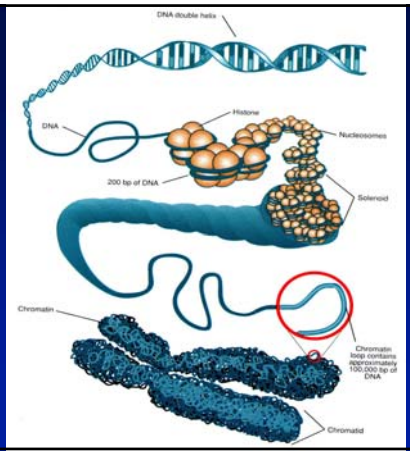
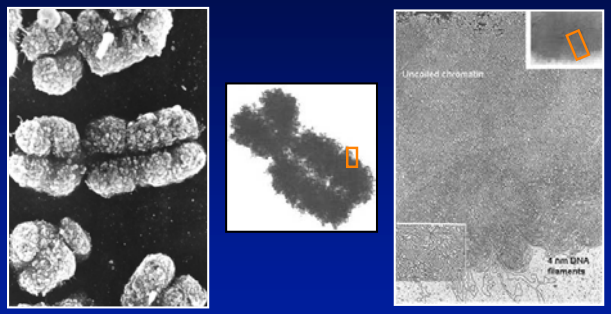
The Human Genome

- 6 billion base pairs of DNA
  - ~ 3 meters of DNA
- Approximately 30,000 – 70,000 genes
  - Approximately 80-100,000 proteins
- These genes are spread across 24 different chromosomes
- One chromosome each from each parent, for a total of 23 pairs (24 different chromosomes) or 46 chromosomes per somatic cell



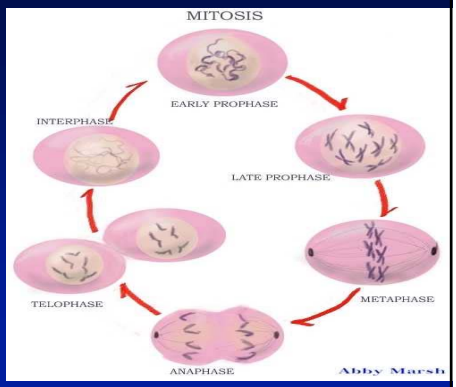
Chromatin Compaction



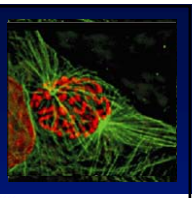
Metaphase chromosome is compacted into a structure that is 50,000 times shorter than its extended length

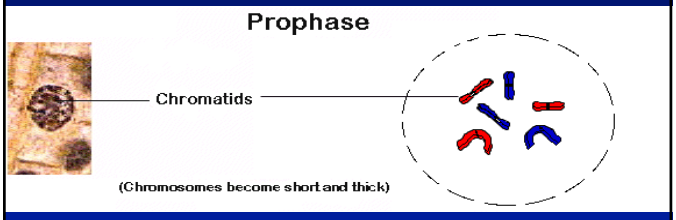
- Cell Cycle ~ 17 – 18 hrs
- Mitosis 1-2 hrs
- DNA is replicated during S-phase in preparation for mitosis

Cell will be visible as Interphase Nucleus Majority of Time

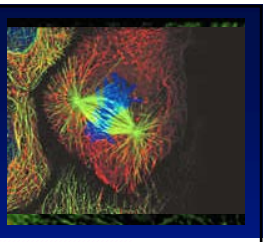


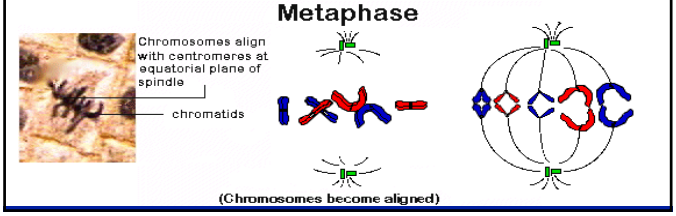
Mitosis - Prophase



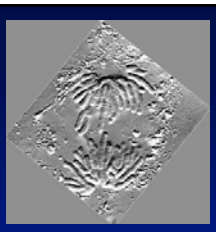


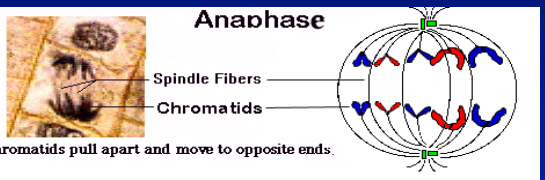
Mitosis – Metaphase



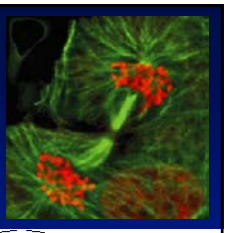


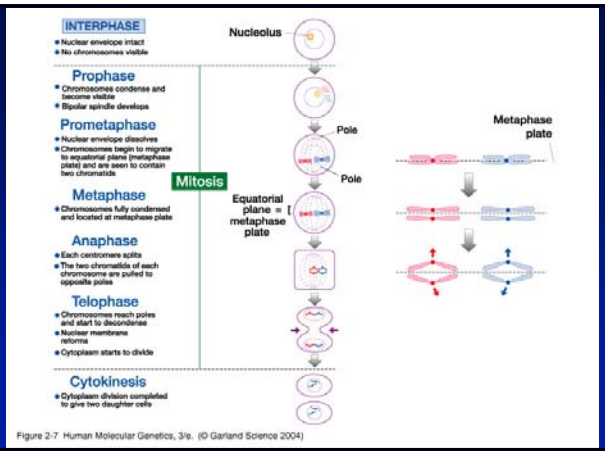
Mitosis – Anaphase





Mitosis - Telophase





Meiosis

- Takes place only in the ovaries and testes
- Reduces the number of chromosomes from diploid (2*n*=46) to the haploid number (*n*=23)
- Fertilization restores the diploid number in the zygote
- Meiosis I is comprised of several substages: Prophase I, Metaphase I, Anaphase I, Telophase I
- Prophase I is a complex stage further subdivided as follows:
  - Leptotene
  - Zygotene
  - Pachytene
  - Diplotene
  - Diakinesis

Leptotene

- There are 46 chromosomes
- Each comprised of 2 chromatids
- Chromosomes begin to condense
  - Not yet visible by light microscopy
- Once leptotene takes place, the cell is committed to meiosis

Zygotene

- Homologous chromosomes pair, locus for locus
  - Pairing is called synapsis
- Synapsis of the X and Y chromosomes in males occurs only at the pseudoatosomal regions (the only regions that contain homologous loci and do not undergo X-inactivation)

Pachytene

- Synapsis is complete
- Paired homologs form structures called bivalents (aka tetrads because there are 4 chromatids)
- Crossing over occurs (recombination)

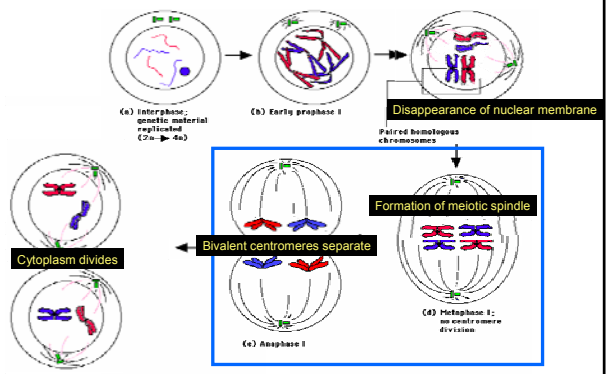
Diplotene

- Homologous chromosomes begin to repel each other
- Only place chromosomes are still attached are at the points where crossing over occurred
  - These points are referred to as chiasmata

Diakinesis

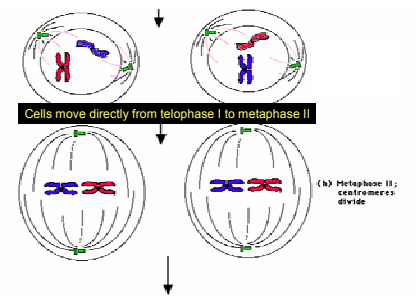
- Chromosomes reach their greatest contraction during this last stage of prophase

Meiosis 1

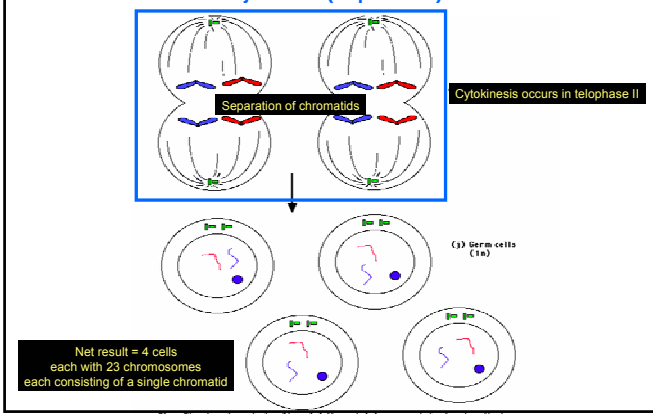


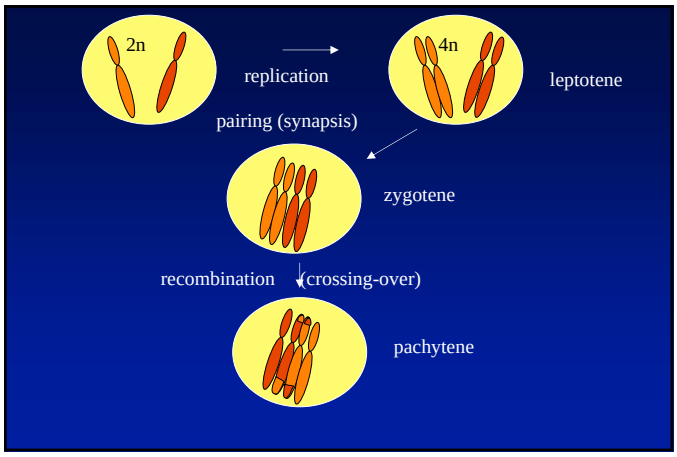
Normal Disjunction (Separation)

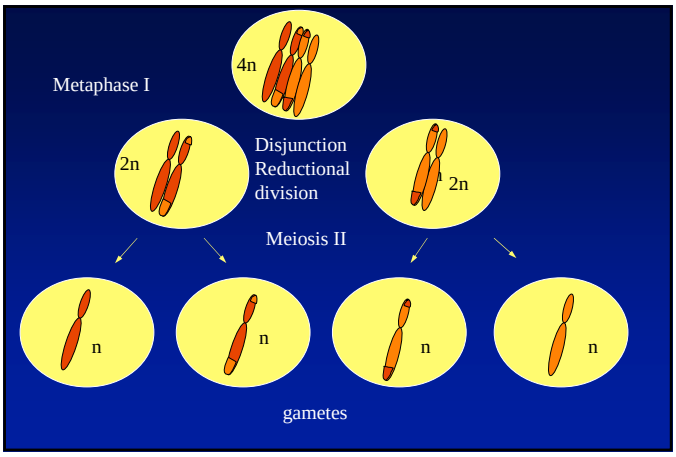
Meiosis II



Normal Disjunction (Separation)







Male Meiosis

- Begins at puberty
- Continues throughout life
- Spermatocytes continually replaced by mitosis
- Sperm maturation involves loss of histones and highly condensed DNA
- Each cycle from spermatocyte to sperm takes about 40 days
- Each meiotic division produces 4 sperm

Female Meiosis

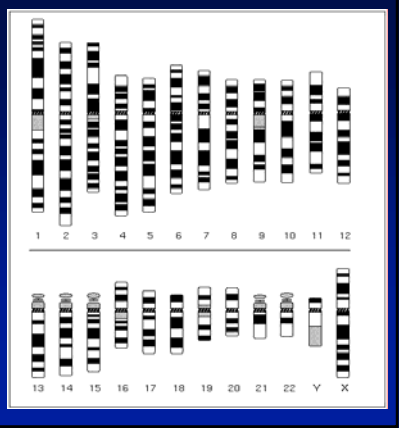
- All oocytes are formed during fetal life: continually lost by apoptosis throughout life
- Meiotic prophase begins at 14 weeks of gestation
- Meiosis is arrested after diplotene and resumes only at the time of ovulation, when meiosis I is completed
- Meiosis II is completed only after fertilization
- Cell division is asymmetrical, producing one large egg and 3 nonfunctional polar bodies

Cytogenetics

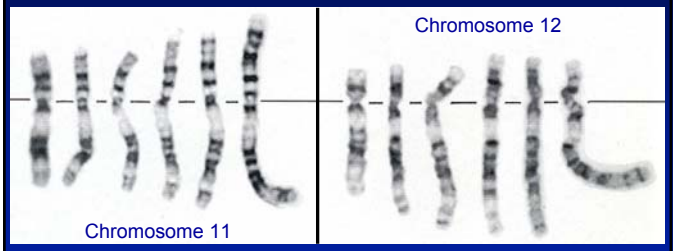
Banding techniques enable identification of chromosomes

Chromosome Identification

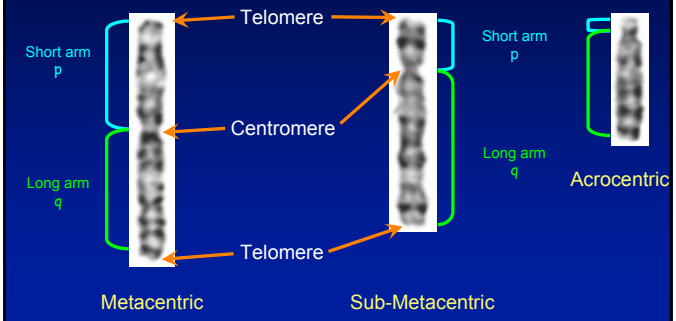
Chromosomes are identified by their size, banding pattern and the position of the centromere



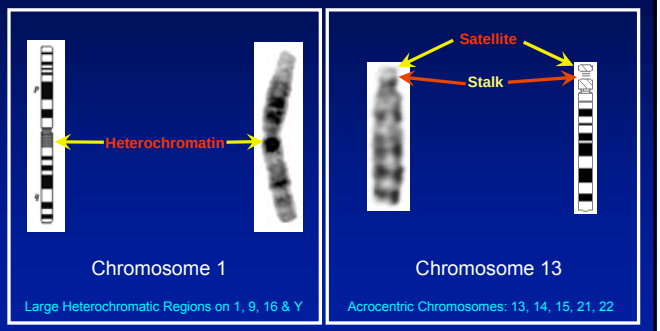
Banding Resolution



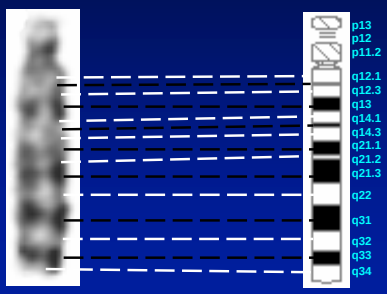
Naming of Parts



Naming of Parts



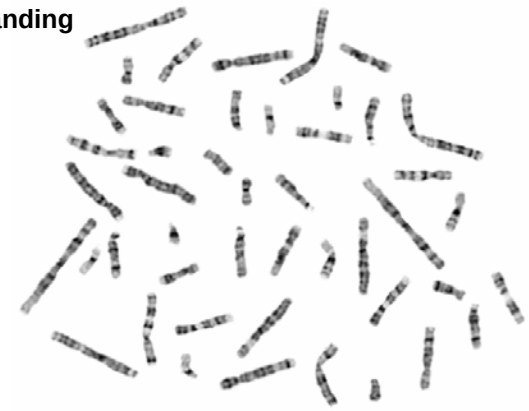
Chromosome Nomenclature & Band Designation



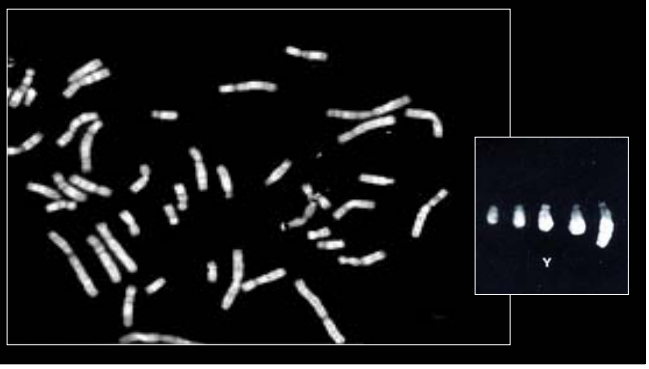
Chromosome Banding

- Various banding patterns can be produced by using different enzymes, chemicals and stains
- G-Banding: Routine banding method in USA
  - GTG: G bands produced with Trypsin using Giemsa
- Q-banding: First banding method developed for Human Chromosomes
  - Certain fluorochromes, such as quinacrine dihydrochloride, will bind DNA & produce distinct banding patterns of bright & dull fluorescence. Requires fluorescence microscope for analysis
- C-banding: Stains constitutive heterochromatin around the centromeres and other heterochromatic regions (1, 9, 16, Y)
  - CBG: C bands produced with Barium hydroxide using Giemsa
- R-banding: Banding pattern produced is the Reverse/Opposite of G-banding

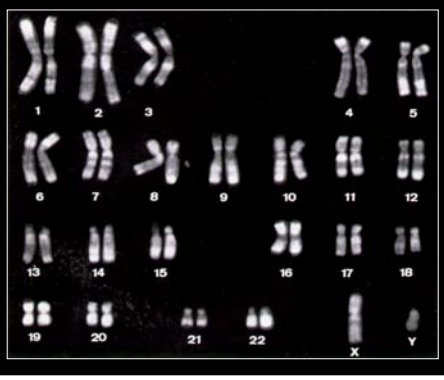
G-Banding



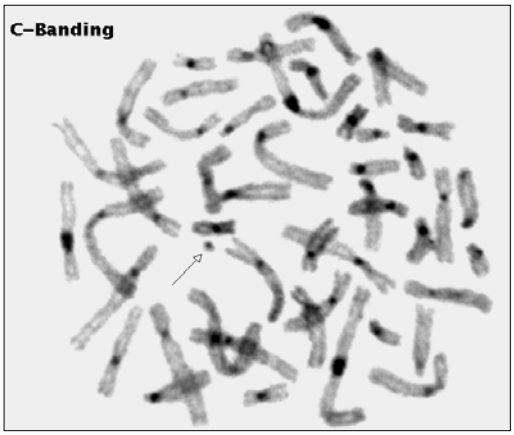
Q-Banding (Quinacrine)



R-Banding



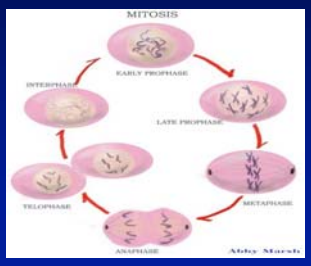
C–Banding



Studying Human Chromosomes

Cell will be visible as an Interphase Nucleus Majority of Time

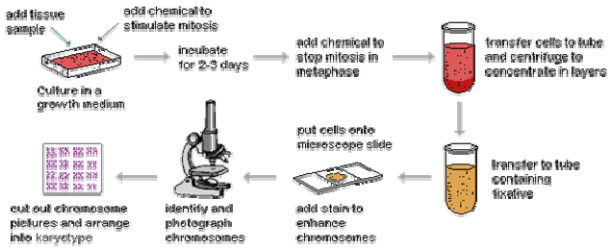
Have to catch cell during active division (METAPHASE) in order to view chromosomes



Technical Advances

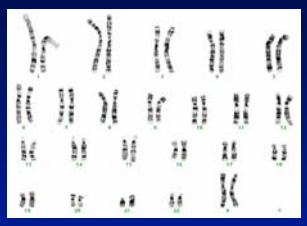
- Mitogens (PHA) to push cell into active cell division
- Spindle poisons (colchicine) produce metaphase arrest
- Hypotonic solution to rupture nucleus
- Differential staining

Preparing chromosomes for cytogenetics



Normal 46,XY Male Karyotype

Normal 46,XX Female Karyotype



- A precise amount of genetic material is required for normal development & functioning

Genetic Imbalance

- An incorrect amount of genetic material in a conceptus may disturb/distort the normal growth pattern

Zygote → Blastula → Embryo → Fetus → Newborn → Adolescent → Adult

Chromosomal Imbalance

- An imbalance in the amount of chromosomal material may involve 100's to 1000's of genes and generally has more catastrophic effects

Zygote → Blastula → Embryo → Fetus → Newborn → Adolescent → Adult

Chromosomal Imbalance

- May involve the gain or loss of a whole chromosome (complete aneuploidy) or of part of a chromosome (partial aneuploidy)
- The abnormality may occur in the non-mosaic or mosaic state (Mosaicism = Various chromosome complements in different cells)
- Monosomy (one missing) is generally more devastating than trisomy (one extra)

Chromosomal Imbalance

- Most (complete) autosomal trisomies & all (complete) autosomal monosomies are so catastrophic that their presence in a conceptus is not compatible with survival
- Trisomies, monosomy X and polyploids are the most common abnormalities observed in spontaneous abortions
- ~ 66% of first trimester spontaneous abortions
- ~20% of 2nd trimester spontaneous abortions

Frequency of Chromosome Abnormalities

Source	%
Sperm	8
Oocytes	20-30
"Good" preimplantation embryos	30-40
"Poor" preimplantation embryos	85
Early recognized conceptions (>4 wks)	8-10
Early miscarriages	60-70
Late fetal deaths and stillbirths	7
Livebirths	0.3
Children with mental retardation	12-15
Infants with congenital heart disease	25

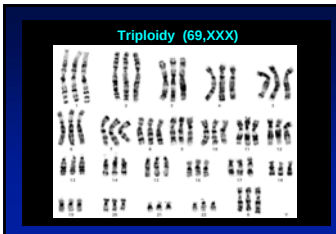
Chromosomal Imbalance and Pregnancy* Loss

- 65% Trisomies
- 11% Monosomies
- 11% Triploidies
- 7.5% Multiple Aneuploidies
- 5.5% Tetraploid and structural

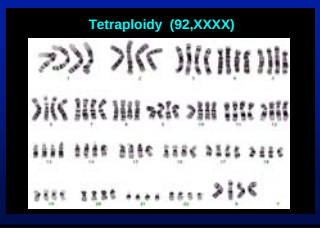
Trisomy 16
- Most common trisomy observed in POC studies
- Never seen in liveborn

Trisomy 21 & 22
- Next most common (equally)

*Recognized pregnancies



Chromosomal



Imbalance

Survivable Chromosomal Imbalance

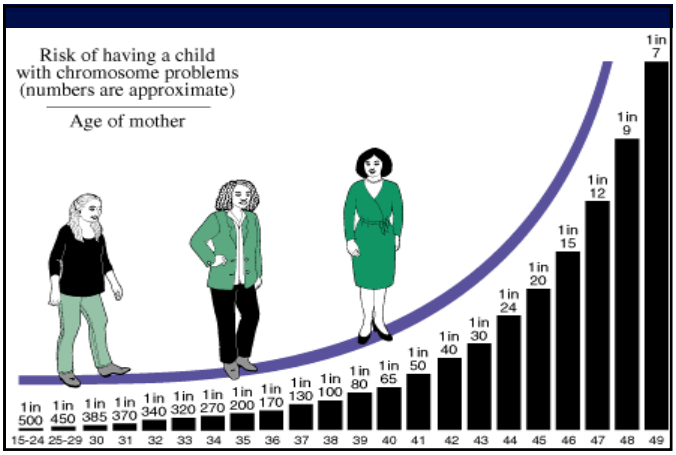
- **Only a few complete non-mosaic aneuploidies are observed in liveborns.**
  - **Down syndrome (Tri 21), Edward Syndrome (Tri 18), Patau Syndrome (Tri13), Turner Syndrome (Mono X)**
- **All other imbalances will contain much smaller chromosomal regions (partial aneuploidy) that would allow for the organisms to survive….Albeit with clinical abnormalities (in most cases)**

Numerical abnormalities

- Ploidy: The category of chromosome changes which involve the addition or loss of **complete sets** of chromosomes.
- Triploidy
  - The possession of one complete extra set of chromosomes.
  - Usually caused by polyspermy, the fertilisation of an egg by more than one sperm.
  - Such embryos will usually spontaneously abort.
- Tetraploidy
  - Usually the result of a failure of the first zygotic division. It is also lethal to the embryo.
  - Any other cell division may also fail to complete properly and in consequence a very small proportion of tetraploid cells can sometimes be found in normal individuals (mosaicism).

Autosomal Numerical abnormalities

- Aneuploidy
  - The category of chromosome changes which do not involve whole sets. It is usually the consequence of a failure of a single chromosome (or bivalent) to complete division.
- Monosomies
  - All autosomal monosomies are lethal in very early embryogenesis. Most abort too early even to be recognised as a conception.
- Down syndrome, trisomy 21
  - The incidence of trisomy 21 rises sharply with increasing maternal age.



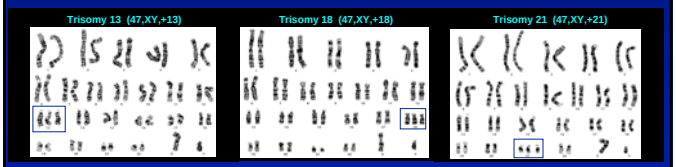
Clinical Phenotypes of Chromosomal Abnormalities

- Associated with Developmental Delay/MR
- Alteration of facial morphogenesis to produce characteristic facial features
- Growth delay
- Malformations of the internal organs - especially cardiac

Indication for chromosome analysis = MCA/MR

Survivable Chromosomal Imbalance

- **Only a few full non-mosaic aneuploidies are observed in liveborns**



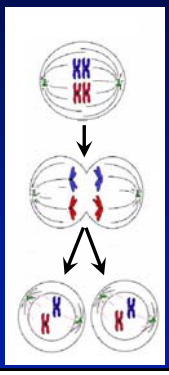
Trisomy 21 (47,XY,+21) – Down Syndrome

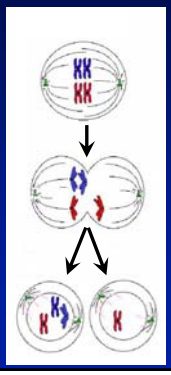
Down syndrome: Cytogenetics

- 95% caused by nondisjunction = Trisomy 21
  95% of trisomies due to maternal nondisjunction
  75% of errors occur during meiosis 1
  MATERNAL MEIOSIS 1 NONDISJUCTION

MEIOSIS-1 NONDISJUCTION

Normal Disjunction





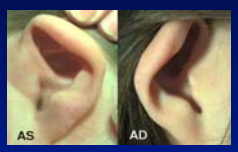
Non-Disjunction

Trisomy 21 – Down Syndrome

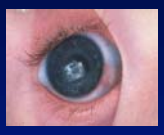
Trisomy 21 Down Syndrome

- Hypotonia
- Redundant neck fold/flat occiput
- Low set ears with characteristic pinnae
- Protruding/large tongue
- Abnormal dermatoglyphics
  - Simian line and clinodactyly
  - Wide space between 1st & 2nd toes

Down Syndrome



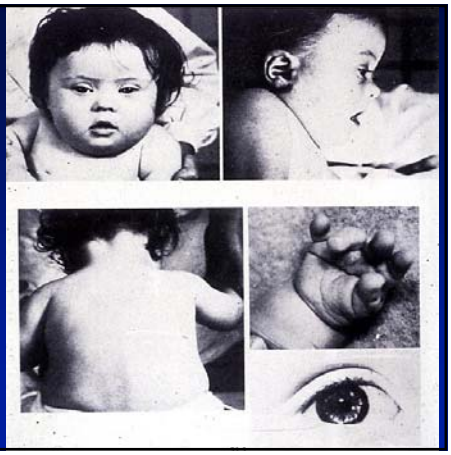
Small overfolding of Angulated upper helix. Small/absent ear lobes



Epicanthal fold

Simian crease

Down Syndrome

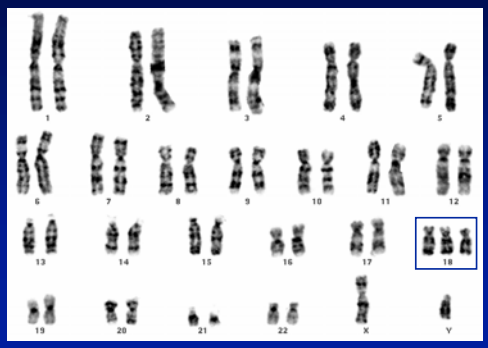


Down Syndrome – Brushfield Spots

Down syndrome: Medical Problems

- Gastrointestinal obstruction 3%
- Respiratory infections Common
- Leukemia 15-20 X
- Congenital heart defect 40%
- Moderate to severe mental retardation 100%
- Development:
  - Early intervention program

Trisomy 18 (47,XY,+18) – Edward Syndrome



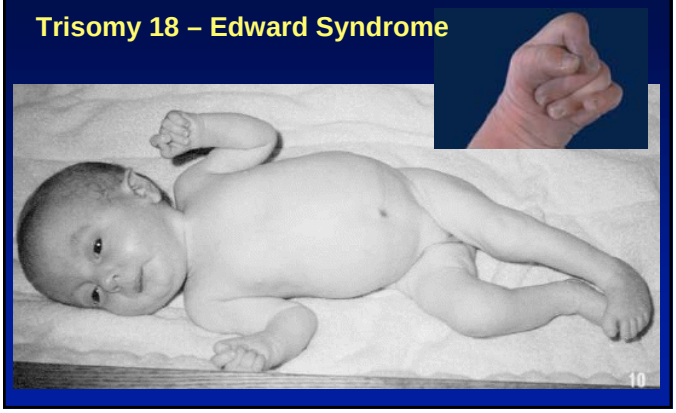
Trisomy 18

- Incidence 1:3333 live births
- Most common abnormality in stillbirths with multiple congenital abnormalities
- Prenatal growth deficiency resulting in a small for gestational age infant (SGA)
- 90% congenital heart defect VSD
- 10% alive at one year
- Marked developmental disability

Trisomy 18 - Physical Features

- Prominent occiput
- Micrognathia
- Microcephaly
- Low set malformed ears
- Characteristic clenched fists
- Rocker-bottom feet
- Short big toe that is dorsiflexed

Trisomy 18 – Edward Syndrome



Trisomy 18

- Prominent Occiput
- Low-set malformed ears
- Small chin
- Clenched fists

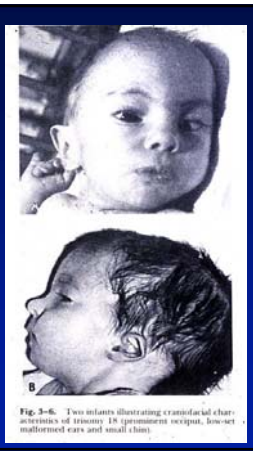


Fig. 3–6. Two infants illustrating craniofacial characteristics of trisomy 18 (prominent occiput, low-set malformed ears and small chin).

Trisomy 18 – Rocker-Bottom Feet

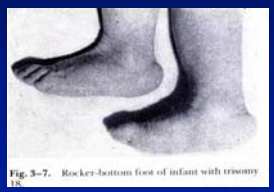
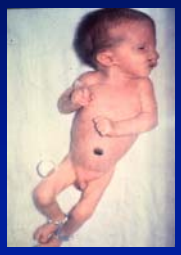


Fig. 3–7. Rocker-bottom foot of infant with trisomy 18.

Trisomy 18

- Ocular manifestations in 10%
- Low-arch dermal ridge pattern
- Underdeveloped nails
- Congenital anomalies of lungs,diaphragm, and kidneys
- Hernias, cryptorchidism, rectus muscle separation

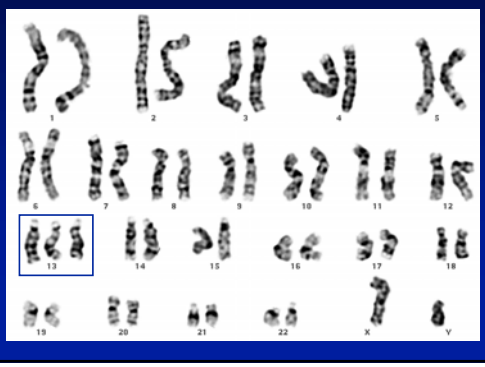
Trisomy 18 – High Morbidity & Mortality



Trisomy 18 - Medical Management

- Feeding difficulties GE reflux
- Apnea
- Seizures
- Slow post natal growth
- Developmental disability/ mental retardation
- Scoliosis

Trisomy 13 (47,XY,+13) – Patau Syndrome



Trisomy 13

- Incidence 1:5,000 births
- Distinctive malformation pattern
  (Craniofacial and Central Nervous System)
- 95% spontaneously aborted
- Survival rate and development similar to Trisomy 18

Trisomy 13 Patau Syndrome

- Microcephaly with sloping forehead
- Holoprosencephaly
- Ophthalmologic abnormalities
  - microphthalmia or anophthalmia
  - Colobomata of iris and ciliary body
- Cleft lip +/- palate
- Low set ears with abnormal helices

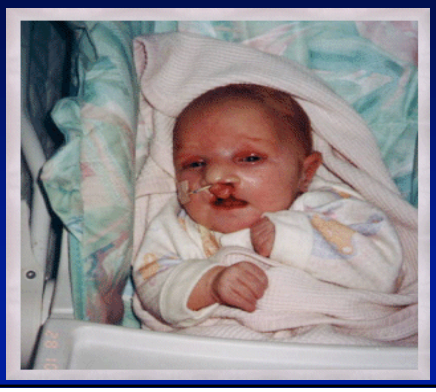
Trisomy 13 Patau Syndrome

- Cardiac defects: ASD, PDA, VSD
- Males: cryptorchidism ; Females: Bicornuate uterus
- Polycystic kidneys
- Aplasia cutis congenita
- Polydactyly of hands +/- feet
- Rockerbottom feet

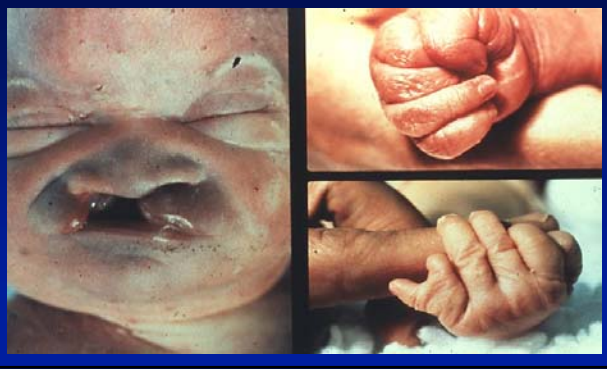
Trisomy 13 - Cytogenetics

- 75% due to meiotic nondisjunction
- 20% arise from translocations
  - 25% are due to familial translocations
- 5% due to mosaicism
  - Mitotic nondisjunction

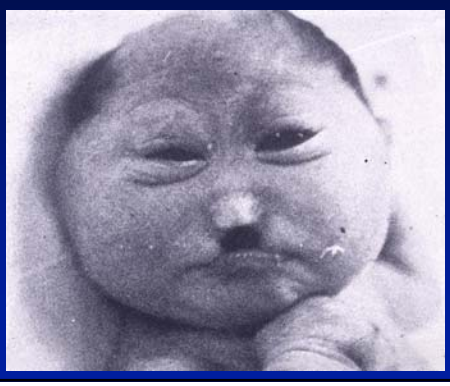
Trisomy 13 - Cleft Lip & Palate



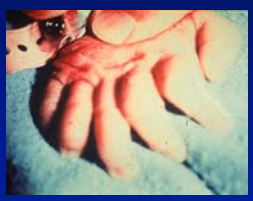
Trisomy 13

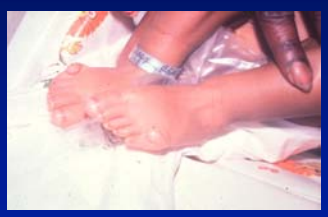


Trisomy 13 - Holoprosencephaly



Trisomy 13 – Polydactyly





Numerical Abnormalities of the Sex Chrms

- Sex Chromosome Aneuploidies
  - Because of X inactivation and because of the paucity of genes on the Y chromosome, aneuploidies involving the sex chromosomes are far more common than those involving autosomes.

Sex Chromosome Abnormalities

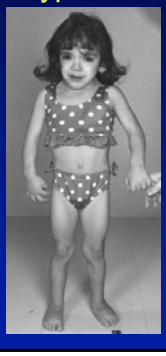
- Turner syndrome
- Klinefelter syndrome (XXY)
- Triple X
- XYY

Numerical Abnormalities of the Sex Chrms

- Turner syndrome 45,X
  - The incidence is about 1 in 5000 female births but this is only the tip of the iceberg because 99% of Turner syndrome embryos are spontaneously aborted.
  - Individuals are very short, they are usually infertile. Characteristic body shape changes include a broad chest with widely spaced nipples and may include a webbed neck.
  - IQ and lifespan are unaffected.

Turner syndrome: Phenotype

- Facies- Triangular shape
  Posteriorly rotated ears
- Webbed neck
- Shield chest
- Lymphedema at birth
- Coarctation of aorta 20%
- Structural kidney defects 50%
- Rx: Growth hormone and estrogen



Turner Syndrome

Turner syndrome

- SHORT STATURE
- OVARIAN DYSGENESIS
  INFERTILITY
- LEARNING DISABILITIES
  SPATIAL PERCEPTION

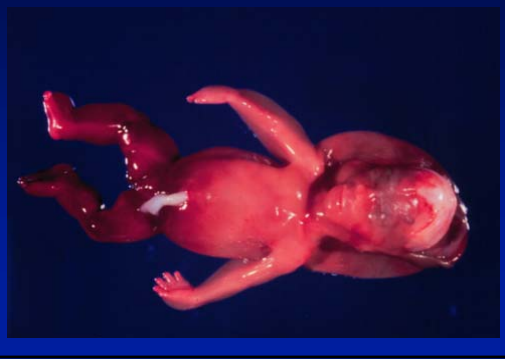
Turner Syndrome

Height

Turner syndrome: Cytogenetics

- 45, X 50%
- 45,X/46,XX
  45,X/46,XY
  Mosaics 30-40%
- Structural X abnormalities 10-20%
- 80% due to paternal meiotic error
- 15-20% spontaneous abortions due to 45,X

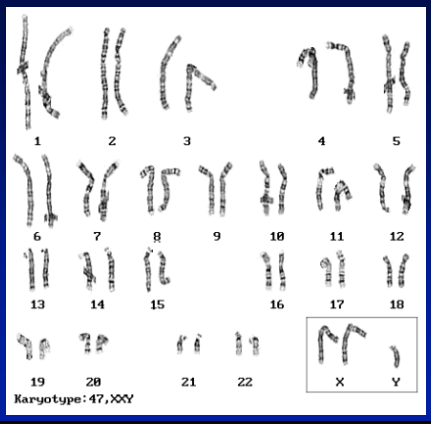
45,X0 in SAB's



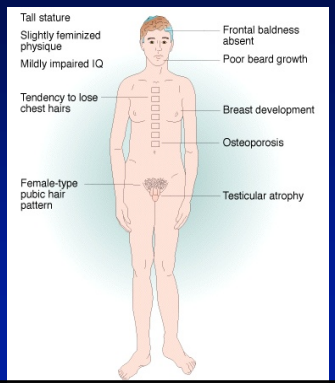
Numerical Abnormalities of the Sex Chrms

- Klinefelter Syndrome 47,XXY
  - The incidence at birth is about 1 in 1000 males.
  - Testes are small and fail to produce normal levels of testosterone which leads to breast growth (gynaecomastia) in about 40% of cases and to poorly developed secondary sexual characteristics. There is no spermatogenesis (Sterility).
  - These males are taller and thinner than average and generally have a slight reduction in IQ (10-15 points below sibs). Many Kleinfelter males lead a normal life.
  - Very rarely more extreme forms of Kleinfelter's syndrome occur where the patient has 48, XXXY or even 49, XXXXY karyotype. These individuals are generally severely retarded.

Klinefelter Syndrome



Klinefelter syndrome stigmata



Triple X – 47,XXX

- 1:1000 female births
- No phenotypic abnormalities
- Variable symptoms:
  REPRODUCTIVE LOSS/ STERILITY
  LEARNING DISABILITIES/ SPEECH
  LANGUAGE

Multi-X: 48,XXXX 49,XXXXX

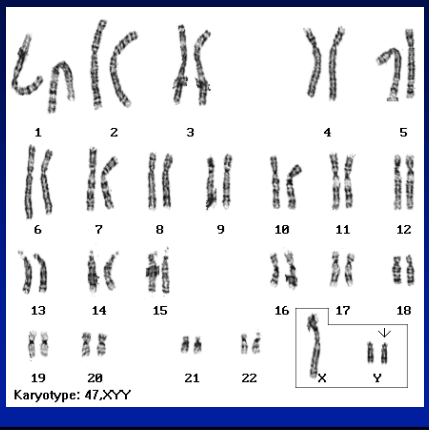
Numerical Abnormalities of the Sex Chrms

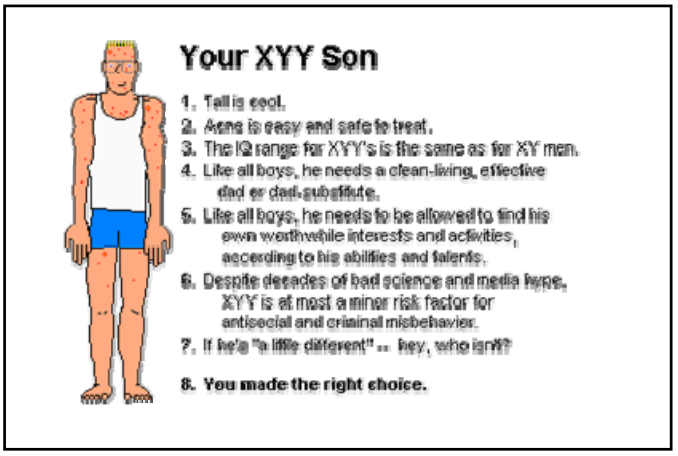
- XXX females
  - About one woman in 1000 has an extra X chromosome. It seems to do little harm, individuals are fertile and do not transmit the extra chromosome.
  - They do have a reduction in IQ comparable to that of Kleinfelter's males (10-15 point below sibs).
  - Variants
    - Multi-X: 48,XXXX 49,XXXXX
    - Mild to moderate MR
    - Variable dysmorphic features

Numerical Abnormalities of the Sex Chrms

- 47,XYY males
  - Incidence 1 in 1000 male births. May be without any symptoms.
  - Males are tall but normally proportioned.
  - 10 - 15 points reduction in IQ compared to sibs? (*IQ*:93-109/109-147)
  - More common in high security institutions than chance would suggest? (Problems with impulse control?)

XYY Syndrome



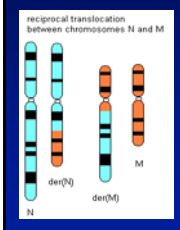


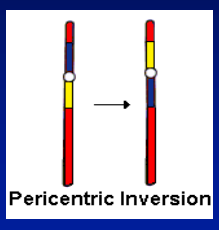
Structural Rearrangements

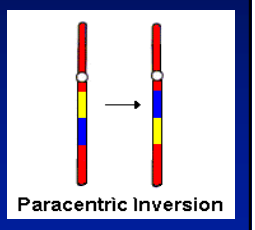
- Translocations
- Inversions

Multiple options for Gametes only 2 of which are balanced

Structural Rearrangements – Translocations & Inversions





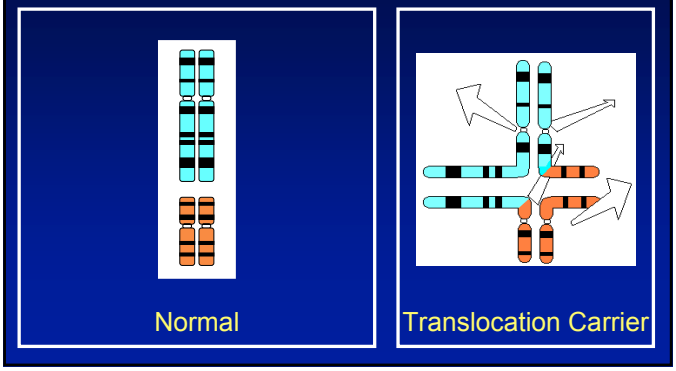


Structural Abnormalities - Translocations

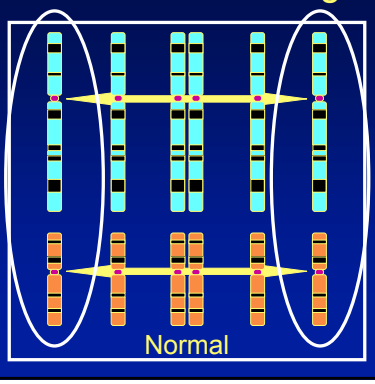
Interchange of genetic material between nonhomologous chromosomes

- RECIPROCAL/BALANCED = Mutual exchange after two breaks
  - Most balanced reciprocal translocations have no phenotype
  - Clinical phenotype may result if there is a disruption of critical genes at the breakpoint regions. Clinical phenotype tends to be more like those observed in single gene defects
- UNBALANCED DERIVATIVE = Partial monosomy & Partial trisomy

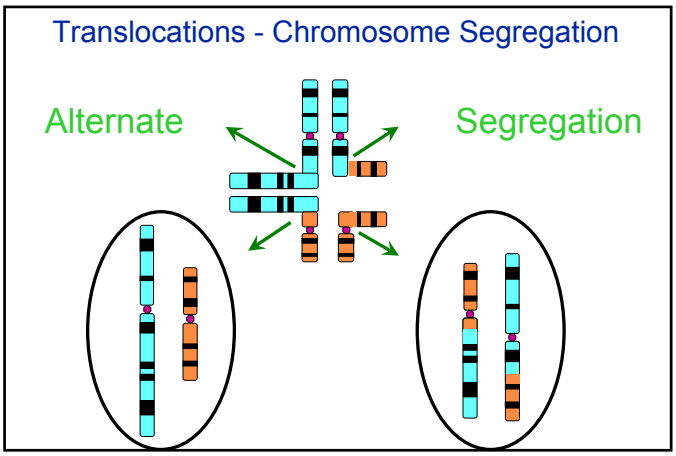
Translocations - Chromosome Pairing Before Segregation



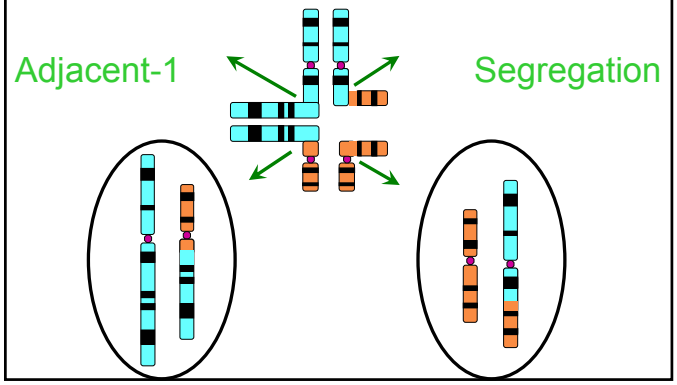
Normal Chromosome Segregation



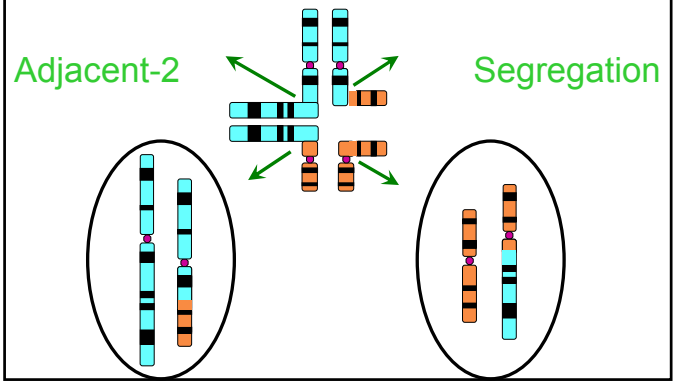
Translocations - Chromosome Segregation

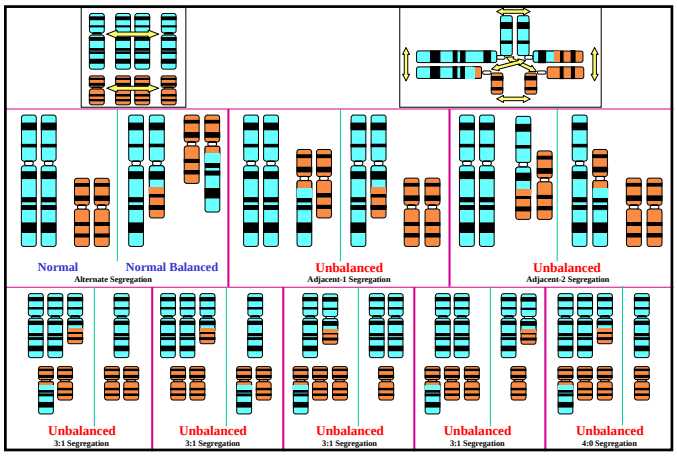


Translocations - Chromosome Segregation

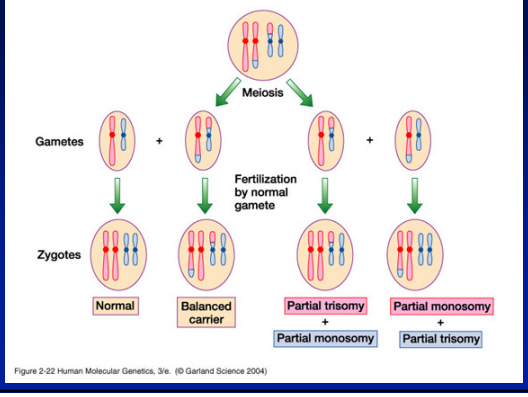


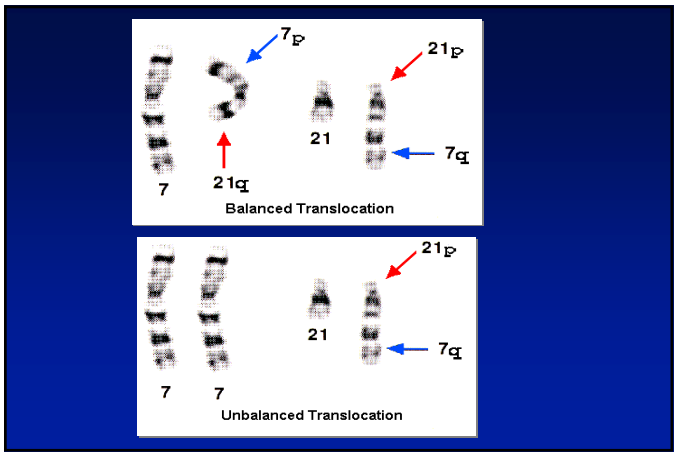
Translocations - Chromosome Segregation





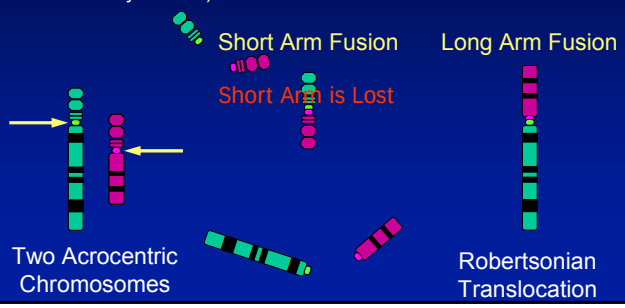
Translocations



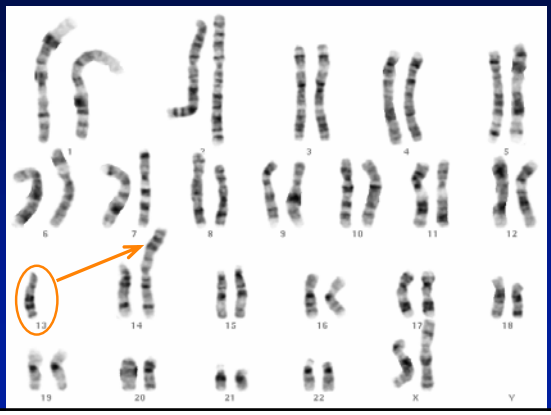


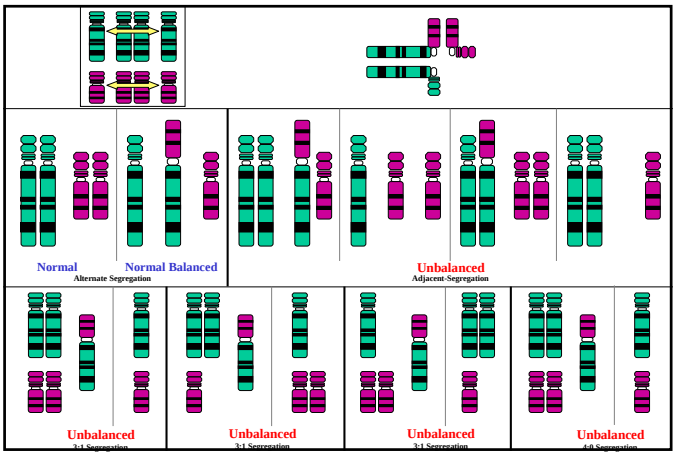
Robertsonian Translocation

- Translocation between acrocentric chromosomes. Short arms are lost and long arms fuse at centromere (5% of Down syndrome)

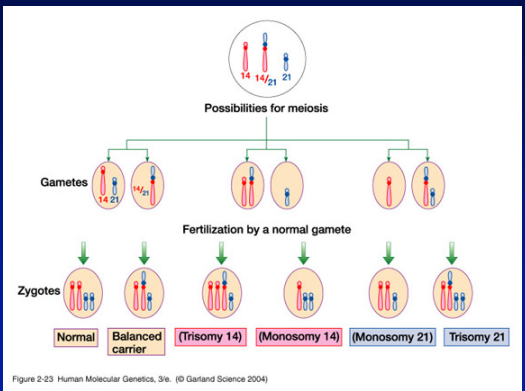


Robertsonian Translocations

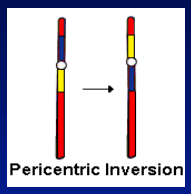


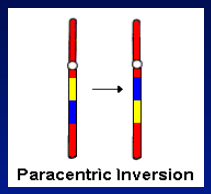


Robertsonian Translocations



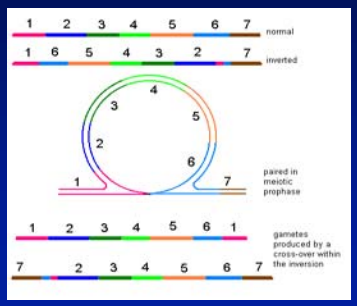
Structural Abnormalities - Inversions

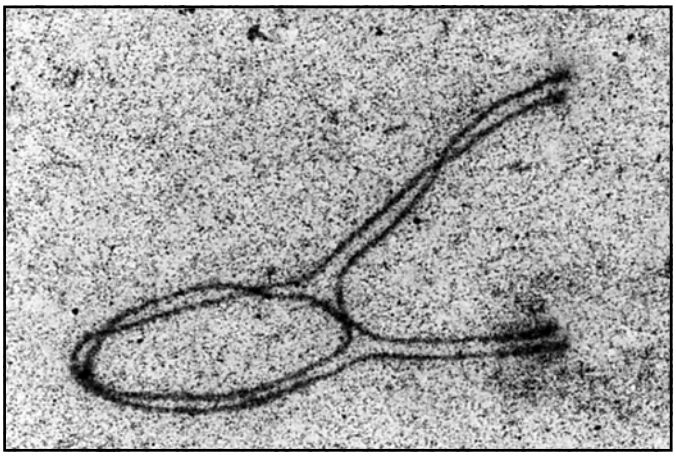




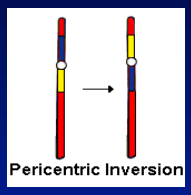
- An inversion consists of two breaks in one chromosome. The area between the breaks is inverted (turned around), and then reinserted and the breaks then unite to the rest of the chromosome. If the inverted area includes the centromere it is called a **pericentric** inversion. If it does not, it is called a **paracentric** inversion.

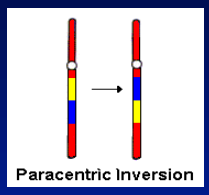
Inversions - Chromosome Segregation





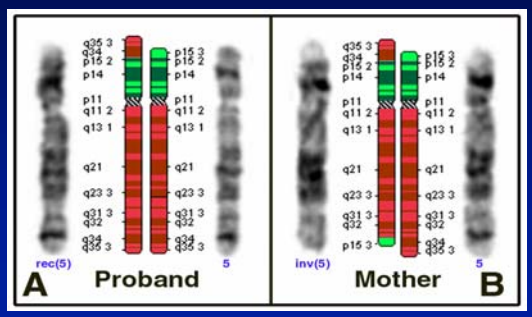
Structural Abnormalities - Inversions

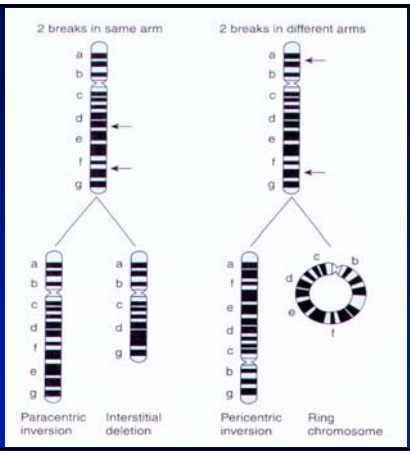




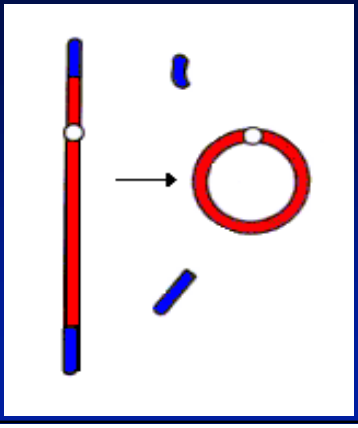
- Pericentric Inversion:
  - A crossover within the inversion loop results in the formation of recombinant chromosomes with duplications/deletions of the material distal to the inversion breakpoints
- Paracentric Inversion:
  - A crossover within the inversion loop results in the formation of an acentric fragment and a dicentric recombinant chromosome

Pericentric Inversion





Ring Chromosomes



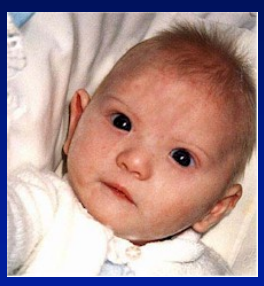
Translocations & Inversions

- BALANCED
  - Most balanced rearrangements have no phenotype
  - Clinical phenotype may result if there is a disruption of critical genes at the breakpoint regions. Clinical phenotype tends to be more like those observed in single gene defects
- UNBALANCED
  - Partial Monosomy/Trisomy

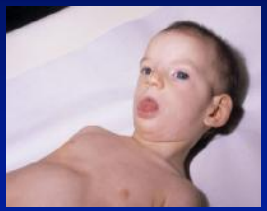
Deletions

- 5p- Cri-du Chat
- 4p- Wolf-Hirschhorn

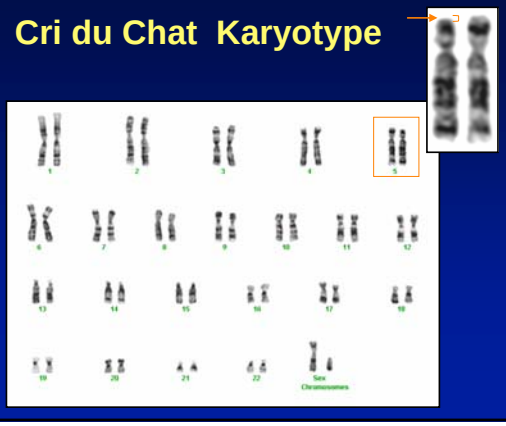
Deletion 5p - Cri Du Chat Syndrome



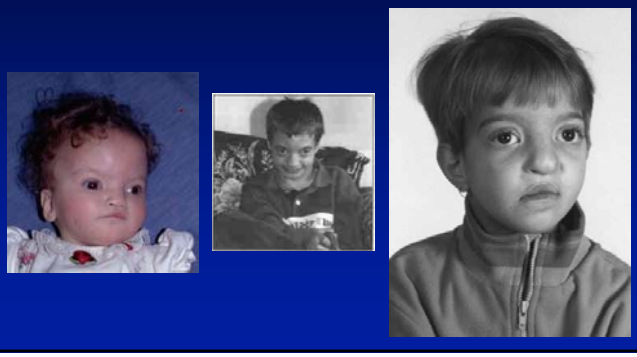
Cri Du Chat Syndrome - **Older childre**



Cri du Chat Karyotype



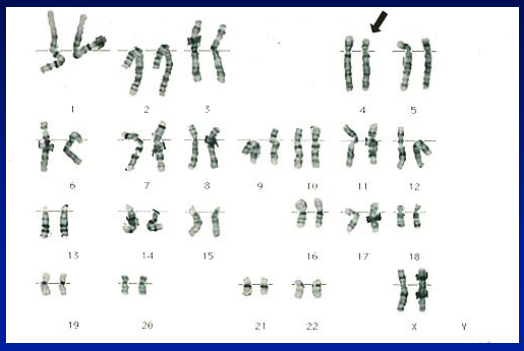
Deletion 4p - Wolf- Hirschhorn Syndrome



Deletion 4p Cytogenetics Wolf Hirschhorn Syndrome

- Deletion in the terminal band 4p16.3
- 87% of cases due to de novo interstitial deletion of paternal origin
- 13% due to unbalanced product of a parental reciprocal translocation

Deletion 4p Karyotype



"Viable" Chromosome Imbalance

- Unbalanced Translocations Partial Monosomy & Partial Trisomy
- Deletions Partial Monosomy
- Duplications Partial Trisomy
- Ring & marker chromosomes Partial Trisomy
- Recombinant Inversion derivatives Partial Monosomy & Partial Trisomy
- Isochromosomes Partial Trisomy/Tetrasomy or Partial Monosomy & Partial Trisomy

"Viable" Chromosome Imbalance

- 1 in 150 Livebirths
- 10-15% Mentally retarded population
  - Higher percentage when cryptic rearrangements are included
- Most case reports involve partial aneuploidy