by many other investigations including ourselves and thus did not calculated the high regression coefficient and the value of average error.

The present investigation used the euchromatin of the long chromosome no. ١ as a reference parameter to reduce the problem of chromosome contraction due to colchicine and cell cycle duration, because of its easy identification under the light microscope and the most affected chromosome by the colchicine treatment.

٤.٢.٢ C – band polymorphism and male infertility:

The effect of C – band variants (length and position) on male infertility has been conducted and grouped in tables (٨,٩,١٠,١١). A significant increase in C – band size (absolute and relative length) of chromosomes number ٩ and ١٦ among the azoospermia and oligospermia patients have been obtained as compared to normal control group ($P < ٠.٠١$). No such observation was noticed with regard to C – band size of chromosome number ١. Indeed, no significant differences were recorded when both infertile groups were compared with normal fertile group ($P > ٠.٠١$).

Furthermore, our results showed a significant increase in the frequency of C-band heteromorphism among the oligospermia and azoospermia as compared to normal control groups ($P < ٠.٠١$) (Table ١٠) (Fig.٥,٦,٧) .It must be mentioned here that the present work first to correlate the C-band heteromorphism and male infertility in Iraq or elsewhere. C – band polymorphism has been carried out elsewhere specially in cancer cells. Many investigations claimed the presence of positive relationship between C – band polymorphism and some type of malignancy (Kivis *et al.,* ١٩٨٠) (Sofia *et al.,* ١٩٩٣) and (Land *et al.,* ١٩٩١). No C – band polymorphism in relation with infertility has been conducted except the present study.

Generally, a C – band polymorphism was thought to have some deleterious effect on some patients and may induce some chromosomal aberration. Chromosome aberration was reported in ١٥٪ of ٤٤ patients who carry $^{١}qh^{+}$, $^{٩}qh^{+}$ C – band variants (Holbek *et al.,* ١٩٧٤) while only

٦٪ of ٣٦ couple who showed no chromosomal aberration, though a spontaneous abortion had occurred (Holbek *et al.*, ١٩٧٤).

On the other hand, Nilson and Wohlert, (١٩٩١) reported in their survey of ٨٧١٢ individuals who carry abnormal karyotype in five population about ٠.٠٥٪ of them showed $^{9}qh^{+}$ (large C – band) while about ٣.٦٪ of their parents and ٢.٨٪ of their relatives showed the C – band variants ($^{9}qh^{+}$).

Based on above data, there were no definitive result could be recorded although most investigation which dealt with C – band related the deleterious aspects with larger C – band size (Erdtmann *et al.*, ١٩٨٢).

Indeed, if we accept that the larger C – band had a deleterious effect so the inevitable question now could be raised that why a high frequency of these C – variants are recorded among normal population even in different races.

The answer to such question is most probably due to the fact that, the C – band variants play a role in evolutionary stability of C – band variation, including the extreme variants. If we accept that the C – band variability is now in equilibrium, then the heteromorphic lost by selection need to be replaced and this can be done by mutation or preferential segregation. The mutation rate has not yet been established exactly because of methodological problems, but it seems to be very low (Balicek *et al.*, ١٩٧٨). Preferential segregation has not been detected, expect for the $^{9}qh^{+}$ (Robinson *et al.*, ١٩٧٧; Maginis *et al.*, ١٩٧٧). When the data of fine reports are combined (Dar and Winder, ١٩٦٩), Plamer and Schroder,

١٩٧١; Fitzgeral, ١٩٧٣; Robinson *et al.,* ١٩٧٦; Carneval *et al.,* ١٩٧٦) the preferential segregation of $^{٩}qh^{+}$ is ٧١٪ (٥٠:٢٠).

With regard to the C-band size using the qualitative method, it must be mentioned here that the C-band is distributed into three levels only (level, ١,٢ and ٣) in the present investigation . No further levels (i.e level ٤ and ٥) have been noticed . The results showed an increase in the C-band large size (level ٣) among the infertile groups (Oligospermia and Azoospermia) as compared to normal control group ($P < ٠.٠٠٥$) . Again, the level ١ small size (level ١) showed a significant increase in the frequency among the infertile patients as well as compared to normal control group ($P<٠.٠٠٥$) (Table ١٢) . Furthermore , a significant decrease in the frequency of C-band size (Level ٢) among the oligospermia and Azoospermia as compared to normal control group . Theses results was confusing and hence we relied on the quantitative study in evaluating the relationship between the C-band polymorphism and infertility . Indeed, the qualitative study depend mainly on the relative measurement of the short arm of chromosome number ١٦ . The short arm of chromosome number ١٦ is small and less affected by cell cycle duration and colchicine treatment. Thus, The present study depends mainly on the measurement of the long arm of chromosome number ١, which is large chromosome and more affected by colchicine treatment and cell cycle duration . It is well known that the large C-band is located on the long arm of this chromosome as well . This conclusion was reached by other authors (Erdtmann et al. ١٩٨٢) .

As stated earlier, our results showed a significant increase in the absolute and relative C – band size in chromosomes ٩ and ١٦ among

infertile groups (Olig, azoo) as compared to normal fertile group. Our results seem to be consistent with other reports which correlate the large C – band with some reproductive fitness as the abortion (Nielon et al, ١٩٧٤; Holbeck et al ١٩٧٤) and oligospermia and azoospermia as in our cases.

It seems that the large C – band on chromosome no. ٩ and ١٦ may have indirect effect on sperm production.

It is obvious that the molecular basis of male infertility or fertility is not a linear order of genetic events but the interaction of complex genetic networks functioning in three main developmental pathways: male germ line development, male gonads development and male somatic development. Consequently, primary genetic switch signals should exist for linking of different gene networks and/or for starting them. There is some evidence that switch signals are concentrated on the sex chromosomes (Vogt ١٩٩٧). Accordingly, multiple genes encoding gene products functional for male fertility exists also on other chromosomes (autosomes). It was suggested here that these genes are located on the C – band region of chromosome number ٩ and ١٦, so any increase in size of the C – band region may have indirect effect on the spermatogenis because it will effect the signals network in general.

٤.٢.٣ C – band inversion and male infertility:

It is a well established fact that the C – band shows both size and position variation, except the heterochromatic region of Y chromosome which is located in the terminal part of the chromosome and chromosome no. ١٦. All other chromosome have been reported to show a pericentric inversion (localization variants). However the most studied C – banded are

those which are located on the long arm (q) of pairs (١q) and (٩q) for the occurrence of pericentric inversion.

In attempt to correlate a possible role of the occurrence of pericentric inversion on chromosome no.٩ and ١ with decrease sperm count. A full assessment has been conducted and the results are grouped in table (١١) (fig. ٨, ٩).

Considered as a whole, no significant differences could be noticed in comparison between the infertile and fertile group with regard to pericentric inversion ($P < ٠.٠١$). So far search in literature concerning this item reveales no previous works published in relation of inversion and oligospermia as well as azoospermia , hence it seems to be first instance.

٤.٢.٤ Y – Chromosome variations and infertility:

As a matter of fact, Y chromosomes study started at the same time when chromosome analysis was started in the begining of ١٩٥٠. Indeed, Y – chromosome abnormality was associated with many conditions, including, low intelligent quotient (IQ), aggressive behavior, mental disorders and psychosis, although a controversy regarding the above disorders is still unfinished yet (Brogger *et al.*, ١٩٧٧) (Beltran *et al.*, ١٩٧٩). Furthermore, many authors reported an association between Yq and male infertility and to some extent, short stature and many dysmorphic features (Langmail and Laurence, ١٩٧٤). All the researchers used the Y/F index. As we stated in the results, the human Y chromosome should be classified into at least five classes (very small, small, medium, large, very large).

The most frequent class was called the average while the least frequent classes were called very small or very large. The frequency distribution of the Y/F index for each class is given in table (١٤) . Only ٧٪ of the Iraqi normal individual had small Y chromosome while this class occurred among ١٥٪ of Caucasians and ٣.٣٪ among American Blacks. The majority of Iraqi people have a medium size chromosome which accounts to about ٦٣٪ which located in middle range between the Caucasian (٦٦.٧٪) and the American Blacks (٥٦.٦٪).

In man, only two genes have been assignated to the Y chromosome (Mckusick and Ruddbe, ١٩٧٧). The TDF (testis determining factor) is located on the short arm while the HY (Y histocompatibility antigen) gene is located in non C–band (nc) segment of the long arm of the chromosome.

This raised the question of whether the size of the Y chromosome or the C – band area or euchromatin region has any phenotypic effect, especially when it is either too long or too short. The present study suggests that the variable in the Y chromosome size or the size of C – band or non C–band region do not have any phenotypic effects.

Furthermore, the comparisons between the Iraqi Y chromosome and that of other country confirmed the average size of the Iraqi Y chromosomes and when the Y chromosome of the fertile group was compared with the infertile group (Table ١٣) a significant different has been obtained in the length distribution of the Y chromosome ($P < ٠.٠٥$). About ١٦٪ of the infertile group had a very small size Y chromosome in comparison with zero percentage among the normal fertile group. Indeed, the percentage of the average size Y chromosome is decreased from ٦٣٪ to

٤٧٪, among the infertile group. The decrease in the size was occurred in both infertile groups (azoospermia and oligospermia) (Table ١٤). About ٨.٣٪ of azoospermic patients had very small and ٢٥٪ has small size Y chromosome and about ٧.٥٪ of oligospermic patients had very small and ١٧.٥٪ had small size Y chromosome (Table ١٤).

The decrease in the size of Y chromosome raised the question of possible deletion which may occurred in the Y-chromosomes. The heteromorphic size of the long arm of the Y-chromosomes is generally thought to be the result of deletion (Cohen *et al.,* ١٩٦١) (Genest *et al.,* ١٩٧٠). Muldal and Ockey, (١٩٦٢) came to this conclusion because of the lack of the distal portion of the Y-chromosome.

The biological significance of variation in the length of the Y chromosome is not clear. The observed group differences might represent true chromosomal heteromorphic, but the factor maintaining these heteromorphisms in the population is not known. Lubs and Patil, (١٩٧٥) suggested that there exist a north/south gradient in the length of Y in Europeans, so that men of Mediterranean origin had a longer.

Furthermore, some investigations found a longer Y in criminals and (Brogger *et al.,* ١٩٧٧), while others did not find any length differences between criminals and non–criminals control (Soudek and Laraya, ١٩٧٤).

It has been suggested that long Y chromosomes may be an important causes of fetal loss (Nilsen, ١٩٧٨) (Patil and Lubs, ١٩٧٧) .

In fact, the terminal C–band of the chromosome Y has an important role, because of its great differences in length and variability among population even of the same race and under the same environmental

conditions (Erdtmann *et al.*, ١٩٨١) and (Monsalve *et al.*, ١٩٨٠) also detected differences in the Y/F index between samples of the same nationality. However the Y is an odd chromosome, and although it stated as a member of regular chromosome pair, its evolution has proceeded independently since it stops to cross with its original homologue, the X chromosome.

The Y chromosome in man, as in the most organisms, lacks functional genes except for those involved in fertilization and males fertility. Muller (١٩١٤) proposed that in the absence of crossing, the Y will tend to accumulate deleterious recessive mutations protected by the normal selection. The subsequent chromosome explains the gradual decrease in its size, that may or may not alternate with duplications or translocations that would tend to reconstitute the original length (Monsalve *et al.*, ١٩٨٠).

In fact, after the first description of large microscopically visible deletion of the Y chromosome in chromosomal spreads of infertile men (Tieplo and Zuffardi, ١٩٧٦), the effects of several discrete tracts on the Y chromosome that contains genes probably involved in male genetic maturation (Simoni *et al.*, ١٩٩٨). Today, it is well known that designated as azoospermia factor (AZF) which contains at least two well-characterized genes familes RBM (Ma et al., ١٩٩٣) and DAZ (Reijo et al., ١٩٩٥) genes, located in the distal part of the long arm of the Y chromosome. DAZ was shown to be transcribed exclusively in testis and possibly needs an RNA binding protein, where as, RBM gene family,incoding testis specific RNA binding protein. At present it seems that deletion of the RBM genes in the Yq١١ (interval ٦) (Reijo et al., ١٩٩٦) is suffacient to impair spermatogenesis.

If microdeletions of Y chromosome are the cause of infertility in the affected men, the diagnosis becomes particularly important. Moreover, the knowledge that azoospermia can be related to microdeletions in other, more proximal area of Y chromosome (Ma *et al.*, ١٩٩٢), speaks for the presence of the other spermatogenesis genes in this areas as recently suggested by Lahn and Page (١٩٩٧). For a particular purposes, the tracts of Y as AZFa, AZFb and AZFc, respectively (Vogt *et al.*, ١٩٩٦). The above findings could undoubtedly lead to normal karyotype.

The length variation (heteromorphisms) of the long arm of the Y chromosome is a well-established fact and by family studies it has been concluded that the Y chromosome is inherited at a constant length. The variation in length of the Y chromosome has previously been reported to be large because of C – band (heterochromatin region of the long arm, while non – C – band (nc) region is relatively stable.

Nevertheless, there are at least three studies, including our own indicating that the nc segment does contribute to variation in Y chromosome length.

Our findings indicate that the length of the Y chromosome is dependent on the euchromatin region as well as the C–band region (heterochromatin) and there is a strong correlation between C-band and euchromatin region and the total length of the Y chromosome (i.e. the decrease in the size of the Y chromosome is due to the decease of both the heterochromatin and the euchromatin regions) .Indeed, our work is consistent with that reported by (Soudek and Stroka., ١٩٧٨) who reached the same conclusion.

٤.٣. Satellite Assocation and made infertility:

٤.٣.١. Satellite Assocation and made infertility:

It is well established that satellite association is present normally in somatic cells metaphase (Patil and Lubs, ١٩٧١; Cook, ١٩٧٢; Mutte, *et al.*, ١٩٧٦, ١٩٨١). So that a numerous work has been conducted to study the relationship between satellite association phenomenon and many well know pathologic conditions. Ravia *et al.*, (١٩٨٥) reported a higher frequency of satellite association in the phytohaemagglutinin (PHA) – stimulated lymphocytes for cystic fibrosis and claimed that this higher frequency may be used as a diagnostic method for early identification of cystic fibrosis. Again, (Nillson *et al.*, ١٩٧٥) reported that the hypothyrodism patients exhibited a higher number of chromosomal association specifically between chromosomal number ١٤ and ٢١ than those usual, and a reduction in the association frequency after the patients were treated for their conditions. Indeed Zank *et al.*, (١٩٨٠) deny this finding and they did not confirm that increased frequency of chromosome ١٤ and ٢١ in hypothyroid patients, Beside all that. It has been found with trisomy ٢١ that the number of associations is significantly higher in the trisomic cells than the diploid cells (Rosenkranz and Fleck, ١٩٦٩). In addition, NORs revealed that there are three chromosome ٢١ contained a relatively inactive NOR (Zankle And Nagl, ١٩٨٠). This phenomenon was interpreted as a result from decreased activity of the NORs in the cells with supernumerary aerocentric chromosome which reduced the tendency to build up large association (containing more than two chromocomes) (Zankle *et al.*, ١٩٧٩). Indeed, a high frequency of specific acrceontric association has been considered as predisposing factor to meiotic and mitotic non–disjunction (Ferguson-Smith, ١٩٦٤) and the close proximity

of short arms of specific D-group and G-group Could explain the occurrence of exchange between them leading to Robertsonian translocation (Guichaoua *et al.*, ١٩٨٦). Although, the Robertsonian translocation (١٣/١٤, ١٣/١٥, ١٤/١٥) did not in any was associated with phenotypic abnormality except for a possible association with in male sterility (Gnterwell and Merrell, ١٩٧٥). The above statement together with recent observation reported by Yasseen and his Co-workers who claimed a high frequency of SA among infertile group leads us to extend their work by using the Geimsa stain and G-banding technique to shed light on the possible relationship between SA and decrease in sperm count than normal. A total of ٣١٢ males (٨٠ oligospermia, ١٢٠ Azoospermia, ١٢ KF syndrome and ١٠٠ normal control) were subjected to the present investigation. As mentioned in the results, the occurrence of SA was studied through the use of eight association parameters (number of cells containing Ass., number of Ass., Per cell, no. of associated chromosomes Per cell, no. of cell with small Ass., no. of cell, with large Ass., no. of cell. With one ass., no. of cells with two Ass., no. of cells with more than two Ass.) (Table, ١٨, ١٩)

Considered as whole, a significant increase in the frequency of SA among infertile patients as compared to normal control patients has been noticed .As grouped in (Table ١٨), the Z-test analysis showed that the difference in the mean number of cells containing association is highly significant among the infertile group in comparison with the normal subjects ($P < ٠.٠١$).

Indeed, the number of cell, containing association among the olig., Azoo, KF patients, are (٣٧,٣٥ and ٢٩) respectively while the number of

cell containing association among the normal control is only ١٠. This sharp difference in the number of cells with association open the door to study the rest of the parameters which has been used for this purpose. To achieve our goal each infertile group studied separately and a comparison has been conducted to pinpoint whether the number of satellite association is increased with decreased sperm count. The result which is cited in table (١٩) shows the satellite association for ١٢٠ Azoospermia patients and ١٠٠ normal control for all the parameters which has been mentioned at the beginning of this chapter.

It is clear that a significant difference has been recorded for all the parameters used, compared to normal control group ($P < ٠.٠١$).

Again when relationship has been studied between the patients with low sperm count (Oligospermia) and satellite association, a significant difference was noticed for all the parameters used (Z-test analysis given ($P < ٠.٠١$) (Table ٢٠). As stated previously the KF group was studied separately, simply because of their distinctive clinical feature and the result are grouped in Table (٢١). However, although there is a significant increase in the frequency of SA for all the parameter used in comparison with the normal control group the increase in the association parameters were less than other infertile groups. The mean number for the parameters (Ass./cell, Ass.chr.s./cell, no. of cell with small Ass., no of cell with large Ass, no. of cell with one Ass., no of with two Ass., no. of cell with were (٠.٨٢,١.٦,٤١,٤.٦,٢.٦,٦.٧,٢.٠٧). While the same parameters among klinefelters syndrome (٠.٨٠٥,١.٦,٨.٧,٠.٥,٢.٤,٥.٧,١.٩) respectively.

Furthermore when the two infertile groups were compared with each other, no significant increase was found. The significant increase in the

frequency of SA occurs only in the comparison between infertile group and normal control group. Obviously there is no direct relationship between sperm count and the frequency of SA, i.e. once there is increase in SA and decrease in sperm count, the number of SA remain at high frequency regardless of sperm count. (Hansson,A., ١٩٧٠)

In fact, the higher frequency may be due to the existence of compensation Mechanism in the acrocenterie chromosomes in which the degree of compensation might depend on the activity of the NOR of supernumerary or missing acrocetric chromosomes. Other possibility is that the acrocentric association might be related to Robertsonian translocation or auenploidy condition in which the special association behavior may depend on the factor which have caused the fusion of the two chromosome or the non- disjunction. Our result is in agreement with that reporeted by (Yasseen *et al*., ٢٠٠١) who claimed a high frequency of SA among different infertile groups.

٤.٣.٢ Specific Acrocentric Association and Male Infertility:

After we have shown that there is high frequency of acrocentric association among the infertile group compared to normal control group, the attention is turned to study whether this association is random or non-random by applying G- banding technique. A total of ٨٧ infertile mals patients (٤٠ oligospermia, ٣٥ Azoospermia and ١٢ KF) were subjected to this study. As grouped in table (٢٤) although all the acrocetric chromosomes tend to show an association tendency but the medium sized chromosome (i.e. chromosomes number ١٣,١٤,١٥) are the most associated chromosome than the a smaller one. Furthermore, the tendency of chromosome no. ١٣ to associate with the rest of the chromosomes are

apparent specially among the infertile group (oligo and azoospem) which reached a total of ٤٦٢ and ٤٩٦ association per total sample while the tendency of the same chromosomes to associate among the normal control was ٧١ association per total sample. The interesting finding was the association ability of chromosome no.١٣ among the klinefelter's syndrome, which showed ١٢٤ association per sample though the association per cell was high. The specific satellited ability for the five acrocetric chromosomes among the normal control was decreased and there is no difference between them.

Generally, the present investigation revealed a high frequency of association between chromosome number ١٣ –١٤ among the oligospermia patients compared to normal control. The Chi-square analysis showed the association is highly significant. Again, the two by two association particularly between chromosome numbers (١٣-١٣) (١٣-١٤) (١٣-١٥) (١٣-٢١) (١٣-٢٢) were significant ($x^{٢}$ =٣٠٣.٧٧, p < ٠.٠٢٥). No such observation has been noticed among both the azoospermia and the klinefelter's syndromes. A high frequency of association was observed between chromosome number ١٣-١٤ among the azoospermia though it is not significant. Based on the above data, the present investigation showed that the association tendency among the oligospermia groups is non-random specially when chromosomes no. ١٣ and ١٤ are involved.

A high frequency of specific acrocentric association has been considered as predisposing factor to mitotic and meiotic non-disjunction (Ferguson – Smith, ١٩٦٤). Furthermore, the result of Mattei *et al.,* (١٩٧٦) also suggested the unequal frequency observed in the distribution of Robertsonian translocation constituted an argument supporting the view

that the association between acrocentic chromosomes did not occur at random. Indeed, mitotic investigations were often inconsistent, some workers reporting random association while other claimed the non random involvement while other claimed the non-random involvement of acrocentric chromosomes (Mattei *et al.,* ١٩٨١). It is also suggested that the close proximity of the short arms of specific D-group and specific G-group could explain the occurrence of exchange between them leading to Robortsonian translocation (Guichaona *et al.,* ١٩٨٦).

It appears that D/D translocation (١٣/١٤ , ١٣/١٥ and ١٤/١٥) are do not in any way to be associated with phenotypic abnormality, except for a possible association with an increase sterility (Centerwall and Merrell, ١٩٧٥).

Two factors are known to play a causal role in the association of acrocentric chromosomes:-

١-The presence of satellite DNA in the short arms of those chromosomes:

The non-random distribution of Robertsoniaon's translocation can be explained by the mieotic distribution of acrocentric associations (Guichaoua *et al.,* ١٩٨٦). The tendency for specific acrocentric chromosomes to be in Robertsonian translocation could result from the homology at a molecular level (Guichaoua *et al.,* ١٩٨٦). It has been demonstrated that breakpoints in Robertsonian's are preferentially located within the satellite DNA constituting the short arms of acrocentric chromosomes (Gosden *et al.,* ١٩٨١). Also, satellite DNA consists of varios families repeated identical or close sequences, each satellite DNA occurs one more than one chromosome (Jones and Corineo, ١٩٧١). In conclusion, that modal they proposed, based on homology at the molecular level, has

the advantage of reconciling the nonrandom distribution of Robertsonial translocation.

٢-NOR activity**:**

It is well known in somatic metaphase there is a good correlation between the tendency for an acrocentric chromosomes to associate and it's NOR activity measured by silver-staining (Miller *et al.*, ١٩٧٧). Moreover, a significant increase of Silver stained NOR in PHA – stimulated lymphocytes has been investigated, there is the same strict correspondence between mitotic association of acrocentric chromosomes and Ag-NOR staining. Also, Schemed *et al.*, (١٩٧٥) suggested that the repetitive sequence play actual role in the heterochromatic attraction. Quantities evaluations of satellite DNA with different probes carryout by Gosden *et al.*, (١٩٧٥) and Jean Pierre *et al.*, (١٩٨٥) support the assumption.

In conclusion, it seems to be a non-random tendency of acrocentric chromosomes is evident particularly between chromosomes number ١٣-١٤, among the oligospermia, this tendency could be consider as predisposing factor to Robertson translocation. This finding is supported by previous work which revealed that the about ٤٪ of the oligospermic patients have ٤٥,XY, t(١٣,١٤) karyotype (Hook and Hamerton, ١٩٧٧) (Page et al., ١٩٩٦).

Conclusions and Recommendations

١- The present investigation stresses the importance of chromosomal analysis for all infertile patients specially those who are not responding to conventional treatments.

٢- The significant increase in the frequency of autosomal C-band polymorphism may require further intensive work to shed the light on the correlation between the C-band polymorphism and male infertility.

٣- The high frequency of specific satellite associations particularly between chromosome numbers ١٣ and ١٤ push us to suggest that this new technique could used for the adequate diagnosis of male infertility specially those who exhibit normal chromosomal karyotype by routine Giemsa stain analysis.

٤- A significant Correlation between Robertsonian translocation and high frequency of satellite association among Oligospermia patient were observed and has been considered as a predisposing factor for centric fusion.

٥- The decrease in the size of the Y chromosome which was observed among the infertile groups required more research using the more advance molecular biology or PCR techniques to assign the region involved.

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Appendix (١):

Materials and their commercial companies used the methods.

Materials	Company
RPMI	Gibco BRL
Foetal bovine serum	Gibco BRL
Phytohaematoagglutinin	Gibco BRL
Colchicine	FLUKA
Giemsa stain	FLUKA
Sidium chloride	FLUKA
Calcium chloride	Merlk
Trisodium citrate	BDH chemicals
Glacial a cetic acid	BDH chemicals
Barium hydroxide	FLUKA
Silver nitrate	FLUKA
Methanol	FLUKA

دراسة التحزم C واشتراك التوابع الكرومرسومي عند المرضى المصابين بانعدام النطفية وقلة النطفية

رسالة مقدمة إلى
مجلس كلية العلوم ـ جامعة بابل
وهي جزء من متطلبات نيل درجة دكتوراه فلسفة في علوم الحياة (الحيوان)

من قبل

صالح مهدي مصطفى الخفاجي

شعبان ١٤٢٢هـ **تشرين الثاني ٢٠٠١م**

((أيحسبُ الإنسان أن يترك سُدًى ألم يَكُ نُطفَةً من منيٍ يُمنى ثم كان علقةً فخلَق فسّوى فجعل منه الزوجين الذكر و الأنُثى))

الاية 39 سورة القيامة

صدق الله العظيم

الخلاصة

ان العمل المنجز في هذه الأطروحة قد تم في جامعة بابل للفترة من تموز ١٩٩٨ إلى تموز ٢٠٠١ وباشراف كل من الأستاذ الدكتور عقيل عبد ياسين والأستاذ الدكتور اسماعيل كاظم عجام. وباستثناء ما مشار إليه بمصدر معين فان المعلومات الموجودة هي من نتاج الباحث وإنها لم تقدم لنيل درجة علمية أخرى.

تم دراسة مائتين وسبع وسبعين مريضا دراسة ووراثية خلوية للذكور العراقيين الذين يعانون من انعدام الخصوبة من خلال التحليل الكروموسومي، من هؤلاء مائتين واثنى عشر مريضا نجحت الخلايا اللمفاوية في الوصول إلى طور الميتافيز- بالاضافة إلى مئة من الاشخاص الاصحاء كمجموعة سيطرة، وتراوحت اعمار المرضى بين ١٩-٥٤ سنة.

واظهرت النتائج ما يلي:

أ. التحليل الكروموسومي بصيغة الكيمزا:

١. من٢١٢ مريضا يعاني من انعدام الخصوبة، تبين ان ١٢ مريضا فقط اظهروا شذوذا كروموسوميا واضحا، وقد كانت نسبة الشذوذ حوالي ٥.٧%.

٢. كانت نسبة الشذوذ في المرضى المصابين بانعدام النطف من العدد الكلي البالغ ١٢٤ وجد ان هناك اربعة مرضى فقط اظهروا شذوذا كروموسوميا واضحا وكانت نسبته حوالي ٣.٢%.

٣. في المرضى المصابين بقلة النطف وجد الشذوذ الكروموسومي بشكل واضح في ٨ مرضى من ٨٨ مريضا وبنسبة ٩.١%.

٤. اربعة مرضى مصابون بانعدام النطف كانت طبعة النواة في اغلب الاحيان هي ٤٧,XXY مترافقة مع متلازمة كلاينفلتر.

٥. سبعة مرضى مصابون بقلة النطف كانت طبعة النواة لهم هي ٤٦,XY/٤٧,XXY.

٦. مريض واحد مصاب بقلة النطف الشديد اظهر شذوذا كروموسوميا في الخلايا الجسمية وهو ٤٦,XY/٤٥,XYt (D;D).

ب. التغايرات في التحزم C في الخلايا الجسمية:

١. تم دراسة التغايرات في تحزم C بشكل مكثف من خلال دراسة التغايرات في الحجم، الموقع وكذلك التشابه وعدم التشابه (في التحزم C للكروموسومات المتماثلة) الواقع على كروموسوم رقم ١، ٩، ١٦%.

٢. وجد ان هناك زيادة معنوية في حجم هذا التحزم على الكروموسوم رقم ٩، ١٦ في خلايا المرضى المصابين بالعقم مقارنة مع الاصحاء٠.٠١ > P.

٣. زيادة معنوية في حجم التحزم(C) المستوى الثالث للمرضى المصابين بالعقم في كروموسوم رقم ١ مقارنة مع مجموعة السيطرة ٠.٠٥ > P.

٤. زيادة معنوية في تكرار التغايرات (اختلاف حجم التحزم في الكروموسومين المتماثلين) عند المرضى المصابين بانعدام الخصوبة ٠.٠١ > P.

٥. ان التغايرات في موقع التحزم C، لم يلاحظ فروق معنوية في المجاميع قيد الدراسة ومجموعة السيطرة ٠.٠٥ < P.

ج. تغايرات كروموسوم Y:

١. طبقا للمعيار Y/F وجد ان هناك تكرار عالي لكروموسوم Y صغير الحجم في الأشخاص المصابين بانعدام الخصوبة، مقارنة بمجموعة السيطرة.

٢. هناك ارتباط واضح وقوي في تحزم C الواقع في الطرف البعيد للذراع الطويل لكروموسوم Y ومنطقة Euchromatin والطول الكلي لكروموسوم Y .

د. اشتراك التوابع: أظهرت الدراسة ما يلي:

١. وجد تكرار ذو مغزى لاشتراك التوابع بين المرضى المصابين بانعدام الخصوبة مقارنة بالناس الاصحاء.

٢. وجد ان اشتراك التوابع بين الكروموسومين (١٣-١٤) اكثر تكرارا عند المرضى المصابين بقلة النطفية.

٣. ان اشتراك التوابع بين الكروموسومات (١٣-١٣) (١٣-١٤) (١٣-١٥) (١٣-٢١) (١٣-٢٢) ذات تكرارات تتوزع بشكل غير عشوائي ٠.٠٠٥ > P.

٤. اما فيما يتعلق بالمرضى المصابين بانعدام النطف وجدت الدراسة ان اشتراك التوابع بين الكروموسومين (١٣-١٤) الاكثر تكرارا. اضافة إلى ان اشتراك التوابع بين الكروموسومات (١٣-١٣) (١٣-١٢) (١٣-١٥) (١٣-٢١) (١٣-٢٢) لها تكرارات تتوزع بشكل عشوائي P .> ٠.٠٠٥

Table (٦): The average error of C-band size in patients suffering from subfertility.

C – bands	No. of patients	C – band mean ±SD (in Mm)	Regression coefficient (١qh – h x h)	Slope/ C – band mean
Larger ١qh	٨٣	٢.٧٦ ± ٠.١٦	٠.١٩٢*	٠.٠٧٤
smaller	٨٣	١.٧٢ ± ٠.٣٦	٠.١٢٣*	٠.٠٧١
larger ٩qh	٨٣	١.٨٢ ± ٠.٣٩	٠.١٣٥*	٠.٠٦٩
smaller	٨٣	١.٧٤ ± ٠.٣٤	٠.٠٩٥	٠.٠٥٤
larger ١٦qh	٨١	١.٥ ± ٠.٦٩	٠.٠٧٣	٠.٠٤٨
smaller	٨١	١.٢٣ ± ٠.٥	٠.٠٥٢	٠.٠٤٢
> ١.٦M Yq١٢ < ١.٦M	٨٠	١.٧١ ± ٠.٣٢	٠.١٨٤*	٠.١٠٧

	٨٠	١.٨١ ± ٠.٤١	٠.٠٨٢	٠.٦٩

* P < ٠.٠١

Table (٧): The average error of C-band size normal fertile group.

C – bands	No. of patients	C – band mean SD (in Mm)	Regression coefficient (١qh – h x h)	Slope/ C – band mean
Larger ١qh	٨٣	٢.٢٩ ± ٠.٦٨	٠.١٨٨*	٠.٠٩٢
smaller	٨٣	١.٧٨ ± ٠.٧٤	٠.١٦٥*	٠.٠٨٢
larger ٩qh	٨٣	٢.٠٣ ± ٠.٥٥	٠.١٦٣*	٠.٠٨٠
smaller	٨٣	١.٥٨ ± ٠.٥	٠.١١٤	٠.٠٧٢
larger ١٦qh	٩٥	١.٤٨ ± ٠.٣	٠.١١٥	٠.٠٧٧
smaller	٩٥	١.١٧ ± ٠.٢٩	٠.٠٨٣	٠.٠٧٠

> ١.٦M Yq١٢	٨٠	١.٩٢ ± ٠.١٣١	٠.١٩١*	٠.٩١
< ١.٦M	٨٠	١.٠٧ ± ٠.١٠١	٠.٠٨٢	٠.٩٤

P* < ٠.٠١

Table (٩): Relative lengths of C- band on chromosomes ١,٩ and ١٦ in patients (Oligospermic, Azoospermia) and the control groups.

Length of C- band Relative %	Groups	chromosomes					
		(١)		(٩)		(١٦)	
		Larger (M ± SD)	Smaller (M ± SD)	Larger (M ± SD)	Smaller (M ± SD)	Larger (M± SD)	Smaller (M ± SD)
	Azoospermic n = ١٢٠	٢٣.١ ± ٣.٨٤	٢١.٥ ± ٣.٥٩٦	١٩.٨ ± ٥.٥٦	١٧.٨ ± ٣.٠٨	١٨.٥ ± ٢.١٢	١٦.٤٦ ± ٢.٧
		٢٢.٣٧ ± ٣.٧٤		١٨.٨١ ± ٤.٣٢		١٧.٧٦ ± ٢.٤١	
	Oligosperamic n = ٨٠	٢٢.٢٣ ± ٣.١٢	٢١.١١ ± ٣.٨٦	٢٠.٤٣ ± ٤.٠٩	١٨.٧١ ± ٤.٧٤	١٧.١ ± ٣.٢٩	١٥.١ ± ٣.٧٣
		٢١.٦٧ ± ٣.٤٩		١٩.٥٧ ± ٤.٤١٥		١٦.١ ± ٣.٥١	

	Control **n = ١٠٠**	٢٠.٩٨ ± ٦.٠٦	١٩.٦٢ ± ٥.٦٣	١٧.٧ ± ٣.٦	١٦.٧ ± ٤.١٣	١٢.٩ ± ٣.٩٨	١١.٩ ± ٣.٦٦
		٢٠.٣ ± ٥.٨		١٧.٢ ± ٣.٨٧		١٢.٤ ± ٣.٨٢	

Comparison // A & O & C there are no significant differences F – Test RCBD ANOVA

df Treatment = ٢ SST = ٤٣.٤ MST = ٢١.٧

Error = ٤ SSE = ١٢٤.٦ MSE = ٣١.١٥

$F_{CAL.}$ = ٠.٦٩٧ $F_{TAB.}$ = ١٩.٢٥ P < ٠.٠٥

df Treatment = ٢ SST = ٤٣.٤ MST = ٢١.٧

Error = ٤ SSE = ١٢٤.٦ MSE = ٣١.١٥

$F_{CAL.}$ = ٠.٦٩٧ $F_{TAB.}$ = ١٩.٢٥ P < ٠.٠٥

Table (٢٦): Distribution of two-by-two associated chromosomes among patient with oligospermia.

Patients No.	Associated Chromosomes per ٢٥ cells														
	١٣-١٣	١٣-١٤	١٣-١٥	١٣-٢١	١٣-٢٢	١٤-١٤	١٤-١٥	١٤-٢١	١٤-٢٢	١٥-١٥	١٥-٢١	١٥-٢٢	٢١-٢١	٢١-٢٢	٢٢-٢٢
١	٢	٦	٢	٠	٣	٤	٥	٤	٠	٢	١	٣	٢	٤	٣
٢	٤	٧	٣	٦	٢	٣	٤	٥	٣	٢	١	١	١	٢	١
٣	٣	٥	٢	٣	٣	٤	٣	٣	١	١	٢	٣	٠	٣	٣
٤	١	٤	٣	٤	٤	٢	٢	١	٢	٠	٣	٢	٠	٢	٣
٥	٢	٥	٤	٤	٢	٣	٣	٤	٣	١	٠	٢	٠	١	١
٦	٣	٤	٢	٠	٠	٠	٢	٠	١	١	٠	٠	٠	٢	٤
٧	٣	٥	٢	٧	٣	١	٣	٤	١	٠	٢	١	٠	٠	٠
٨	٧	٨	٧	٤	٢	٥	٢	٢	٣	٠	٣	٢	٣	٣	٢
٩	٠	٨	٠	٠	٢	٣	٥	٦	٤	٠	٣	١	٢	٤	١
١٠	٨	٦	٥	٩	٤	٤	٣	٤	٤	٠	٢	٢	٢	٣	٠
١١	٦	١	٧	٥	١	٠	١	٣	٠	١	٠	٠	٠	٠	١
١٢	٠	٨	٥	٠	٨	١	٥	٤	٣	٢	٢	٤	٠	٣	٢
١٣	١٤	٧	٠	٠	٨	٣	٤	٥	٤	٣	٣	٣	٢	٤	٢
١٤	٥	٨	٠	٠	٠	٢	٤	٤	٥	٤	٤	٣	٣	٤	٢
١٥	٠	٩	٩	٠	٠	٤	٧	٦	٦	٠	٠	٦	٢	٤	٠
١٦	٤	١٠	٠	٠	١	٣	٣	٥	٤	٢	٣	٣	٢	١	١
١٧	٩	٦	٧	٠	٨	٤	٢	٣	٥	٢	٣	٤	٢	٤	٢
١٨	٥	٤	٩	٨	٨	٦	٥	٤	٦	٢	٤	٢	٢	٤	٢
١٩	٠	٧	٩	٨	٠	٢	٣	٤	٤	١	٣	٢	١	٢	٣
٢٠	٨	٧	٠	٠	٠	٤	٣	٣	٥	٣	٤	٥	٢	٣	٢
٢١	٠	٨	٦	٠	٧	٢	١	٤	١	٤	٠	٢	٢	٤	٥
٢٢	٨	٦	١٠	٦	٦	٢	٢	٥	٢	٢	٤	٣	٤	٤	٧
٢٣	٠	٩	٠	٠	٠	٣	٣	٦	٤	٣	١	٢	٥	٤	٤
٢٤	٤	٥	٤	٣	١	٢	٢	٤	٣	٤	١	٢	٤	٣	٣
٢٥	٣	٣	٣	٢	٤	٢	٤	٤	٤	٢	٣	٣	٣	٣	٣
٢٦	١	٤	٣	٤	٤	٢	٣	٤	٤	٢	٣	٣	٣	٣	٣
٢٧	٣	٥	٤	٣	٣	٣	٣	٤	٤	٢	٣	٣	٣	٣	٣
٢٨	٣	٥	٣	٣	٣	٣	٣	٤	٤	٢	٣	٣	٣	٣	٣
٢٩	٣	٥	٣	٣	٣	٣	٣	٤	٤	٢	٣	٣	٣	٣	٣
٣٠	٣	٥	٣	٣	٣	٣	٣	٤	٤	٢	٣	٣	٣	٣	٣

	١٣٣	٢١٦	١٤٢	٩٥	١١٠	٨١	١٢١	١٣٩	٩٩	٧٤	٨٠	٩٥	٨٥	١٠٣	٨٨

p< ٠.٠٠٥ ١٣-١٤ & ١٣-١٣ ١٣-١٤ & ١٣-١٥ ١٣-١٤ & ٢١-٣١ ١٣-١٤ & ١٣-٢٢

x^2 = ٥٦.٣ x^2 = ٥٥.٧٠ x^2 = ٥٥.٩٨ x^2 = ٥٣.٦٢ x^2 total = ٣٠٣.٧٧ df = ١٣٦

Table (٢٨): Distribution of two-by-two associated chromosomes among Azoospermia patients(continued)

Patients No.	Associated chromosomes														
	١٣-١٣	١٣-١٤	١٣-١٥	١٣-٢١	١٣-٢٢	١٤-١٤	١٤-١٥	١٤-٢١	١٤-٢٢	١٥-١٥	١٥-٢١	١٥-٢٢	٢١-٢١	٢١-٢٢	٢٢-٢٢
١	٢	٤	٤	٣	٣	١	٢	٢	٢	٤	٣	٥	٣	٤	٤
٢	٢	٦	٥	٥	٣	٠	٤	٢	٣	٥	١	٣	٤	٢	٣
٣	٤	٥	٤	٤	٢	٤	٥	٠	٦	٤	٠	٤	٦	٣	١
٤	٤	٥	٢	٢	٤	٤	٢	٢	٤	٣	٣	٤	٢	٤	٢
٥	٢	٤	٦	٥	٠	٥	٢	١	٤	٣	٣	٢	٤	٤	٢
٦	٥	٥	٤	٥	٦	٣	٥	٤	١	٣	٥	٦	٢	٢	٤
٧	٥	٤	٦	٤	٢	٦	٥	٣	٤	٢	٢	٧	٥	٤	٤
٨	٥	٤	٣	٢	١	٦	٢	١	٤	٤	٠	١	٢	١	٢
٩	٣	٦	٠	٣	٥	٤	٢	١	٢	٥	٢	٧	٤	٢	٢
١٠	٤	٦	١	٣	٢	٢	٣	٢	٢	٤	٣	٥	٤	٣	٣
١١	٤	٥	٥	٥	٣	٣	٣	٣	٤	١	١	٣	٢	٣	٠
١٢	٣	٤	٢	٣	٦	٣	٤	١	٣	٥	٤	٥	٣	٤	٣
١٣	٣	٤	٤	٢	٣	٤	٤	٦	٣	٤	٣	٤	٣	٥	٢
١٤	٧	٢	٣	٠	٠	٤	٣	٤	٢	٢	٥	٣	٠	٤	٢
١٥	٦	٥	٢	١	٤	٥	٤	١	٥	٣	٦	٢	٦	٣	٢
١٦	٥	٧	٥	١	٤	٣	٥	٣	٣	٣	٣	٤	٢	٠	١
١٧	٥	٠	٣	٢	٥	٢	٤	٢	٤	٢	٣	٤	٢	٢	٠
١٨	٢	٥	٣	٢	٣	٦	٢	٠	١	٣	٤	٣	٤	٣	٠

٩	٥	٢	٤	٤	٤	٣	٦	٥	٧	٤	٥	٥	٤	٣	١
٠	٢	٦	٤	٣	٥	٧	٢	٤	٥	٢	٢	٤	٥	٢	٢
Total	٧٨	٨٩	٧٠	٥٩	٦٥	٧٥	٦٨	٤٧	٦٩	٦٦	٥٨	٨١	٦٧	٥٨	٤٠

Table (٢٨): Distribution of two-by-two associated chromosomes among Azoospermia patients

Patients No.	Associated chromosomes													
	١٣-١٣	١٣-١٤	١٣-١٥	١٣-٢١	١٣-٢٢	١٤-١٤	١٤-١٥	١٤-٢١	١٤-٢٢	١٥-١٥	١٥-٢١	١٥-٢٢	٢١-٢١	٢١-٢٢	٢٢-٢٢
١	٣	٣	٤	١	٣	١	٢	٣	٢	٣	٣	٥	٤	١	٢
٢	٤	٢	٤	٢	٢	٠	٣	٢	٢	٢	١	١	٣	٢	٢
٣	٤	١	٣	٢	٢	٢	٣	١	٤	٣	٢	٣	٢	٠	٣
٤	٣	٧	٣	٣	٢	٢	٣	٢	٤	٤	٢	٤	٤	٢	٢
٥	١	٠	٤	٦	٥	٤	١	٢	٥	٣	٣	٢	٢	٤	٢
٦	٦	٣	٣	٠	٥	٢	٣	٢	٢	٤	١	٤	١	٤	٣
٧	٥	٦	٤	٥	٤	٤	٤	٥	٤	٤	٥	٦	٦	٤	٤
٨	٢	٤	٣	٥	٤	٠	٣	٢	٥	٢	٢	٥	٢	٤	٢
٩	٠	٧	٥	٣	١	١	٤	٣	٢	٢	٤	٥	٥	٣	٢
١٠	٢	٥	٣	٤	٥	٢	٣	٣	٧	٤	٤	٤	٤	٣	٣
١	٥	٨	٥	٣	٣	٢	٤	٢	٢	٥	٤	٥	٤	٤	١
٢	٢	٢	٢	٣	٣	٢	٢	١	٣	٢	٢	٢	٤	١	٢
٣	٥	٧	٣	٥	٤	٣	٢	٢	٢	٣	٣	٣	٣	٣	٢
٤	٦	٨	٥	٥	٤	٣	٣	٣	٥	٠	٢	٦	٣	٢	٥
١٥	٦	٦	٣	٢	٣	٣	٣	٢	٢	٢	٤	٣	٢	٢	١

٦	١	٢	٥	٤	٢	٢	٣	٢	٢	٤	٠	٠	٢	٢	٢
٧	٥	٥	٤	٤	٣	١	٢	١	٤	٠	١	١	٢	٢	٢
٨	٢	٦	٥	٤	١	١	٠	١	٣	٠	١	٤	٤	٤	٤
٩	٤	٤	٣	٥	٢	٣	٢	٢	٤	١	٢	٢	٢	٢	٣
٠	٣	٣	٢	١	٣	٠	٢	٢	٢	١	١	٦	٣	٣	١
Total	٦٩	٨٩	٧٣	٦٧	٦١	٣٨	٥٢	٤٣	٦٦	٤٩	٤٧	٧١	٦٢	٥٢	٤٨
Pooled	١٤٧	١٧٨	١٤٣	١٢٦	١٢٦	١١٣	١٢٠	٩٠	١٣٥	١١٥	١٠٥	١٥٢	١٢٩	١١٠	٨٨

$X^{٢}$ = ١٢٨ NON SIGNIFICANT

Table (٣٠): The distribution of different types of two-by-two associated chromosomes among Klinefelter's syndrome patients.

Patients No.	Associated Chromosomes														
	١٣-١٣	١٣-١٤	١٣-١٥	١٣-٢١	١٣-٢٢	١٤-١٤	١٤-١٥	١٤-٢١	١٤-٢٢	١٥-١٥	١٥-٢١	١٥-٢٢	٢١-٢١	٢١-٢٢	٢٢-٢٢
١	٢	٢	٤	٣	٥	٠	١	٢	٥	٣	٣	٤	٣	٤	٣
٢	٤	٣	٤	٤	٤	٣	٠	٢	٣	٢	٢	٣	١	١	٣
٣	٢	٣	٢	٠	٥	٤	٢	٤	٢	٢	١	٠	٢	٢	١
٤	٢	١	٣	٢	٠	٤	٢	٢	٢	١	٢	٢	٥	٤	٣
٥	٣	٦	٣	١	٣	٣	١	٣	١	٢	٣	٠	٢	٣	٣
٦	١	٤	٥	٢	٠	٠	١	١	٢	٣	٢	٥	٣	٥	٢
٧	٤	١	١	٣	٢	٢	١	٤	٣	٢	١	٤	٤	٦	٤
٨	٣	٥	٣	٥	٢	١	٣	٠	٤	١	٣	٣	٥	٣	٢
٩	٣	٠	٢	٥	٢	٢	١	٣	٥	٣	٣	٢	٤	٥	١
١٠	٤	٣	٤	٠	٤	٣	٢	١	١	٢	١	٥	٤	٠	٢
١	٥	٤	٠	٤	٥	٠	٢	٠	٣	١	١	٤	٥	٢	٢

٢	٣	٥	٥	٣	٢	٤	٢	١	٢	١	٢	٢	٥	٣	٢
	٣٦	٣٧	٣٦	٣١	٣٤	٢٦	١٩	٢٣	٣٣	٢٣	٢٤	٣٤	٤٢	٤٤	٢٨

$p < ٠.٠٠٥$ $df = ١٣$ $x^{٢}$ tab.$= ٨١٩$

١٣-١٤ & ١٣-١٣ ١٣-١٤ & ١٣-١٥ ١٣-١٤ & ٢١-٣١ ١٣-١٤ & ١٣-٢٢

$x^{٢} = ١٠.٦٧$ $x^{٢} = ٩.٩٨$ $x^{٢} = ١٥.٩٤$ $x^{٢} = ١٠.٥٩$ $p < ٠.٠٢٥$

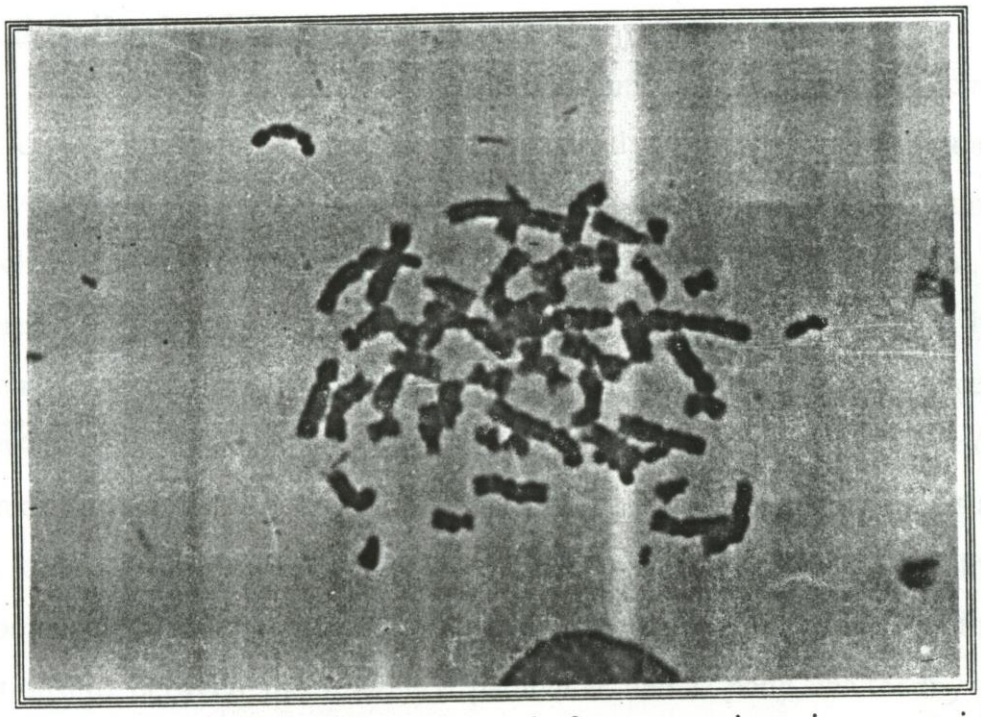
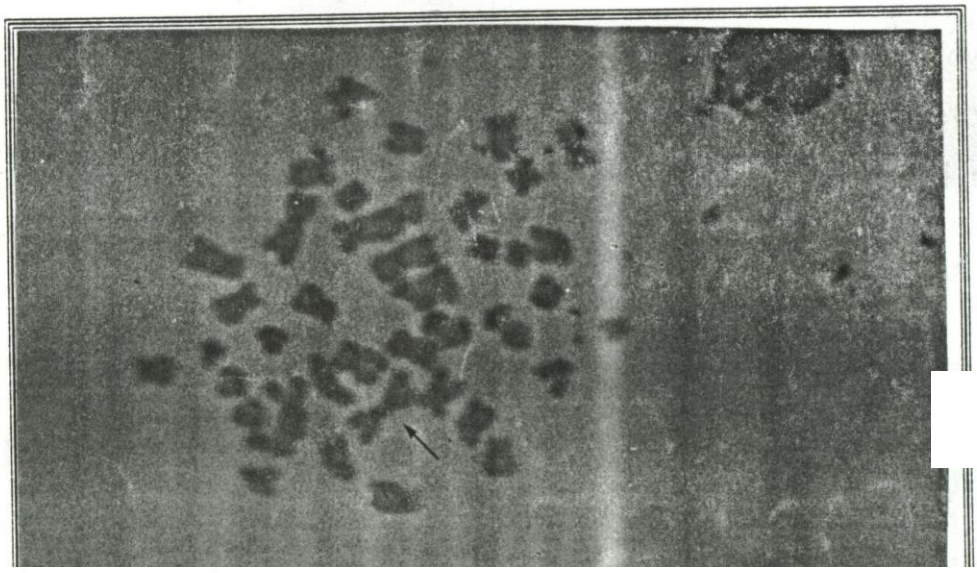
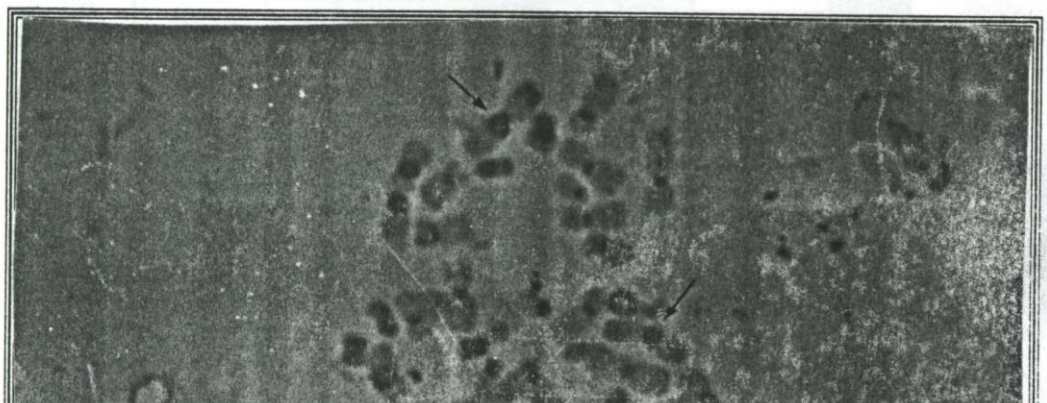


Fig. (1) G- banded metaphase spread of azoospermia patients associated with Klinfelter's syndromes (47, XXY)



e- A significant increase in C – band size of chromosome number 9 among the oligospermia compared to azoospermia patients ($z=6.08$, $P < 0.01$).

f- As far as chromosome no. 16 is involved, a significant increase in the C–band size was noticed among both the oligo and azoospermia patients compared to control group ($z=7.08$, 6.13, $P < 0.01$). Indeed no significant differences was recorded when both the infertile group was compared with each other ($z=1.04$, $P < 0.01$).



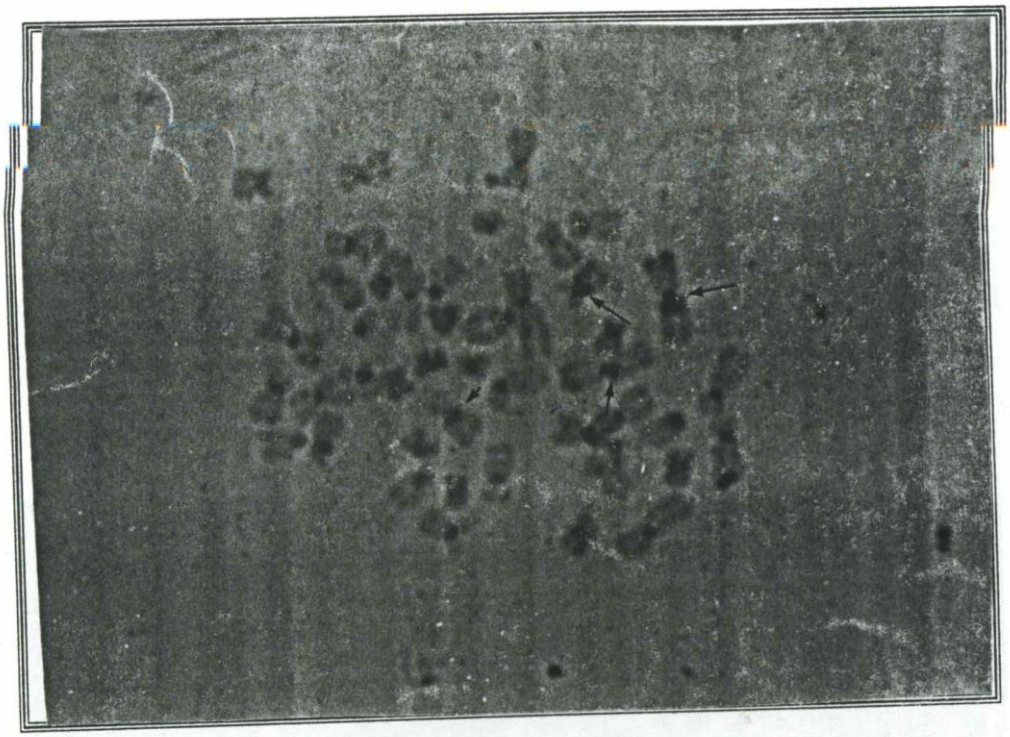
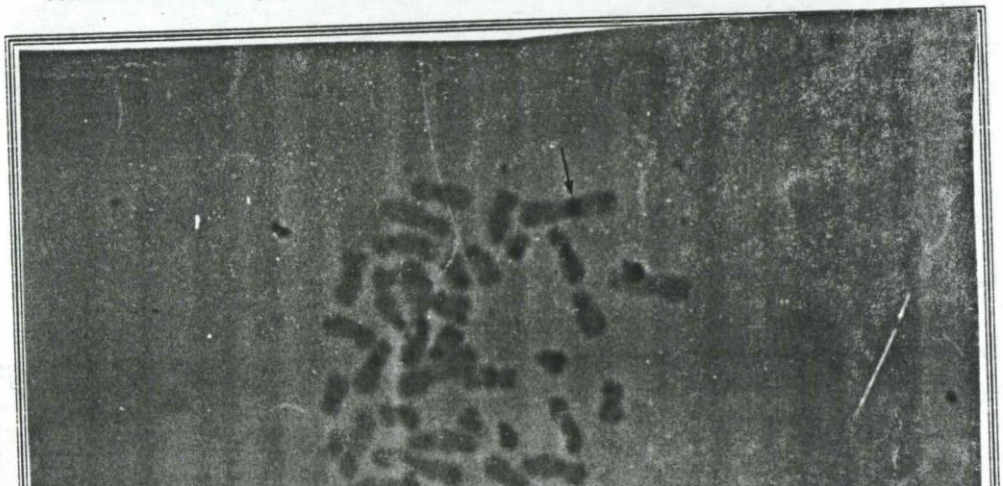


Fig. (5) Representative C- banded metaphase spread of patient with oligospermia note, C- segment a symmetry (heteromorphism) on chromosomes (1) and (9) (arrowed)



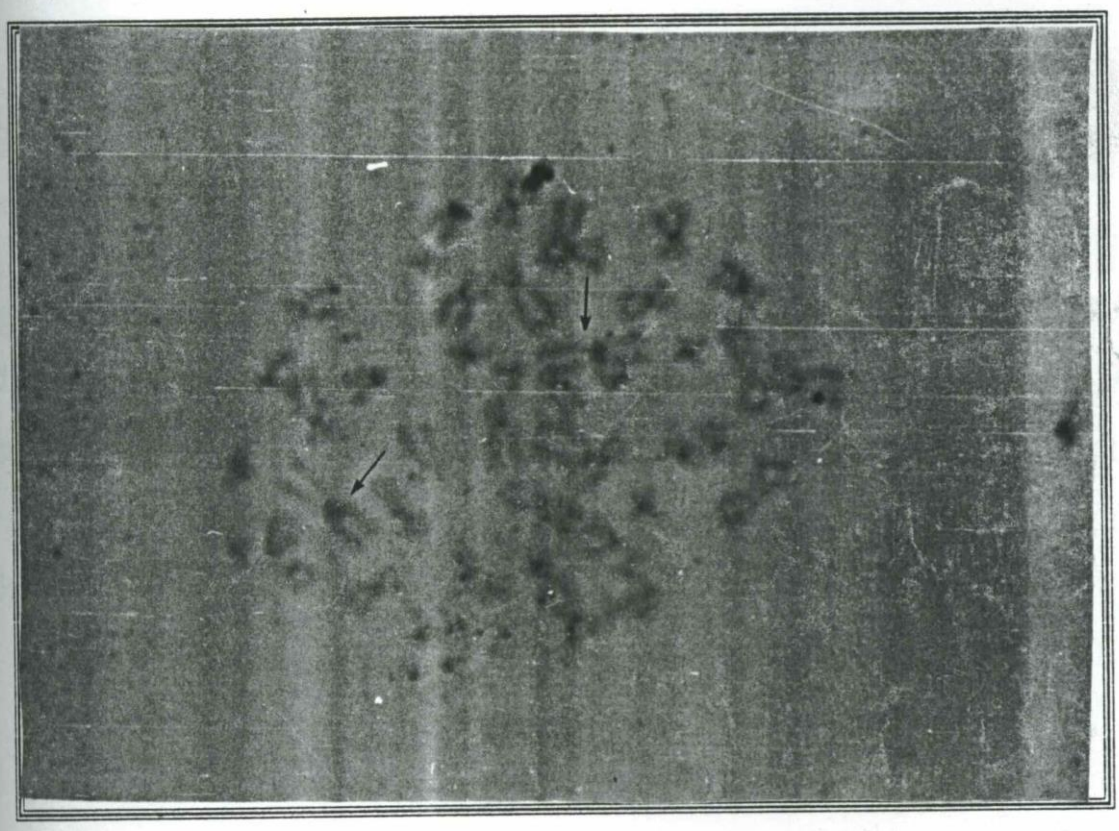
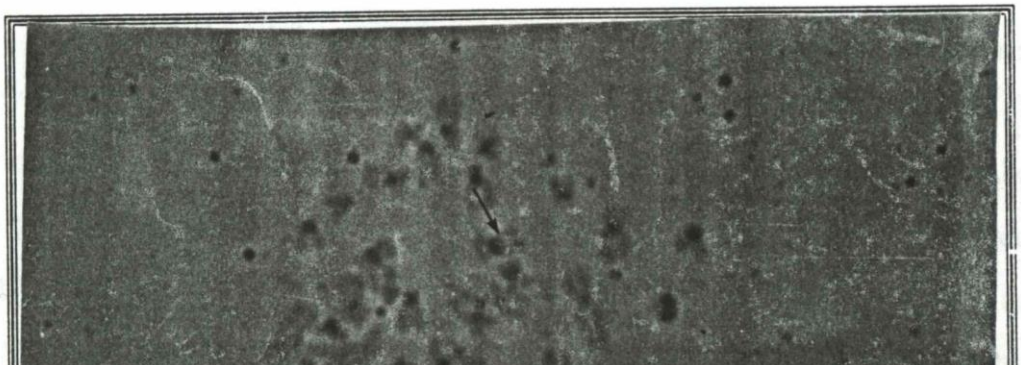
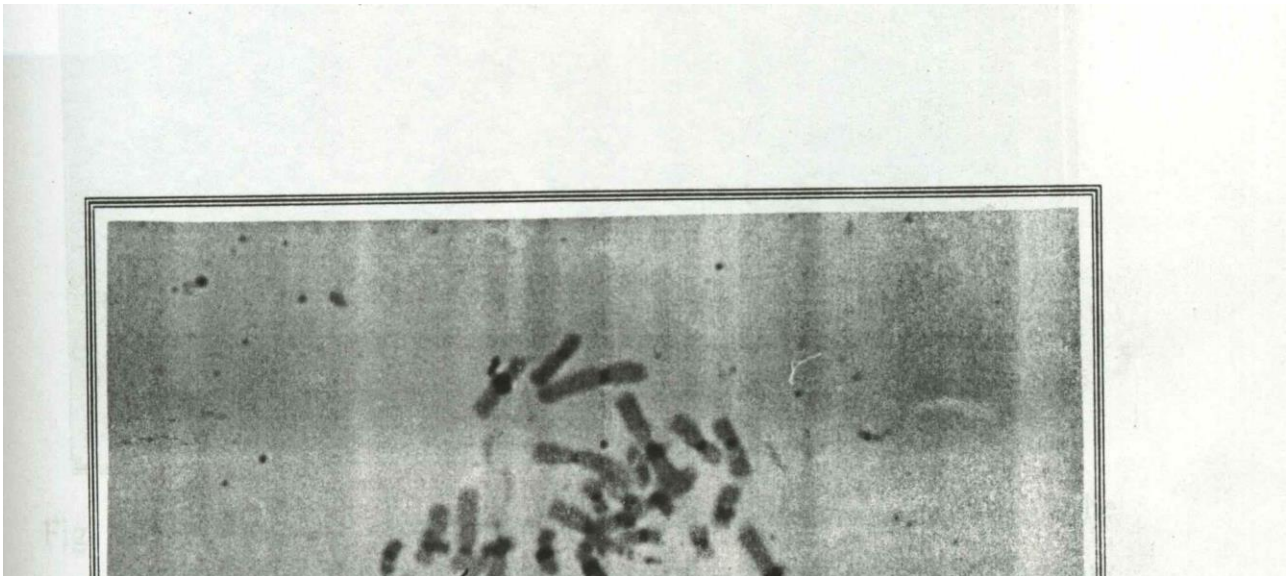


Fig. (7) Representative C- banded metaphase spread of patient with oligospermia note, C- segment asymmetry (heteromorphism) on chromosomes (1) (arrowed)





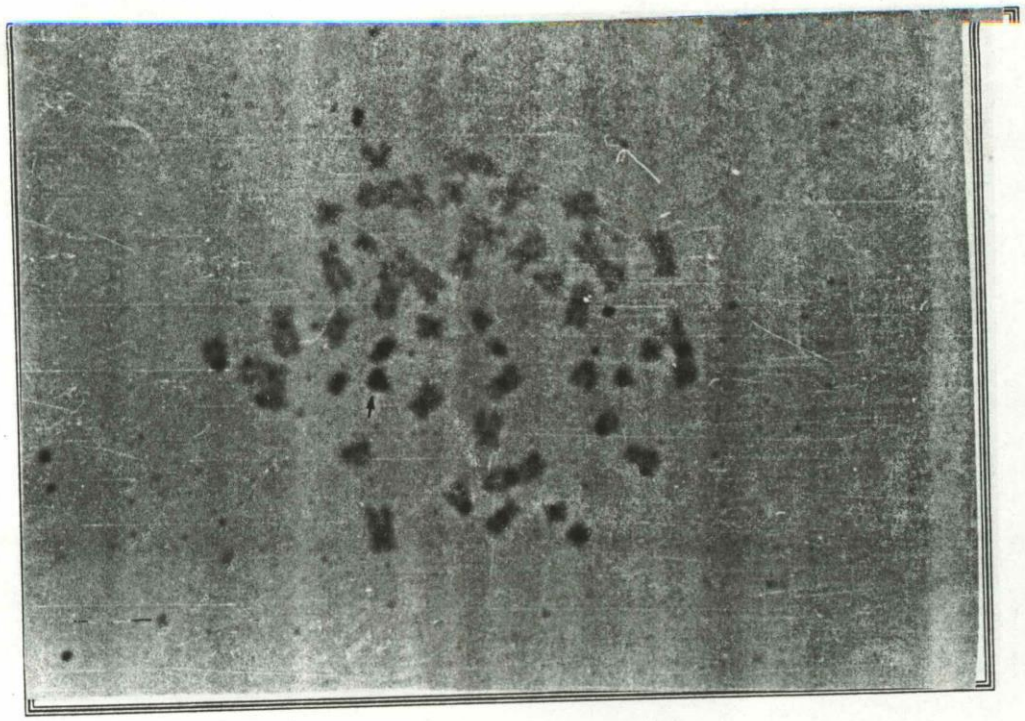


Fig. (11) shows a C- banded metaphase spread of normal in which the heterochromatic in the distal part of Y chromosome is seen as dark stained region (arrowed) small Y chromosome.

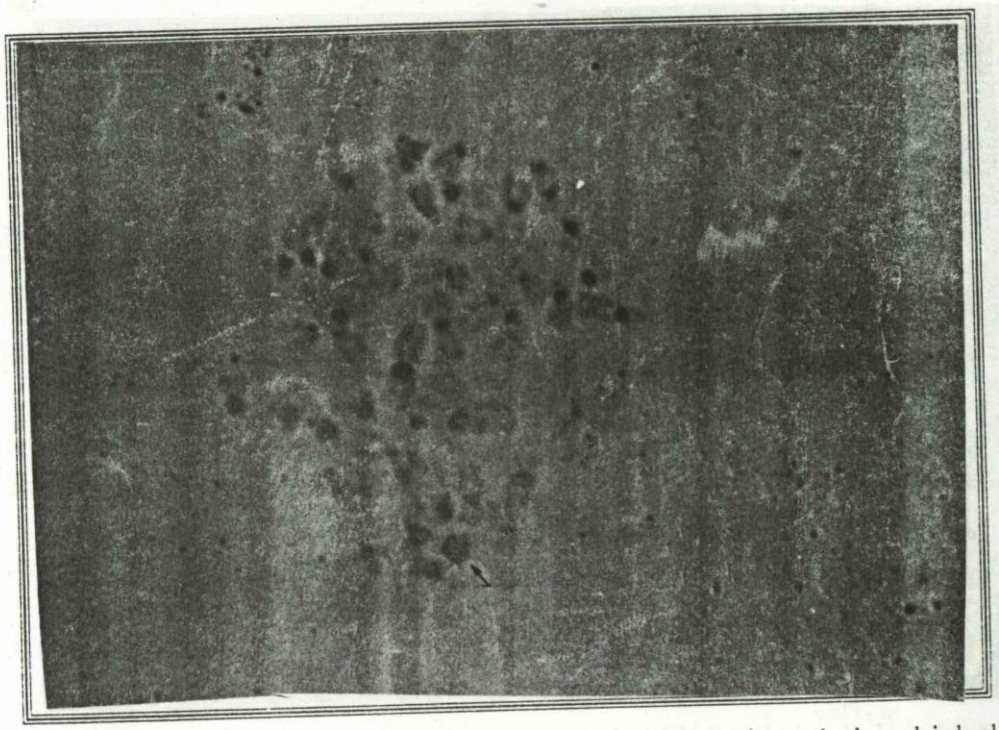


Fig (12) are C- banded metaphase spread of infertile male in which the heterochromatic distal part showed a heteromorphism.

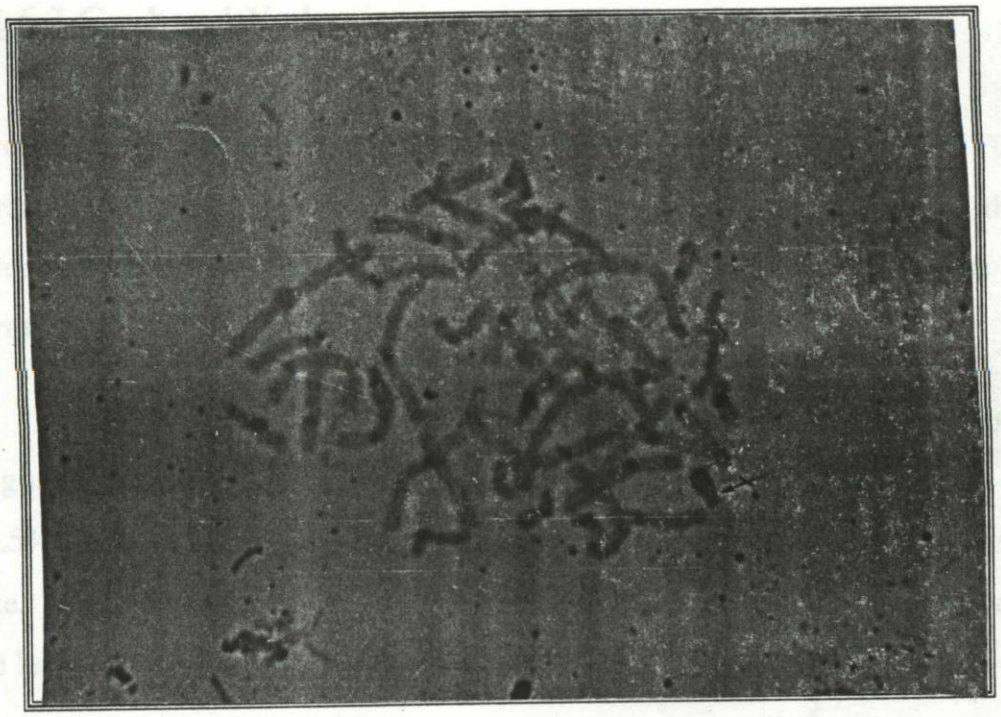
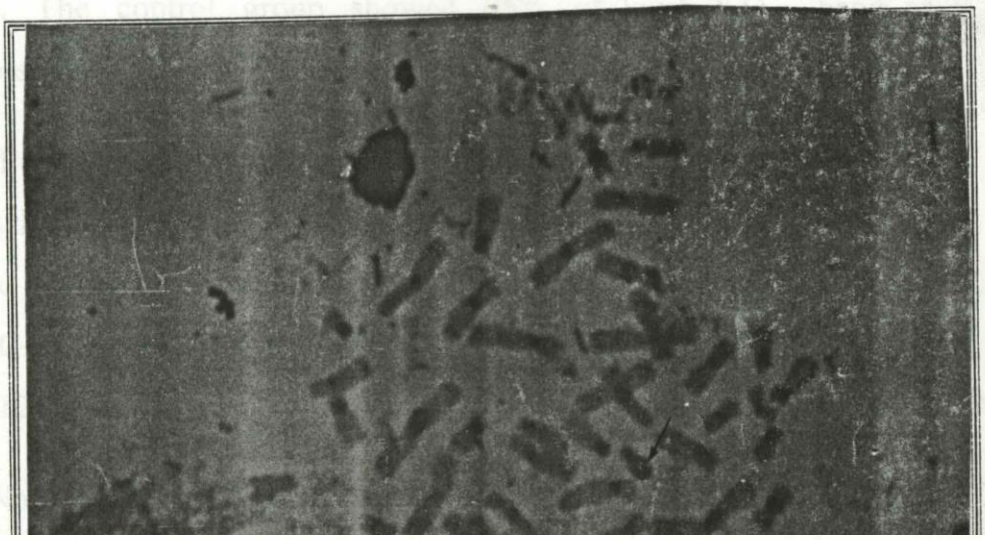
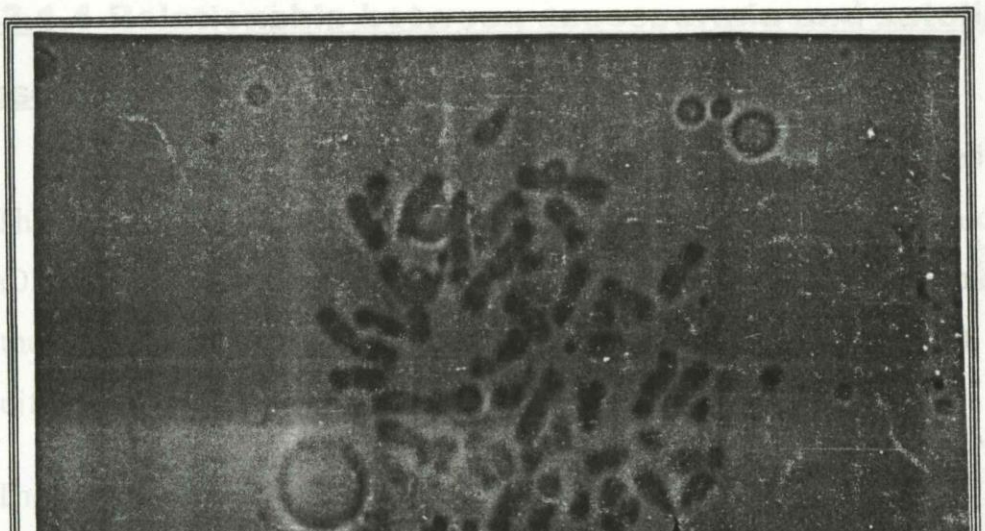


Fig (13) a C- banded metaphase spread of infertile male in which the proximal Euterochromatic part showed a heteromorphism.





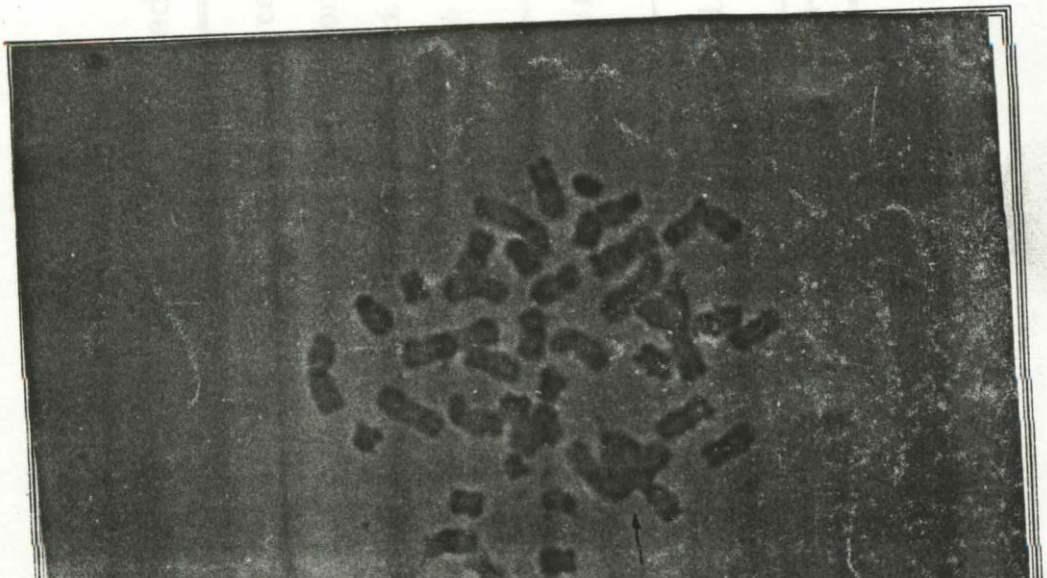


Fig (18) G- banded metaphase spread and the acrocentric association D/D chromosome is arrowed.

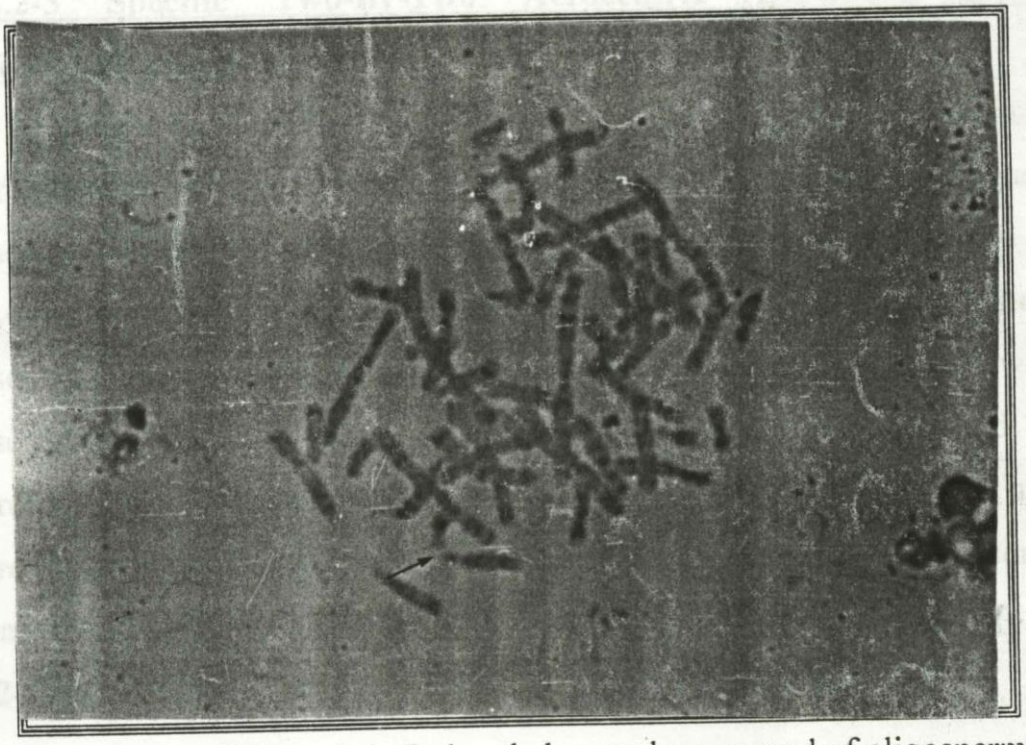
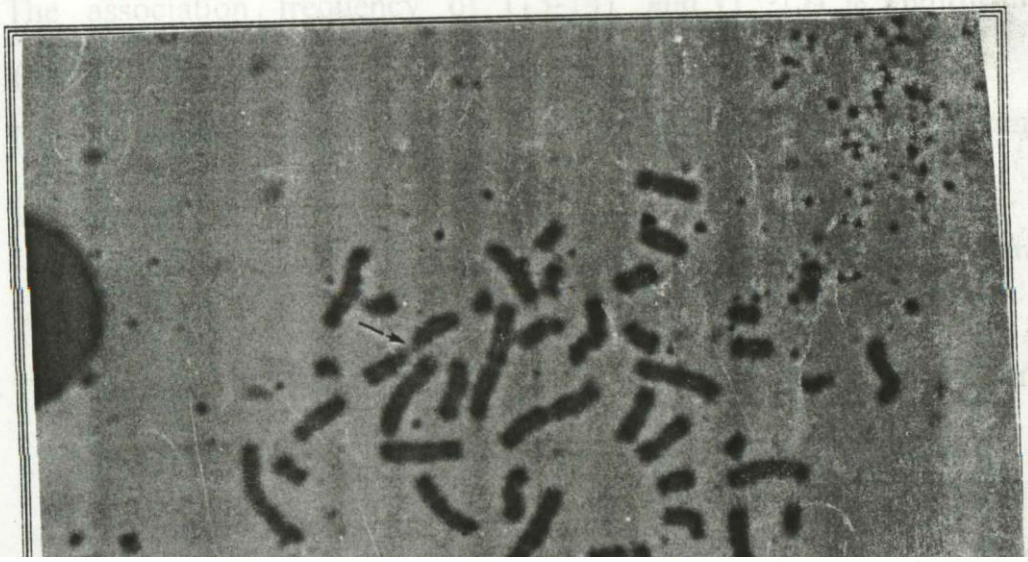


Fig. (19) a representative G- banded metaphase spread of oligospermia patient showed with 13/22 association. The arrow indicates the associated chromosomes.



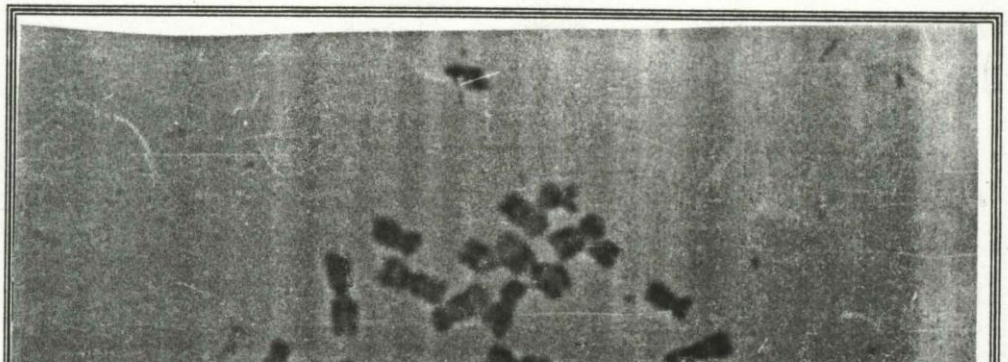


Fig. (24) is a G- banded metaphase of azoospermia patient with 47, XXY, klinfelter's syndromes, the arrow indicate the association including D/G.