Introduction in medical genetics 2

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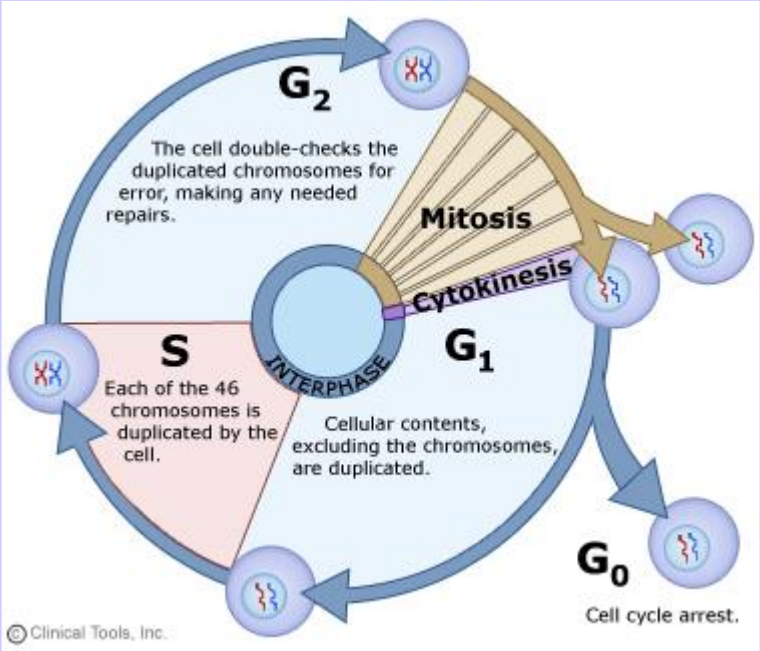
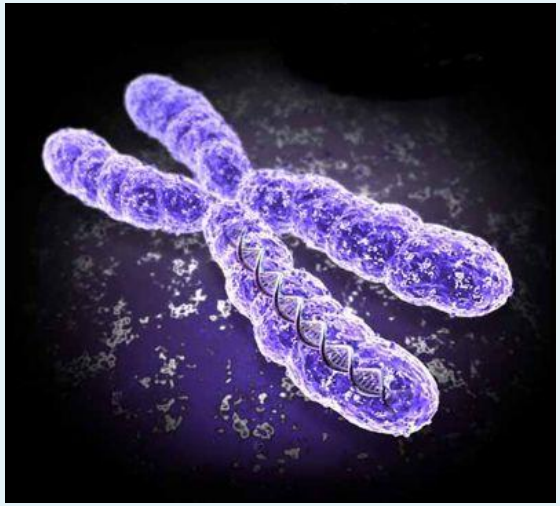
Bratislava, 27.2.2017

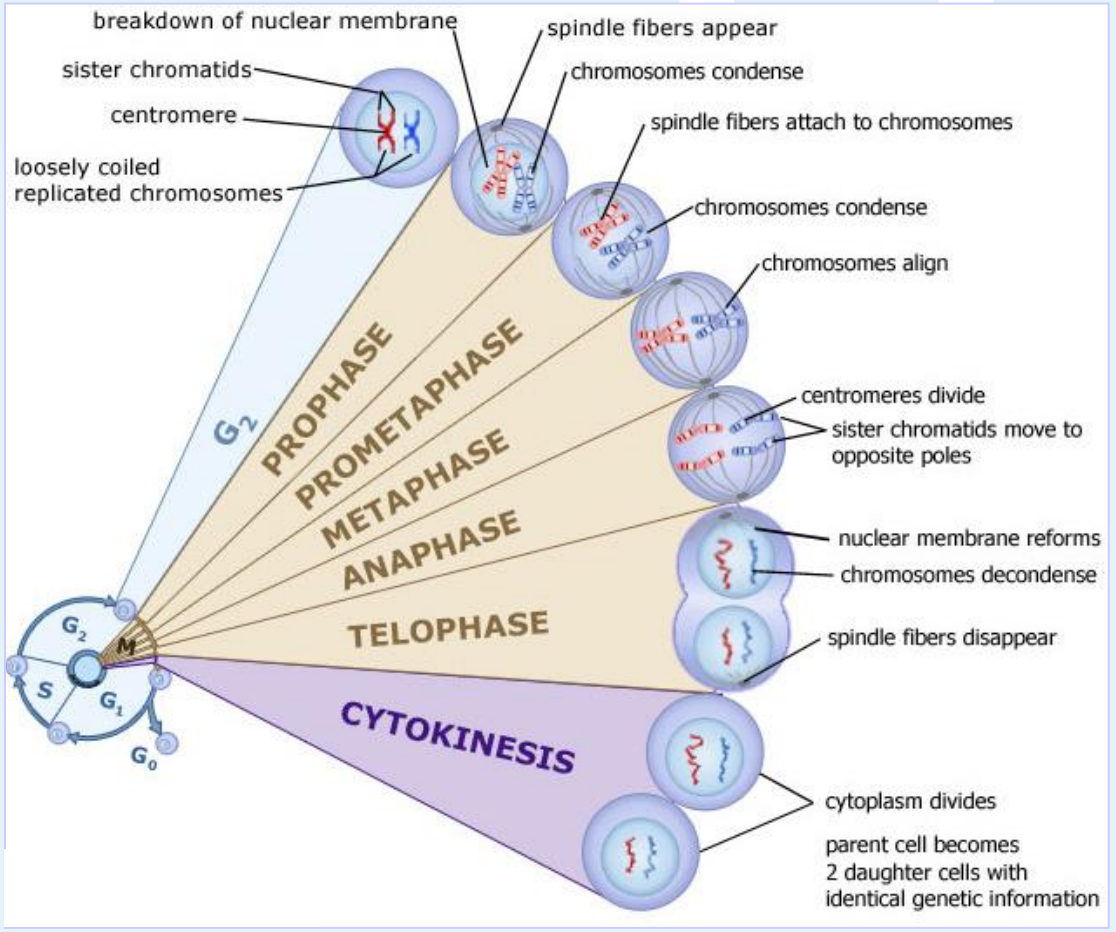
reprogen
REPRODUKČNÁ GENETIKA

Mitóza, meióza.
Mutácie a chromozómové aberácie

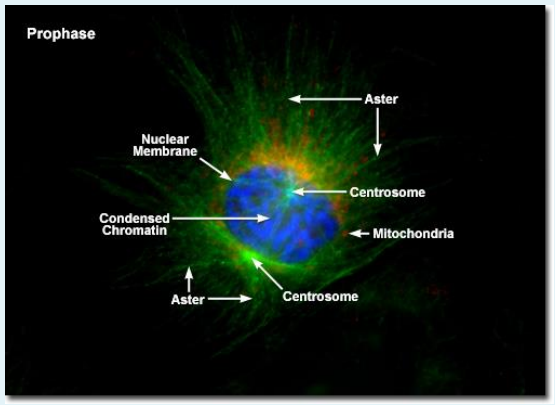
Mitosis and cell division



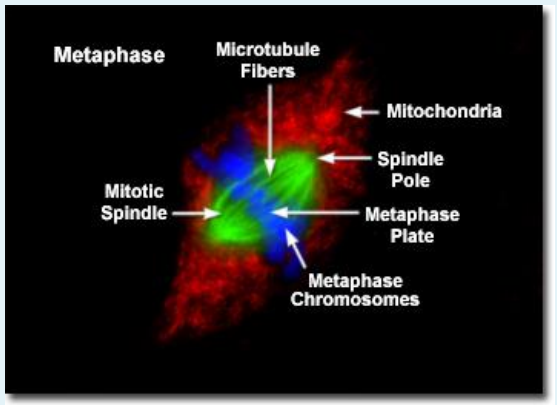




Mitosis and cell division

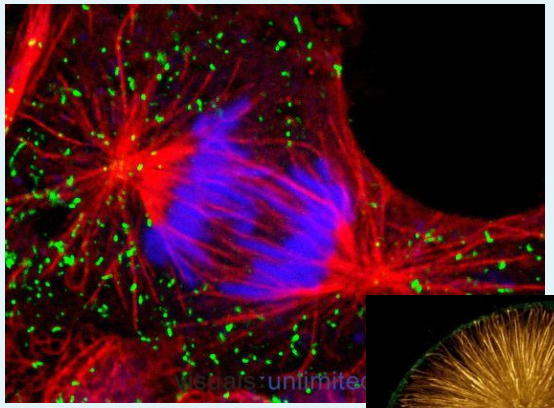


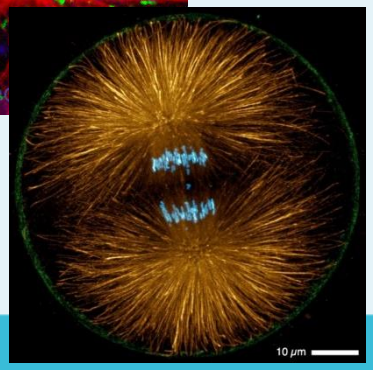
Prophase



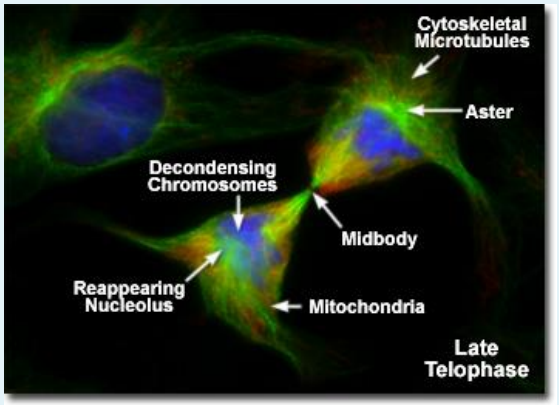
Metaphase

Metaphase plate

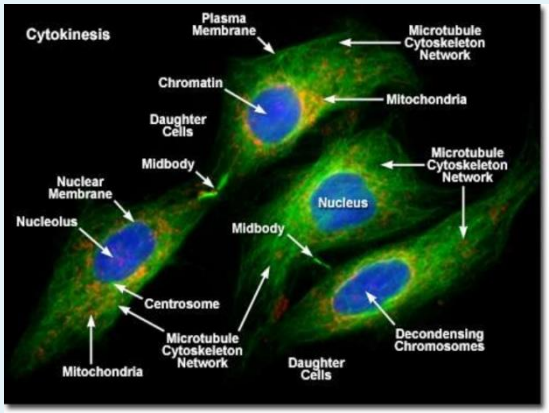




Anaphase



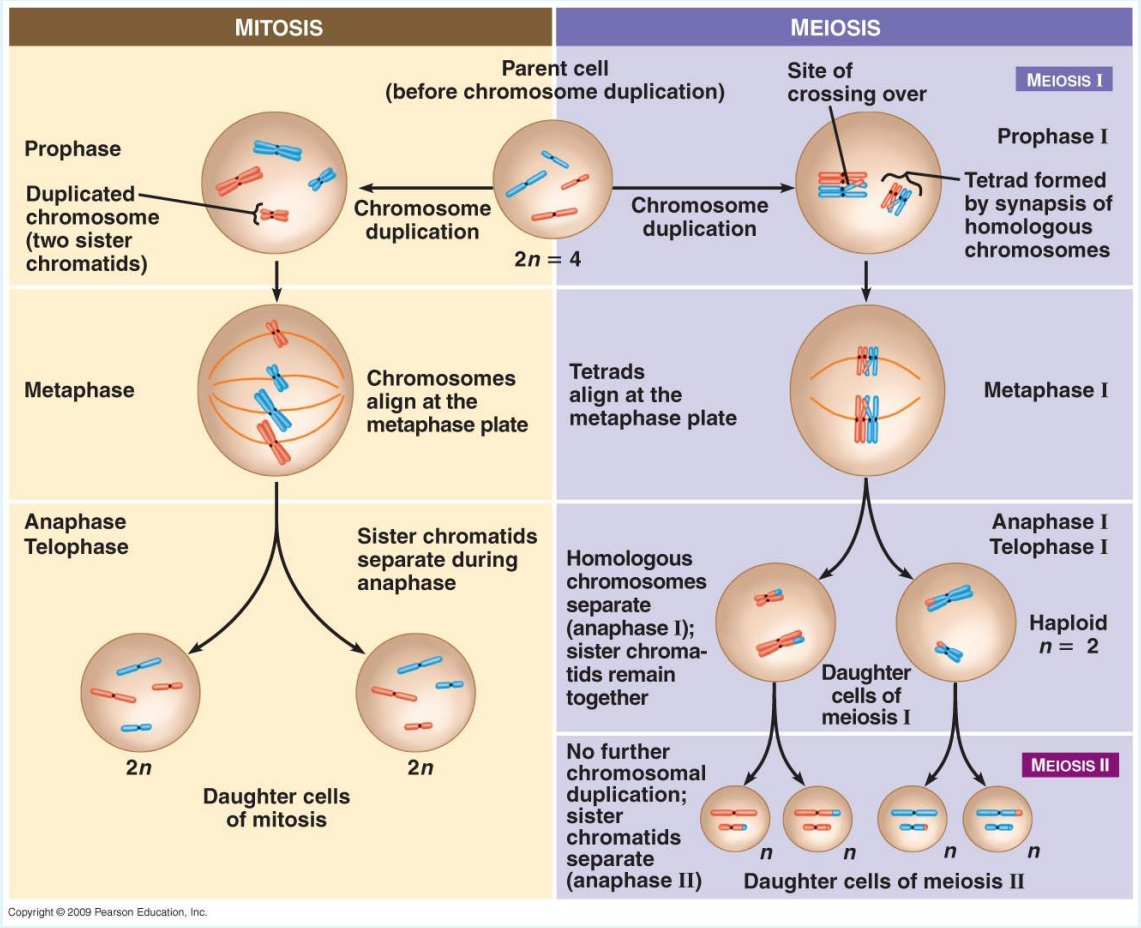
Telophase



Cytokinesis

Lagging chromosome ⇨ aneuploidy

Meiosis


MITOSIS
MEIOSIS
Parent cell (before chromosome duplication)
Site of crossing over
MEIOSIS I
Prophase
Prophase I
Duplicated chromosome (two sister chromatids)
Chromosome duplication
Chromosome duplication
Tetrad formed by synapsis of homologous chromosomes
2n = 4
Metaphase
Chromosomes align at the metaphase plate
Tetrads align at the metaphase plate
Metaphase I
Anaphase Telophase
Sister chromatids separate during anaphase
Anaphase I Telophase I
Homologous chromosomes separate (anaphase I); sister chromatids remain together
Haploid n = 2
Daughter cells of meiosis I
2n
2n
Daughter cells of mitosis
No further chromosomal duplication; sister chromatids separate (anaphase II)
MEIOSIS II
n
n
n
n
Daughter cells of meiosis II
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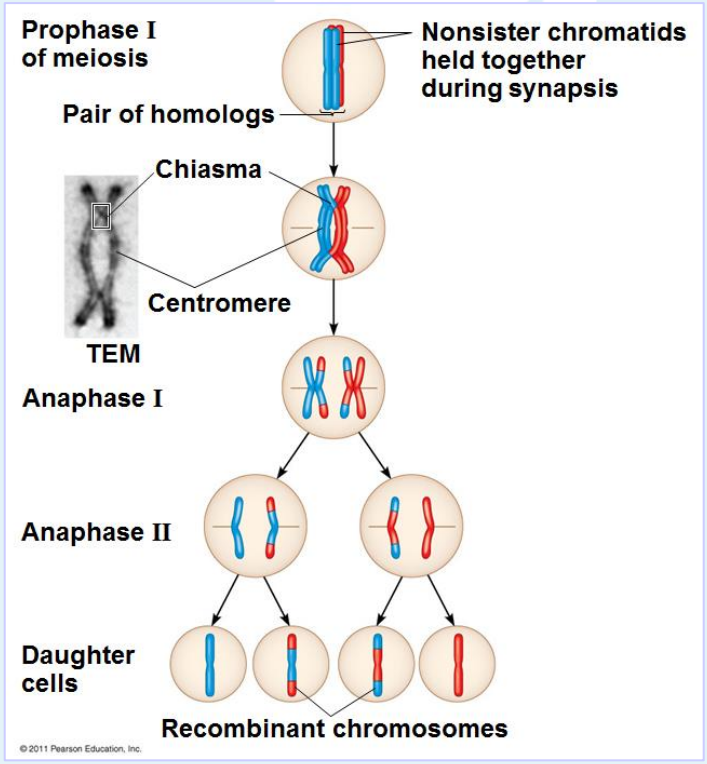
Prophase I of meiosis
Nonsister chromatids held together during synapsis
Pair of homologs
Chiasma
Centromere
TEM
Anaphase I
Anaphase II
Daughter cells
Recombinant chromosomes
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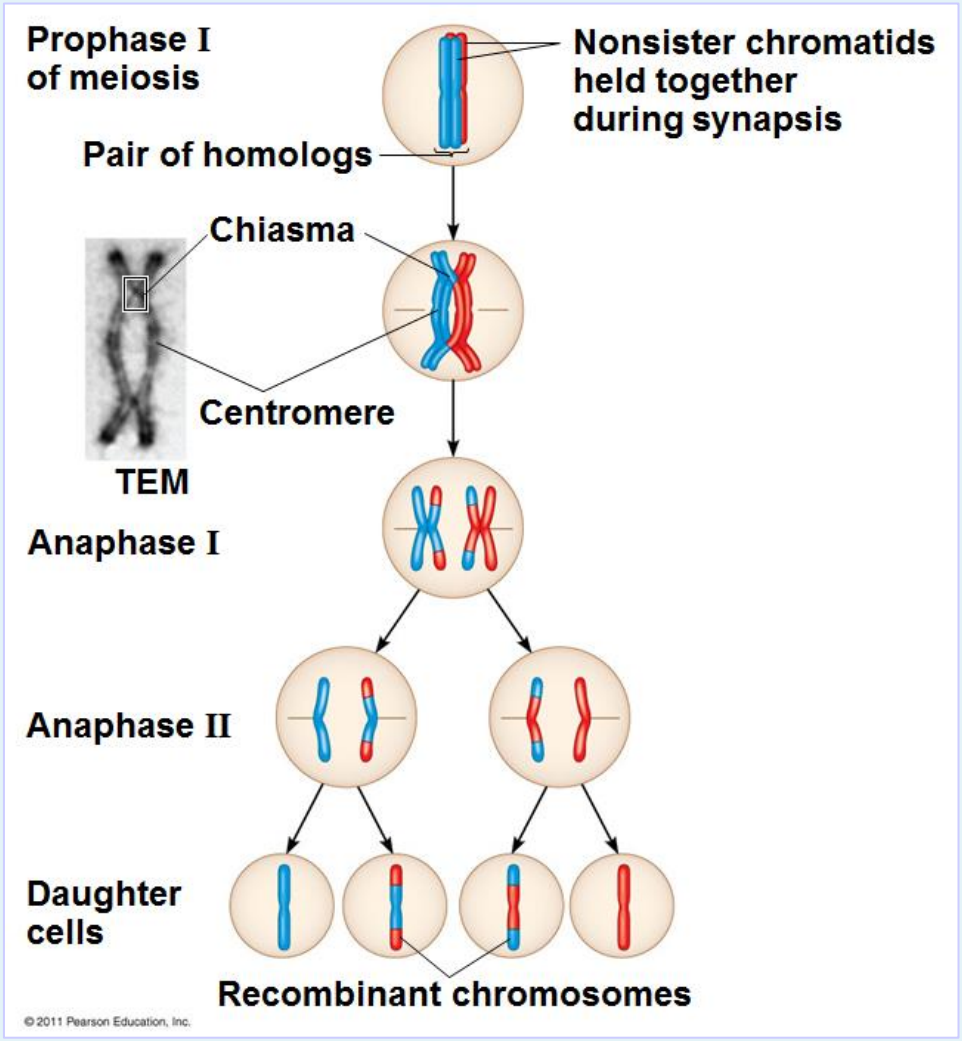
Meiosis: crossing-over (recombination)

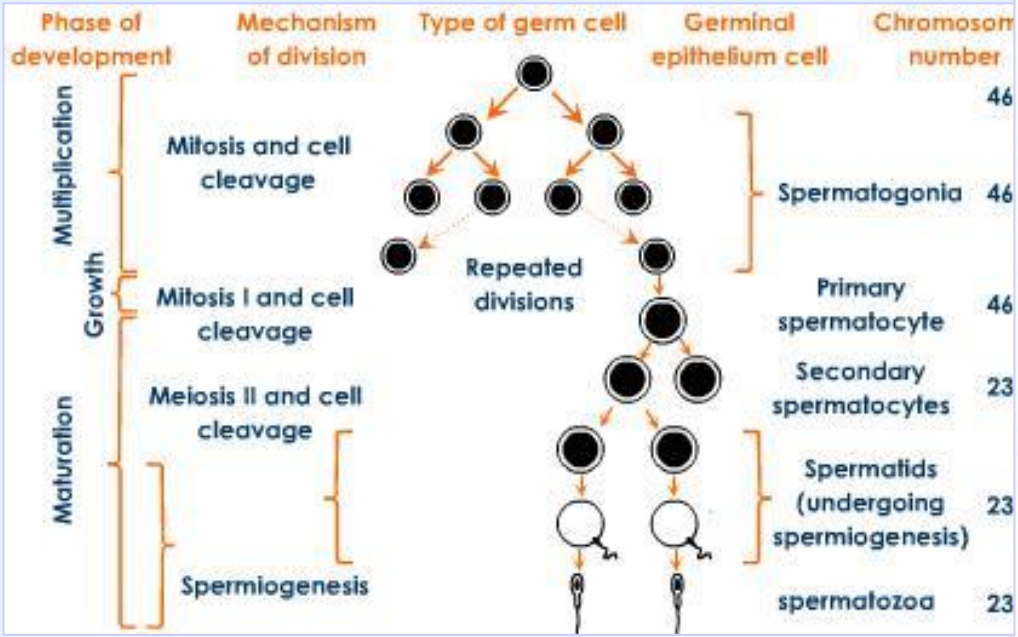


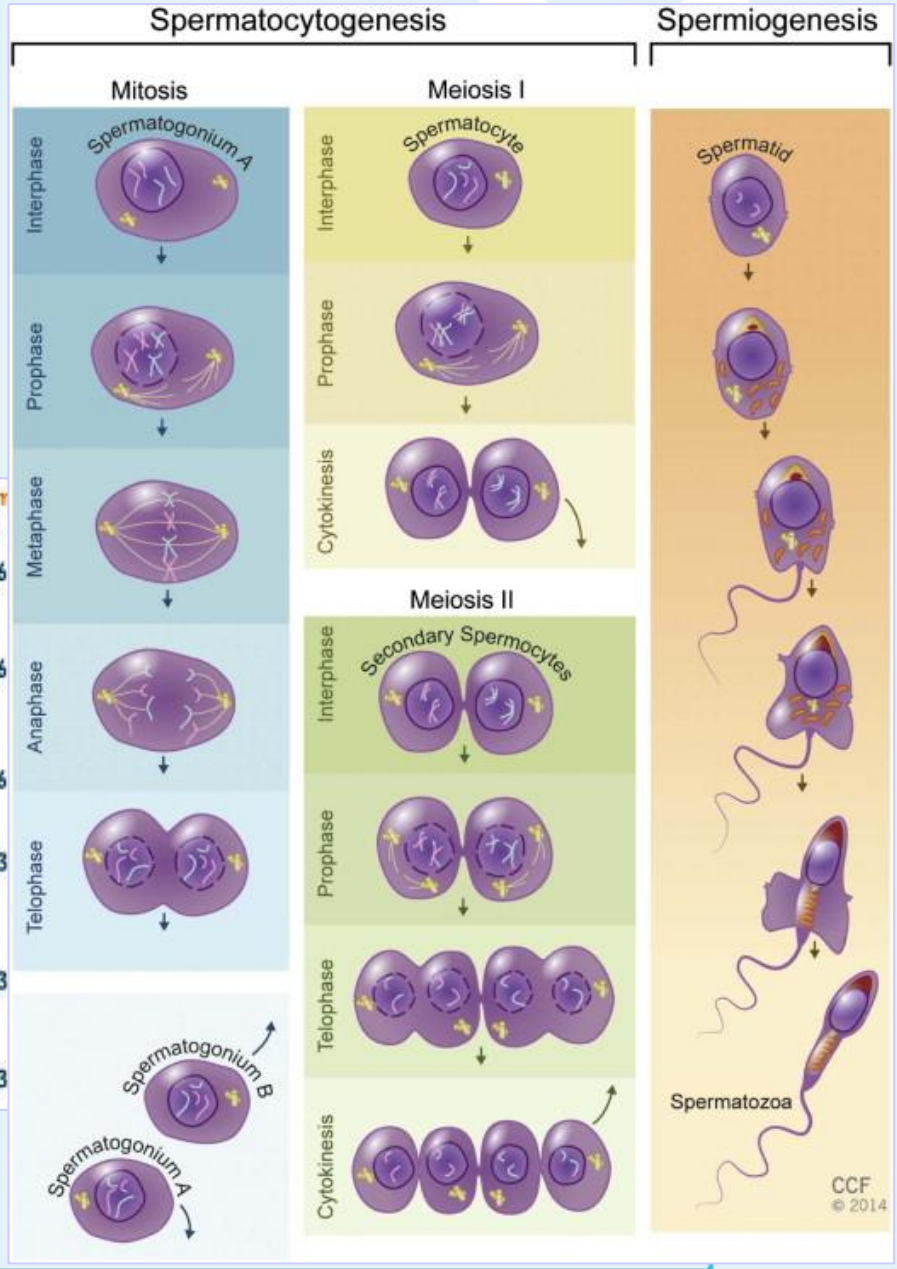
Crossing-over (recombination) - exchange of segment of chromosomal material between two homologous chromosomes. The chromatids held together by centromere are no longer identical.

Crossing-over is important for the normal segregation of chromosomes during meiosis. It produces new combinations of alleles in the cell – important for genetic variation.

Chiasma – point where the non-sister chromatids exchange

Spermatogenesis





Primary Spermocyte

Spermatogonia

Basal lamina

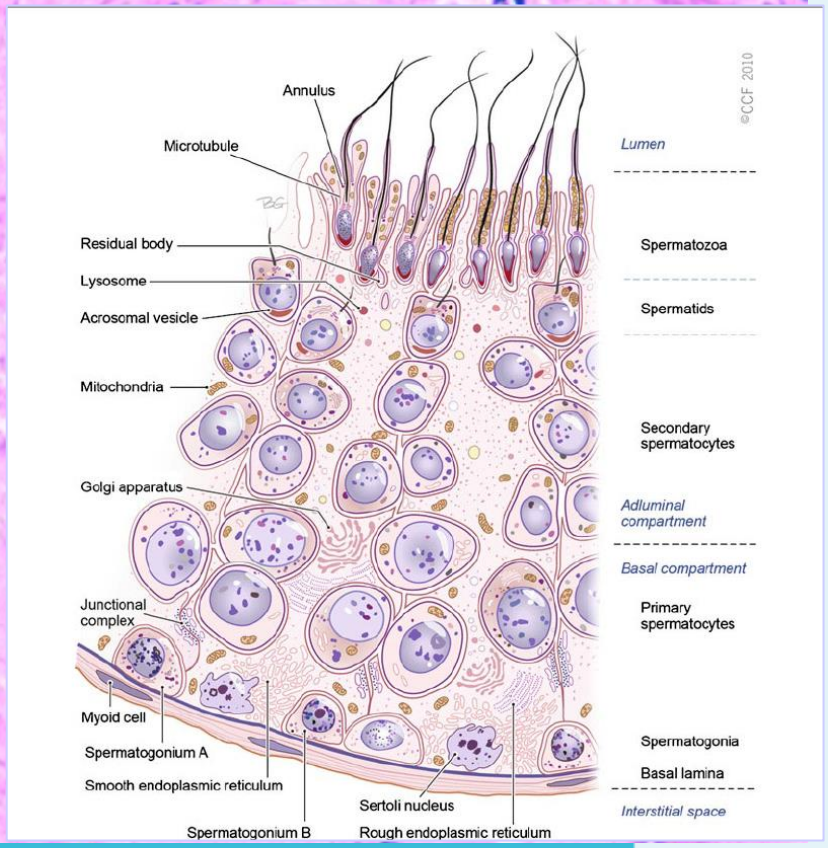
Spermatid

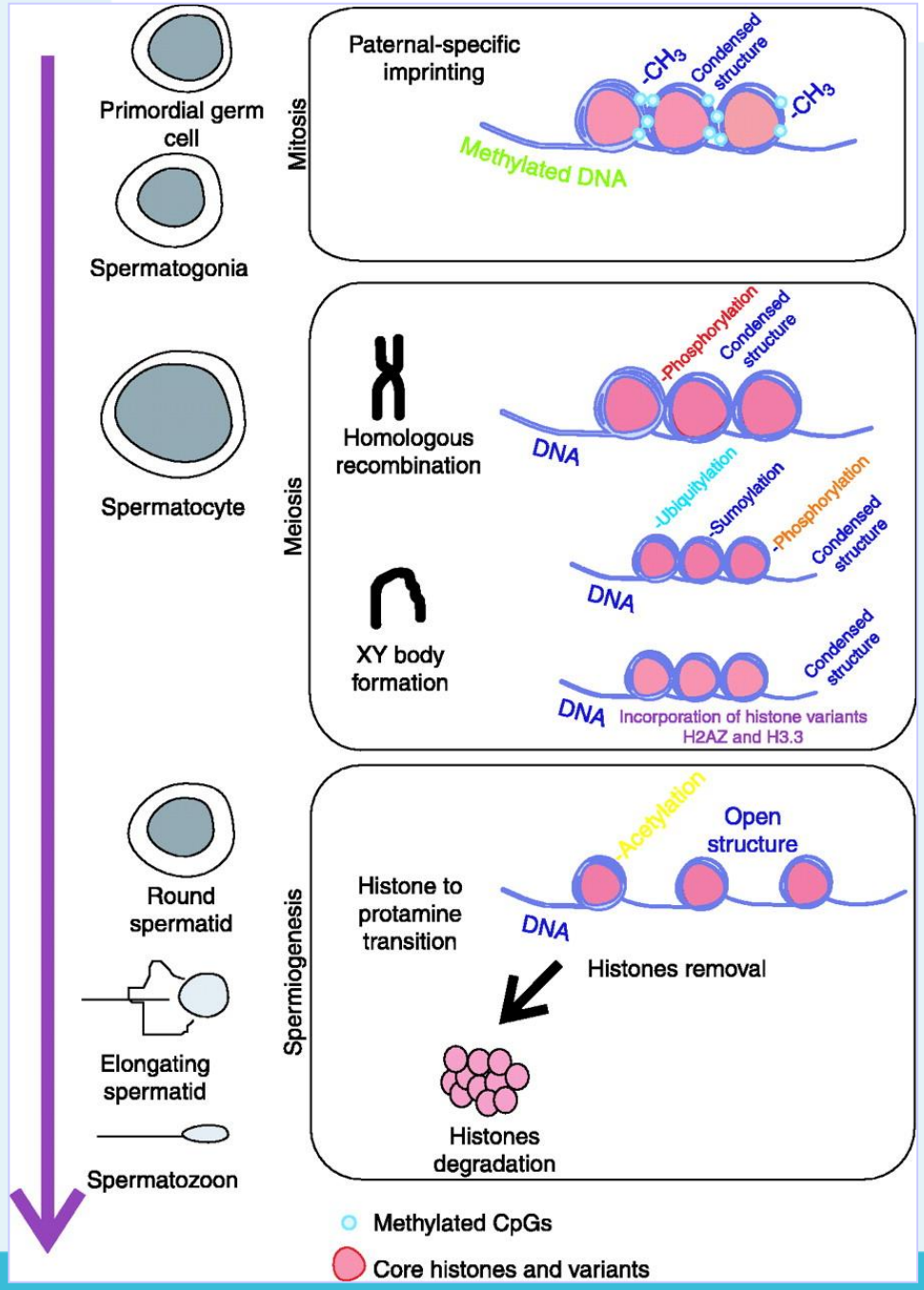
Myoid Cells

Spermatozoa

Lumen

Sertolli cell





Changes and damage of sperm

Inherited paternal imprinting

Meiosis - recombination

Chromosomal aneuploidy /structural changes

Abnormal morphology

Decreased vitality **(partial loss of mitochondria)**

Cytoplasma rests

Protamine insufficiency

Oogenesis

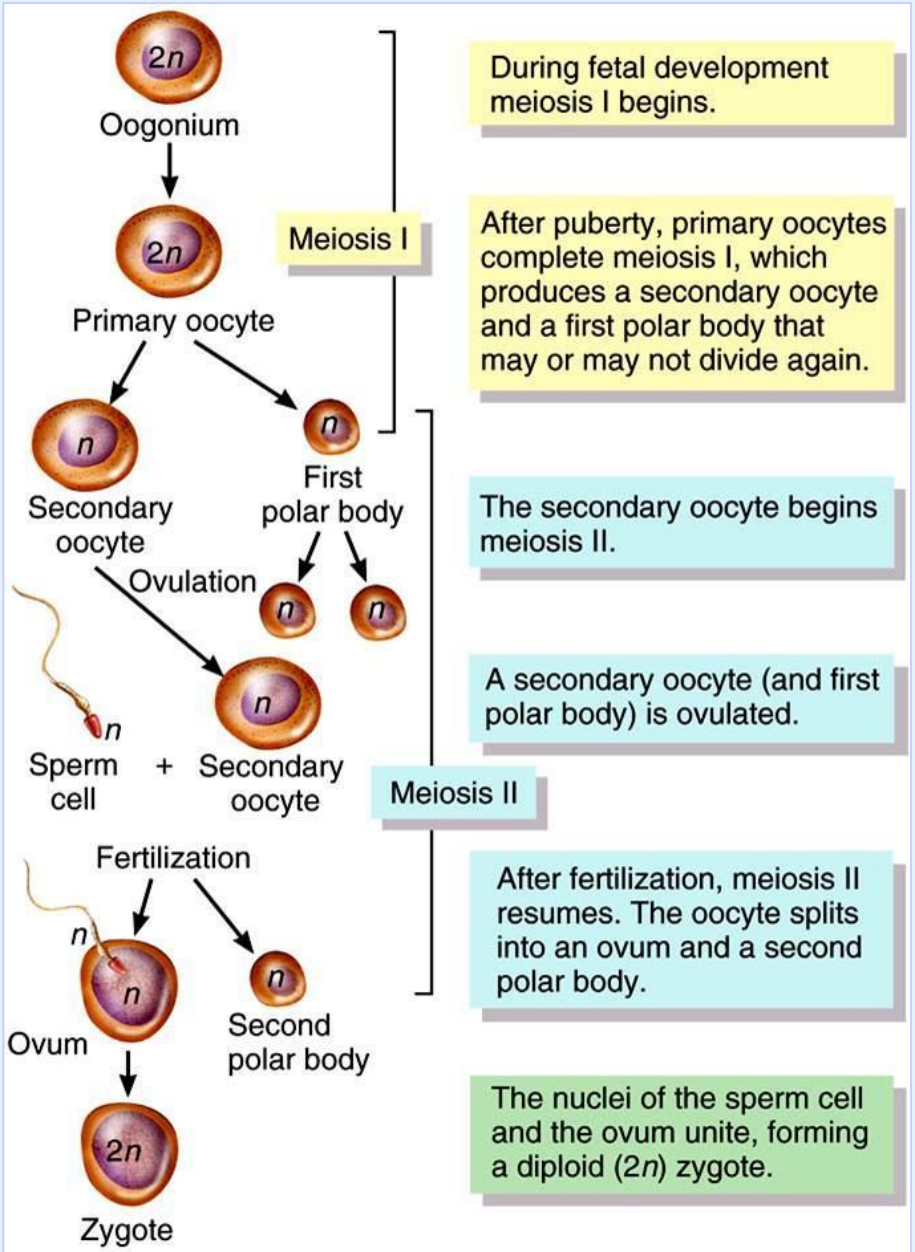
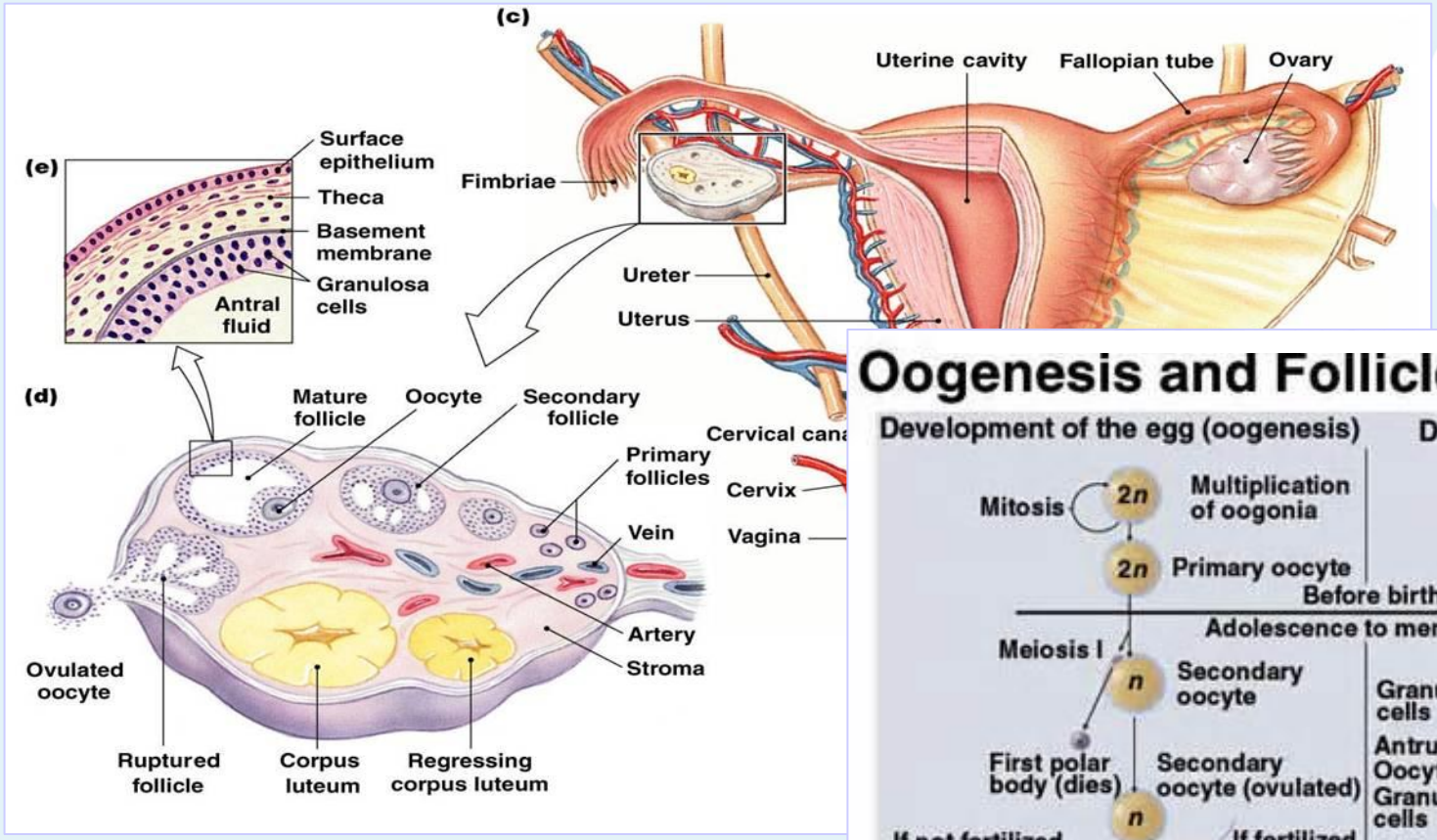
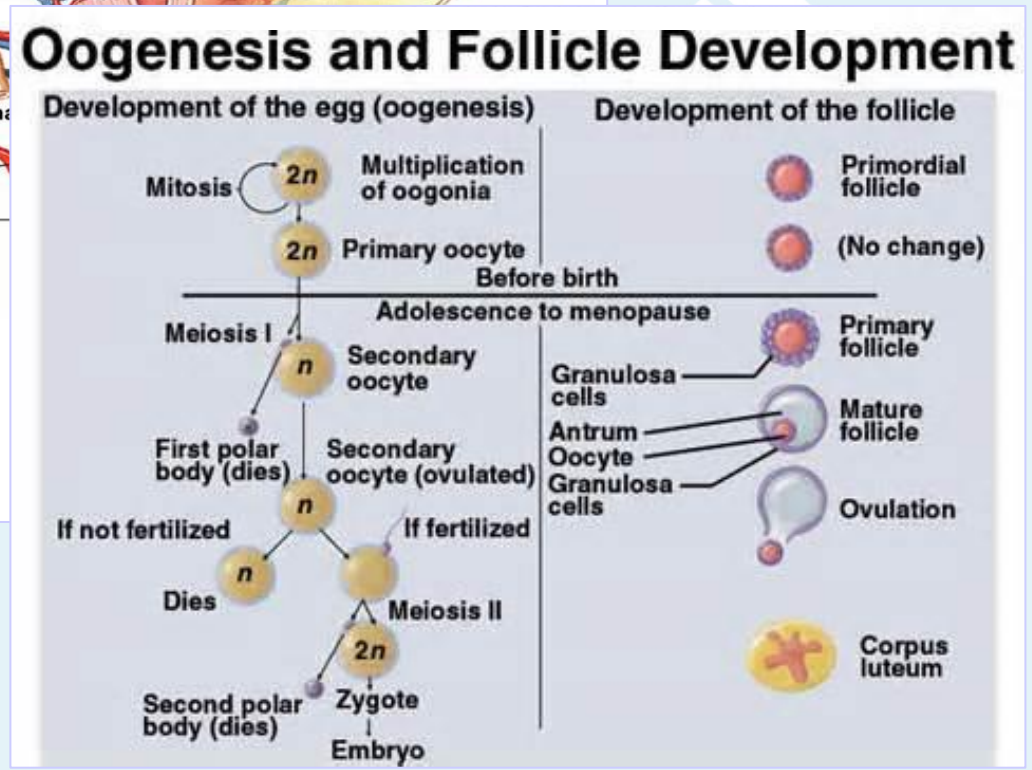


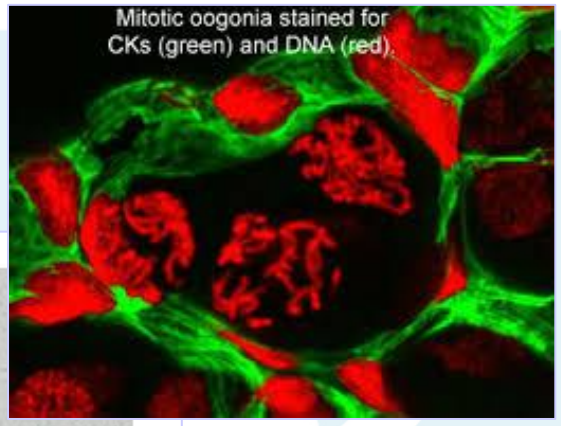
Figure 28.15 Tortora - PAP 12/e

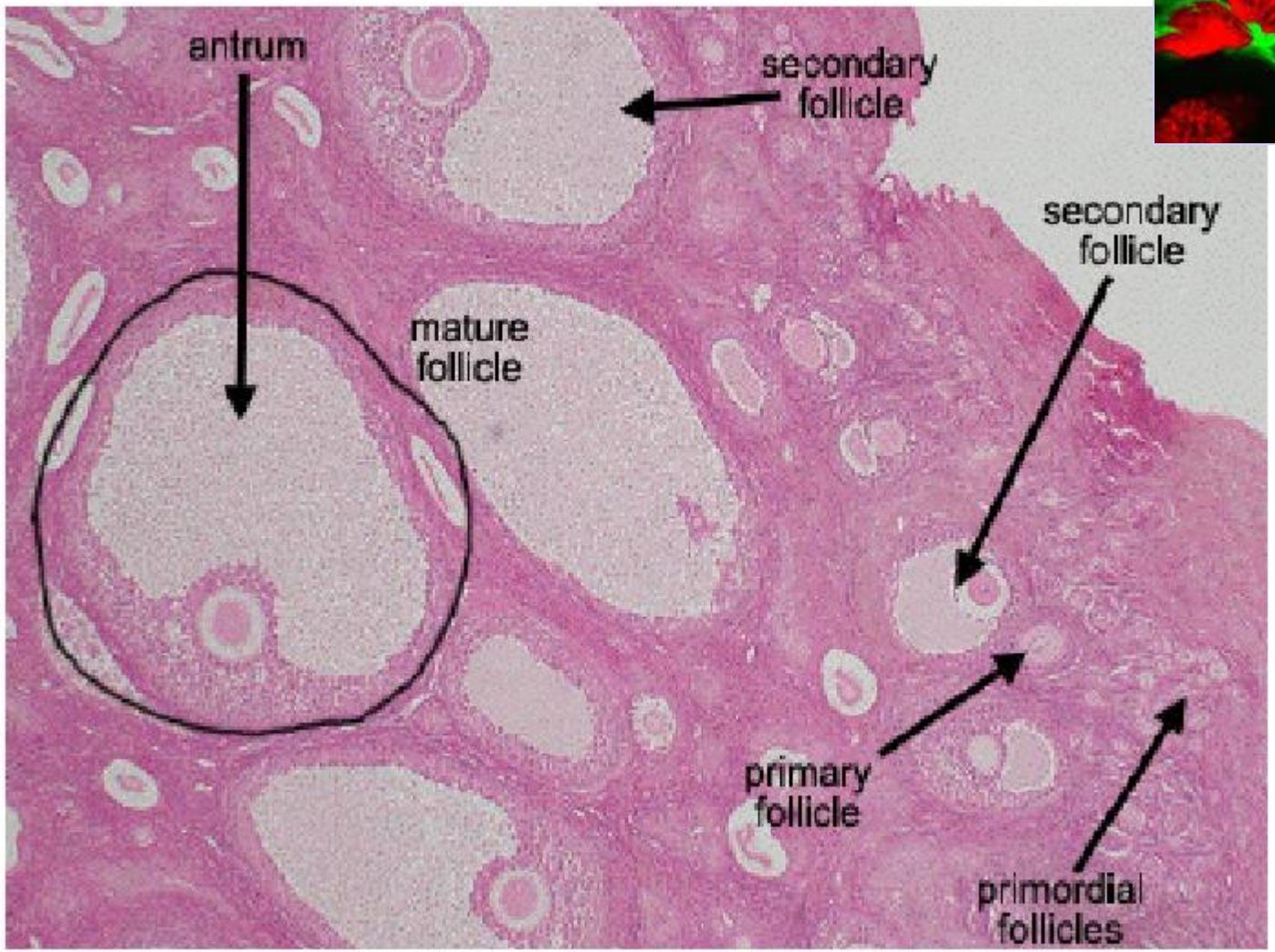
Oogenesis


(c)
Uterine cavity
Fallopian tube
Ovary
Fimbriae
Ureter
Uterus
(e)
Surface epithelium
Theca
Basement membrane
Granulosa cells
Antral fluid
(d)
Mature follicle
Oocyte
Secondary follicle
Primary follicles
Vein
Artery
Stroma
Ovulated oocyte
Ruptured follicle
Corpus luteum
Regressing corpus luteum
Cervical cana
Cervix
Vagina

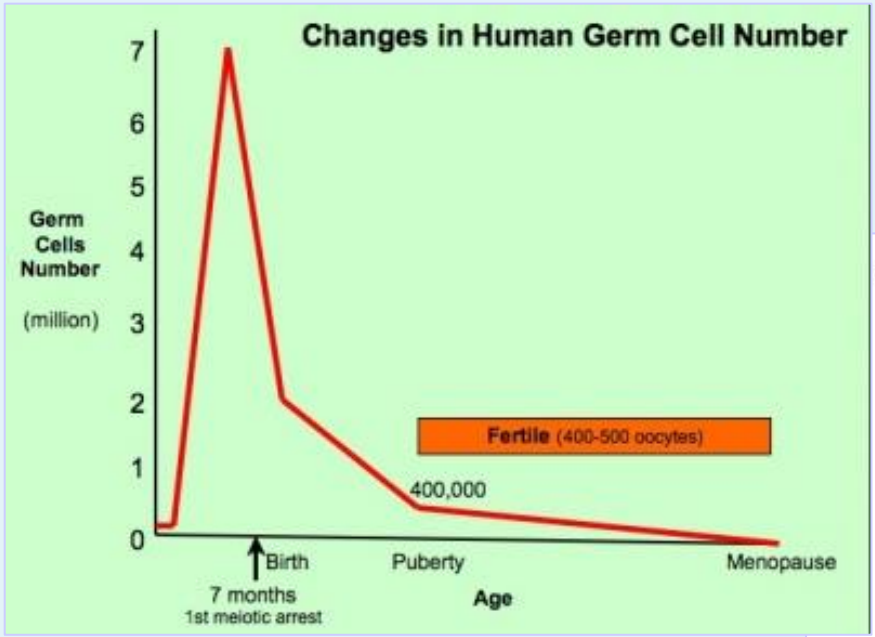

Oogenesis and Follicle Development
Development of the egg (oogenesis)
Development of the follicle
Mitosis
2n
Multiplication of oogonia
2n
Primary oocyte
Primordial follicle
(No change)
Before birth
Adolescence to menopause
Meiosis I
n
Secondary oocyte
Primary follicle
Granulosa cells
Antrum
Oocyte
Granulosa cells
Mature follicle
First polar body (dies)
Secondary oocyte (ovulated)
n
Ovulation
If not fertilized
If fertilized
n
Dies
Meiosis II
2n
Corpus luteum
Second polar body (dies)
Zygote
Embryo

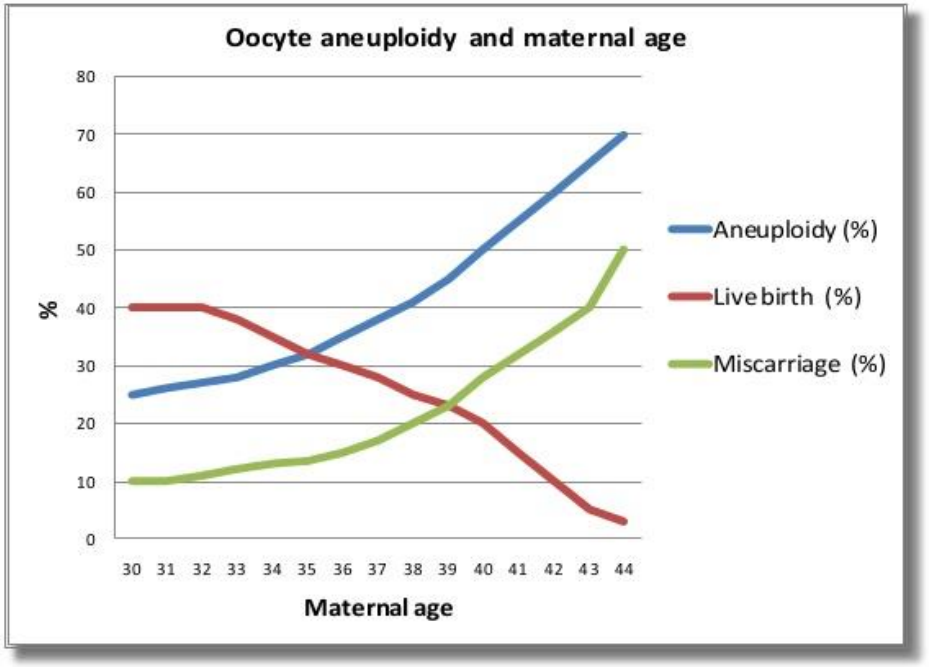
Oogenesis


Mitotic oogonia stained for
CKs (green) and DNA (red)


antrum
secondary follicle
secondary follicle
mature follicle
primary follicle
primordial follicles

Changes based on aged of woman

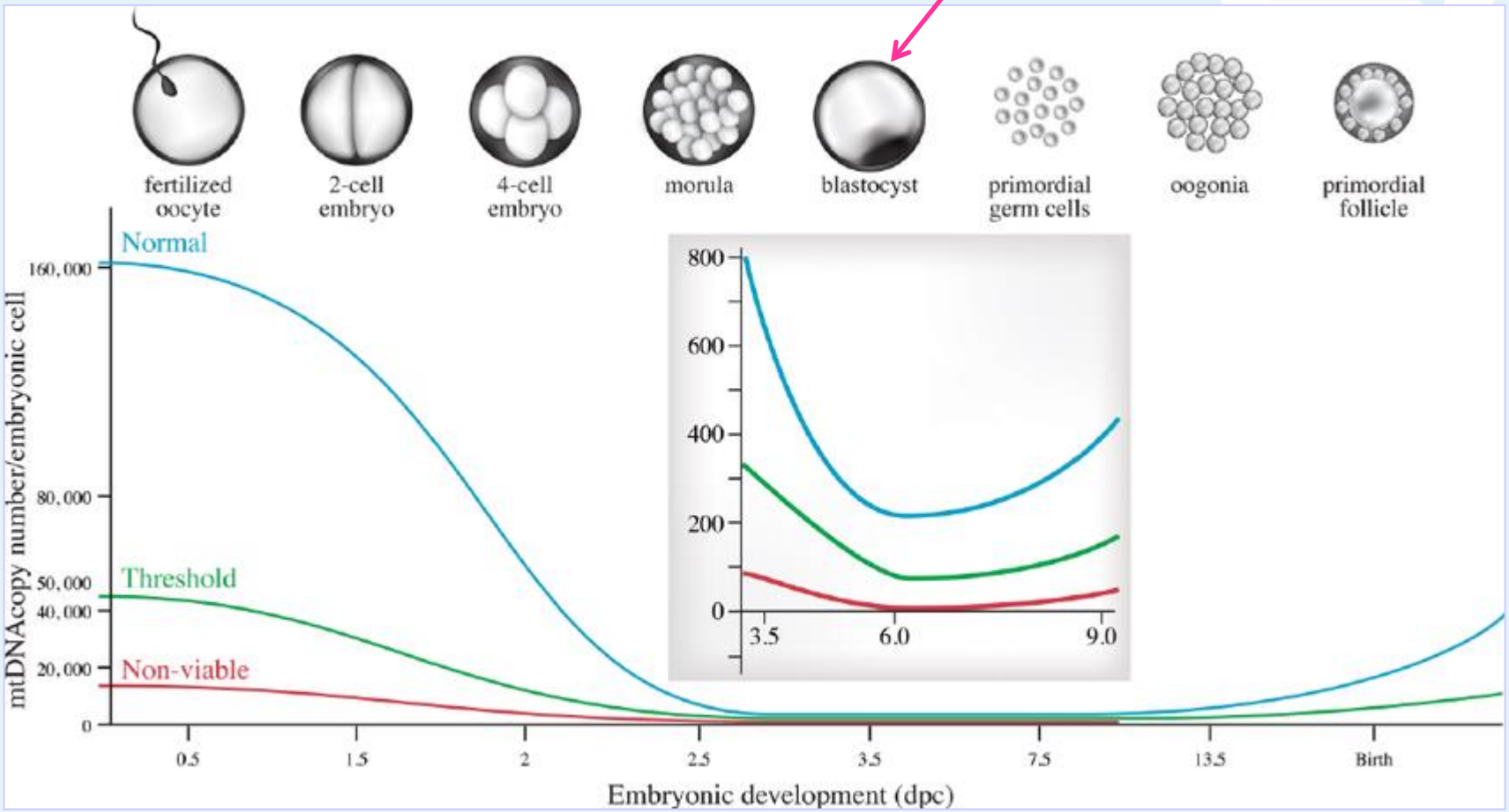




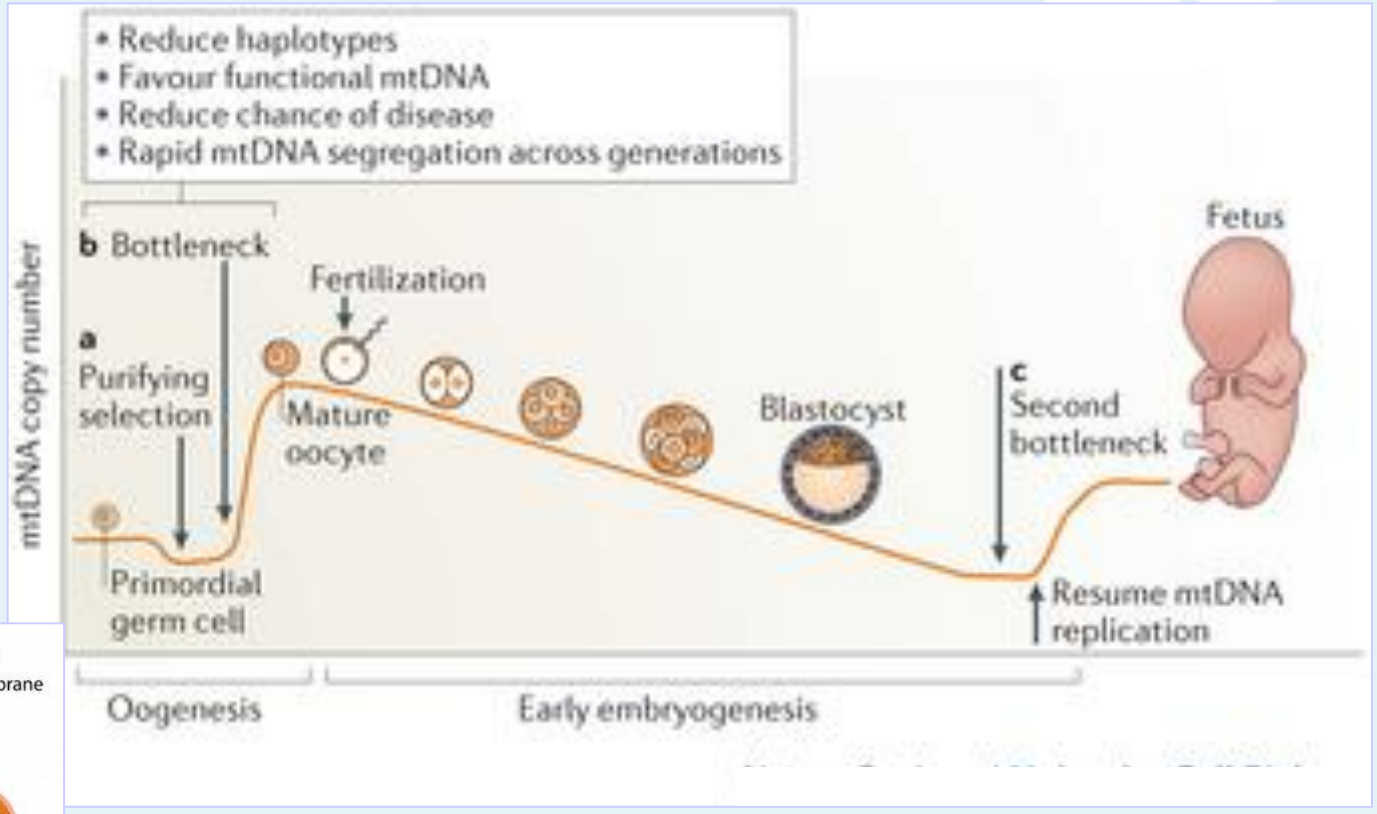
US CDC/SART data

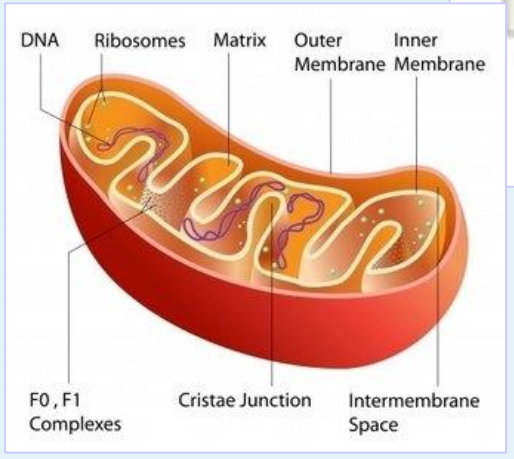
Mitochondria during embryogenesis

Mitoskóre	Implantation rate %
< 18	81%
18-24	50%
24-50	65%
> 50	18%
> 160	none



Mitochondria during embryogenesis





Comparison of spermatogenesis and oogenesis

	Aspect	Spermatogenesis	Oogenesis
Process	Location	Entirely in the testes	Mostly in the ovaries
	Cells produced	Sperm	Oocytes
	Cell structure	Head, middle piece and tail	Round cell
	Meiotic division	Equal division of cells	Unequal division of cytoplasm
	Germ line epithelium	Is involved in gamete production	Is not involved in gamete production
Gametes	Number of gametes produced	Four functional cells	One functional cell and 2-3 non-functional polar bodies
	Size of gametes	Sperm smaller than spermatocytes	Oocytes largeg than
		Cytoplasm is reduced in sperm	Cytoplasm is enhanced in oocyte
		Sperm are motile	Oocytes are immotile
Timing	Duration	Uninterrupted process	In arrested stages
	Onset	Begins in puberty	Begins in foetus (prenatal)
	Release	Continuous	Monthly from puberty
	End	Lifelong (but reduces with age)	Terminates with menopause

Reasons behind genetic diversity

Meiosis

- **crossing-over**
- **independent assortment**

Mutations

- **produces new alleles of genes** to increase variation

Random fertilization of the sperm and ovum

- **mixes up existing combinations** of the alleles of all the genes to increase the range of genotypes to increase variation

Mutation: The source of genetic variation

- Some mutations consist of an alteration of the number or structure of chromosomes in a cell. These major chromosome abnormalities can be observed **microscopically – chromosomal aberrations**

- Mutations that affect only single genes and are not microscopically observable – **gene mutations**

- **Mutation** could occur anywhere in genome but mutations that **take place in coding DNA or in regulatory sequences may have clinical consequences**

Types of mutations and their estimated frequencies

Class of Mutation	Mechanism	Frequency (Approximate)	Examples
Genome mutation	Chromosome missegregation	$2\text{-}4 \times 10^{-2}$/cell division	Aneuploidy
Chromosome mutation	Chromosome rearrangement	6×10^{-4}/cell division	Translocations
Gene mutation	Base pair mutation	10^{-10}/base pair/cell division	Point mutations
		10^{-5}-10^{-6}/locus/generation	

Types of mutations

Substitutions – replacement of a single nucleotide by another

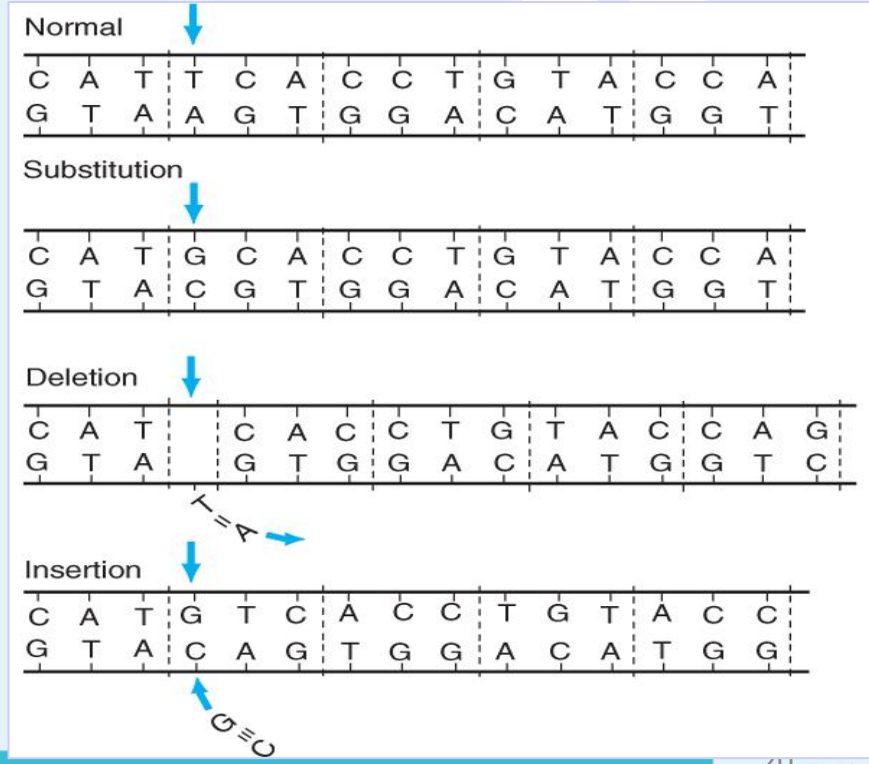
- most prevalent
- transition: replacement a pyrimide for a pyrimidine (C for T and vice versa) or a purine by a purine (A for G and vice versa)
- transversion: substitution of a pyrimidine by a purine and vice versa)

Deletions – loss of one or more nucleotides

- if it occurs in coding region and involves one, two or more nucleotides that are not multiple of three, the reading frame will be disrupted

Insertions – the addition of one or more nucleotides into a gene.

- If it occurs in coding region and involves one, two or more nucleotides that are not multiple of three, the reading frame will be disrupted.



Mutation: The source of genetic variation

- Deletions or insertions, which can result in extra or missing amino acids in a protein, are often detrimental.
- Deletions and insertions tend to be especially harmful when the number of missing or extra base pairs is not a multiple of three.
- Because codons consist of groups of three base pairs, such insertions or deletions can alter all of the downstream codons. This is a **frameshift mutation**.
- Often, a **frameshift mutation produces a stop codon downstream of the insertion or deletion, resulting in a truncated polypeptide.**

Structural effects of mutations on the protein

Silent mutation doesn´t alter a polypetide product of the gene

- Usually when a substitution occurs in the third position of the codon because of degeneracy of the genetic code.

  New triplet codes for the same amino acid with no alteration in the

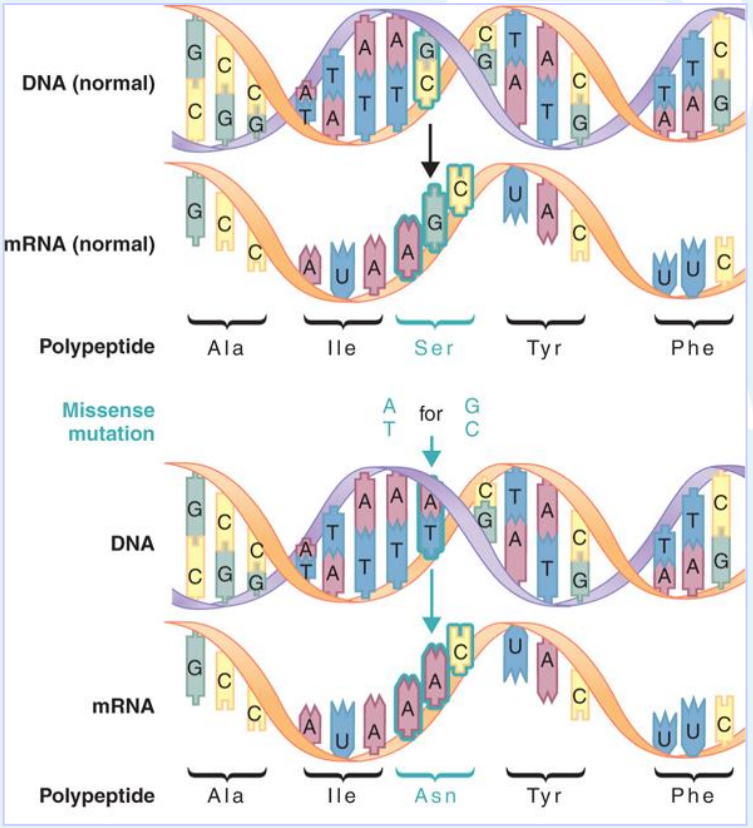
  properties of the resulting protein

Structural effects of mutations on the protein

Missense mutation results in **coding for a different amino acid** and the **synthesis of an altered protein.**

- When new amino acid is chemically similar, usually has no functional effect.
- When **new amino acid is chemically dissimilar** (has a different charge), **the structure of protein will be altered** (usually gross reduction or complete loss of biological/enzymatic activity

  - many abnormal hemoglobins

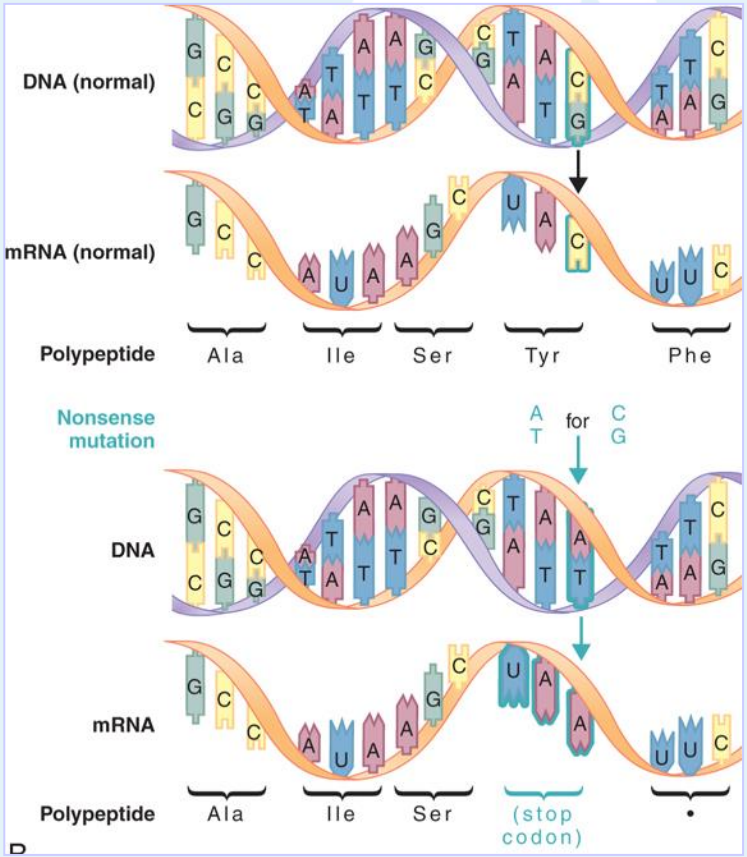


Structural effects of mutations on the protein

Nonsense mutation - a substitution that leads to the **generation of one of the stop codons** will result in **premature termination of translation** of a polypeptide chain.

The shortened chain is unlikely to retain normal biological activity

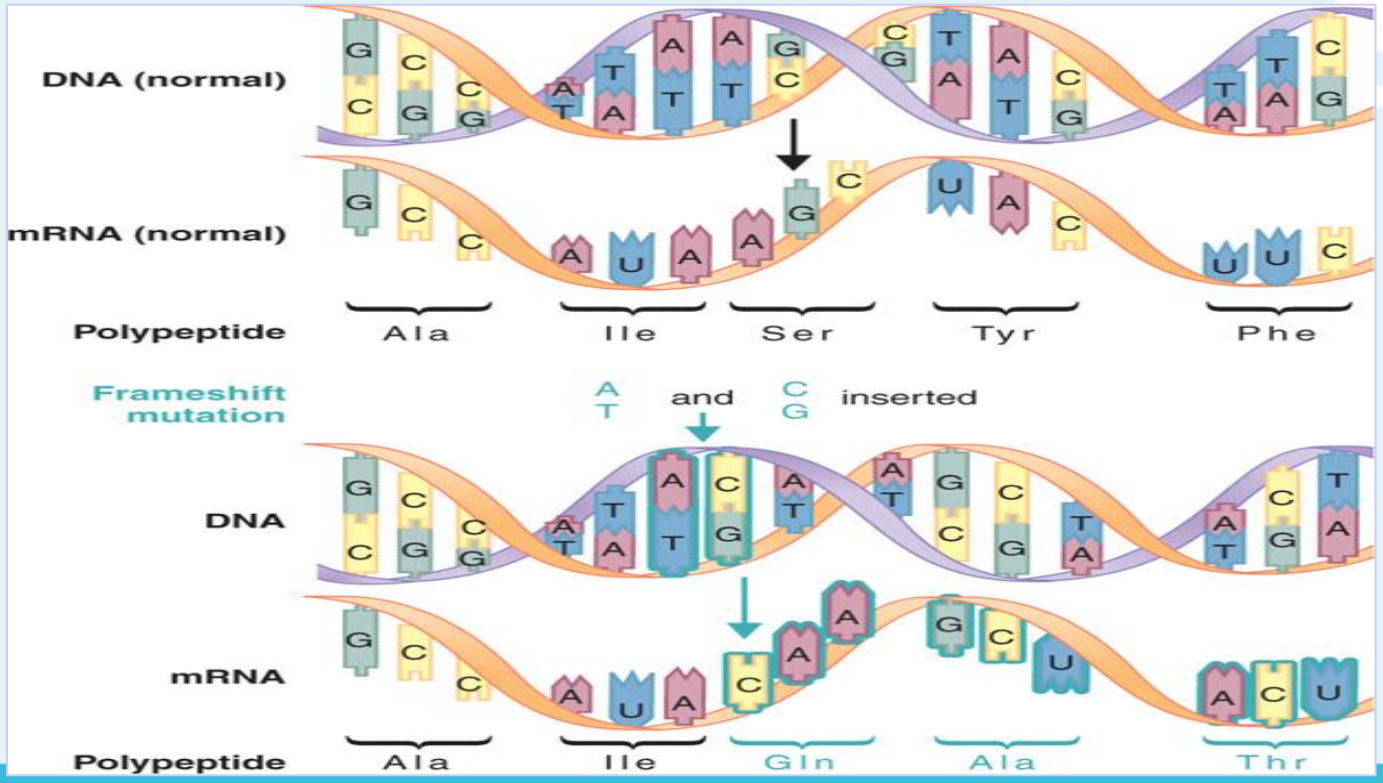
(loss of important functional domain of the protein)



Structural effects of mutations on the protein

Frameshift mutations result from the addition or deletion of a number of bases that is not a multiple of three. This alters all of the codons downstream from the site of insertion or deletion.

- Often, a frameshift mutation produces a stop codon downstream of the insertion or deletion, resulting in a truncated polypeptide.



Structural effects of mutations on the protein

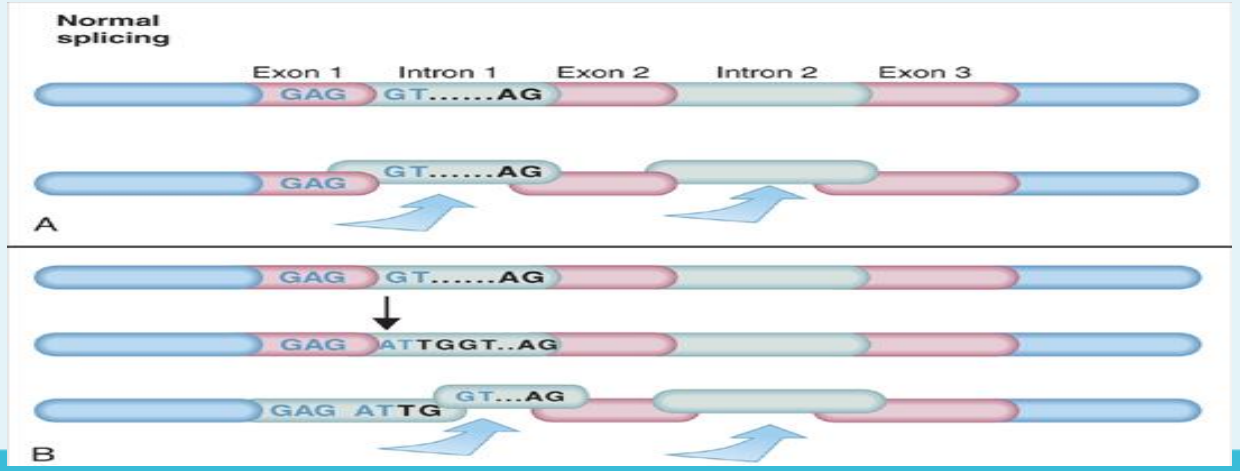
- **Mutation in non-coding DNA** – they are less likely to have phenotypic effect

Exception: mutations in promotor of the gene or other regulatory regions
– the affect of the level of gene expression

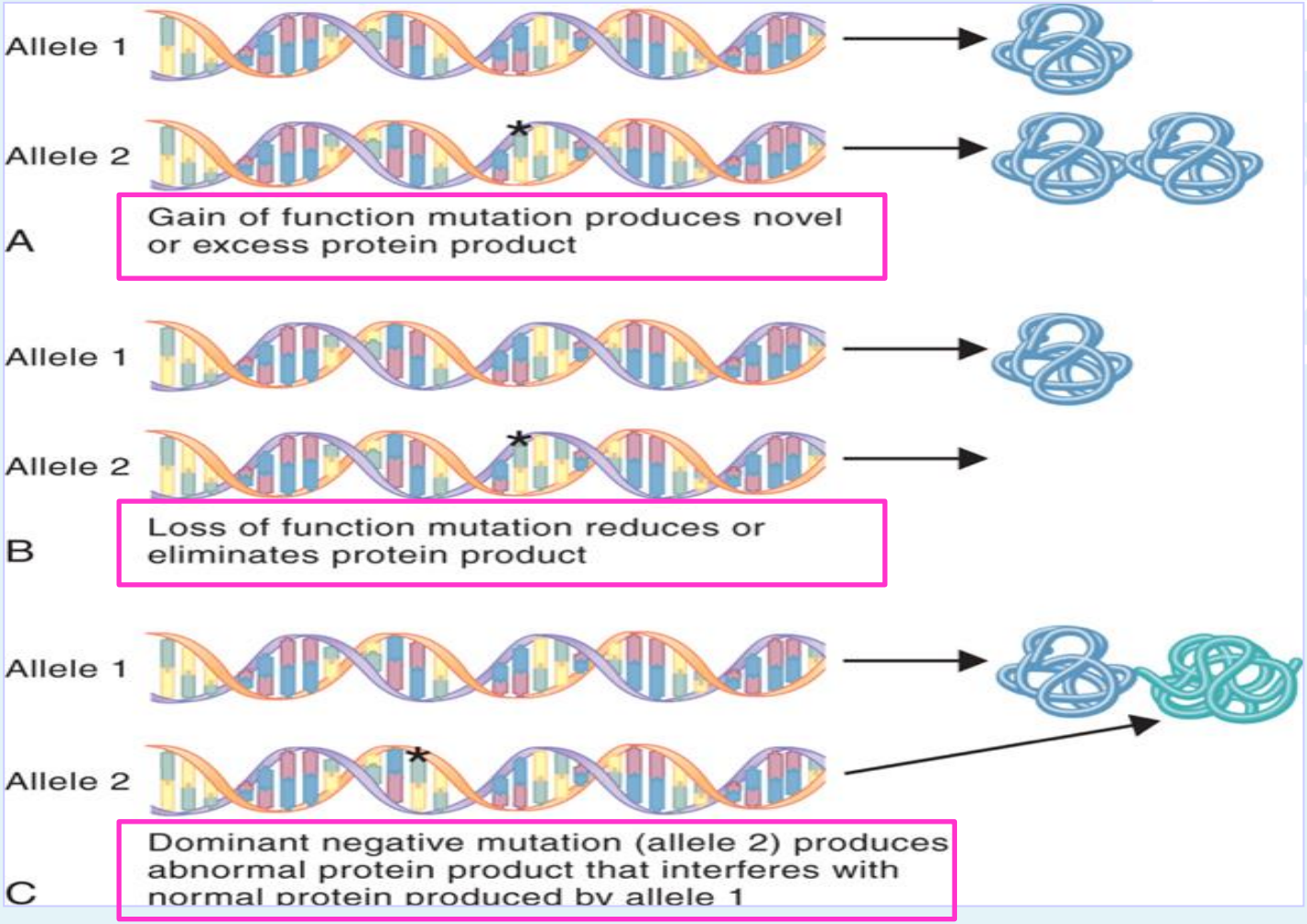
Promoter mutation - can decrease the affinity of RNA polymerase, it results to decreased activity of the gene

Splice-site mutations - those that occur at intron-exon boundaries, alter the splicing signal that is necessary for proper excision of an intron

- Splice-site mutations can occur at the GT sequence that defines the 5' splice site (the **donor site**) or at the AG sequence that defines the 3' splice site (the **acceptor site**)



Functional effects of mutations on the protein



Functional effects of mutations on the protein

Loss-of-Function mutations can result in:

- **reduced activity of protein or decreased stability of the gene product** (hypomorph)
- **complete loss of the gene product** (null allele or amorph)
- **usually autosomal recessive or X-linked recessive inheritance** – catalytic activity of the product of normal allele is more than adequate to carry out the reactions of most metabolic pathways

Haplo-insufficiency – in heterozygous state the half normal levels of the gene product result in phenotypic effect

- homozygous mutations result in more severe phenotypic effects
- *genes for receptors*
- *familial hypercholesterolemia, acute intermittent porphyria*

Functional effects of mutations on the protein

Gain-of-Function mutations result in:

- **increased levels of gene expression** *(Charcot-Marie-Tooth disease – hereditary motor and sensory neuropaty type I, Huntington disease)*
- **development of a new function of the gene product** - *chromosomal rearrangements that result in the combination of sequences from two different genes in specific tumors*
- **autosomal dominant inheritance, in homozygous state – much more severe phenotype, which is often prenatally lethal disorder** *(achondroplasia)*

Dominant-Negative mutations

- **mutant gene in the heterozygous state results in the loss of protein activity or function as a consequence of the mutant gene product interfering with the function of the normal gene product of the corresponding allele**
- common in proteins that are dimers or multimers
  (structural proteins – collagens : mutation can lead to osteogenesis imperfecta)

Types of mutations and their consequences

Types of Mutation in Human Genetic Disease

Nucleotide Substitutions (Point Mutations)	Percentage of Disease-Causing Mutations	Deletions and Insertions	Percentage of Disease-Causing Mutations
Missense mutations (amino acid substitutions)	50%	Addition or deletion of a small number of bases	25%
Nonsense mutations (premature stop codons)	10%	If the number of bases involved is not a multiple of 3, a frameshift results with likely premature termination downstream.	
RNA processing mutations (destroy consensus splice sites, cap sites, and polyadenylation sites or create cryptic sites).	10%		
		If the number of bases involved is a multiple of 3, amino acids in the translated product are either lost or gained.	
Splice-site mutations leading to frameshift mutations and premature stop codons	10%		
		Larger gene deletions, inversions, fusions, and duplications (may be mediated by DNA sequence homology either within or between DNA strands)	5%
Regulatory mutations affecting transcription factor binding, transcriptional control, or other aspects of gene expression	Rare		
		Insertion of a LINE or *Alu* element (disrupting transcription or interrupting the coding sequence)	rare
		Expansion of trinucleotide repeat sequences	rare

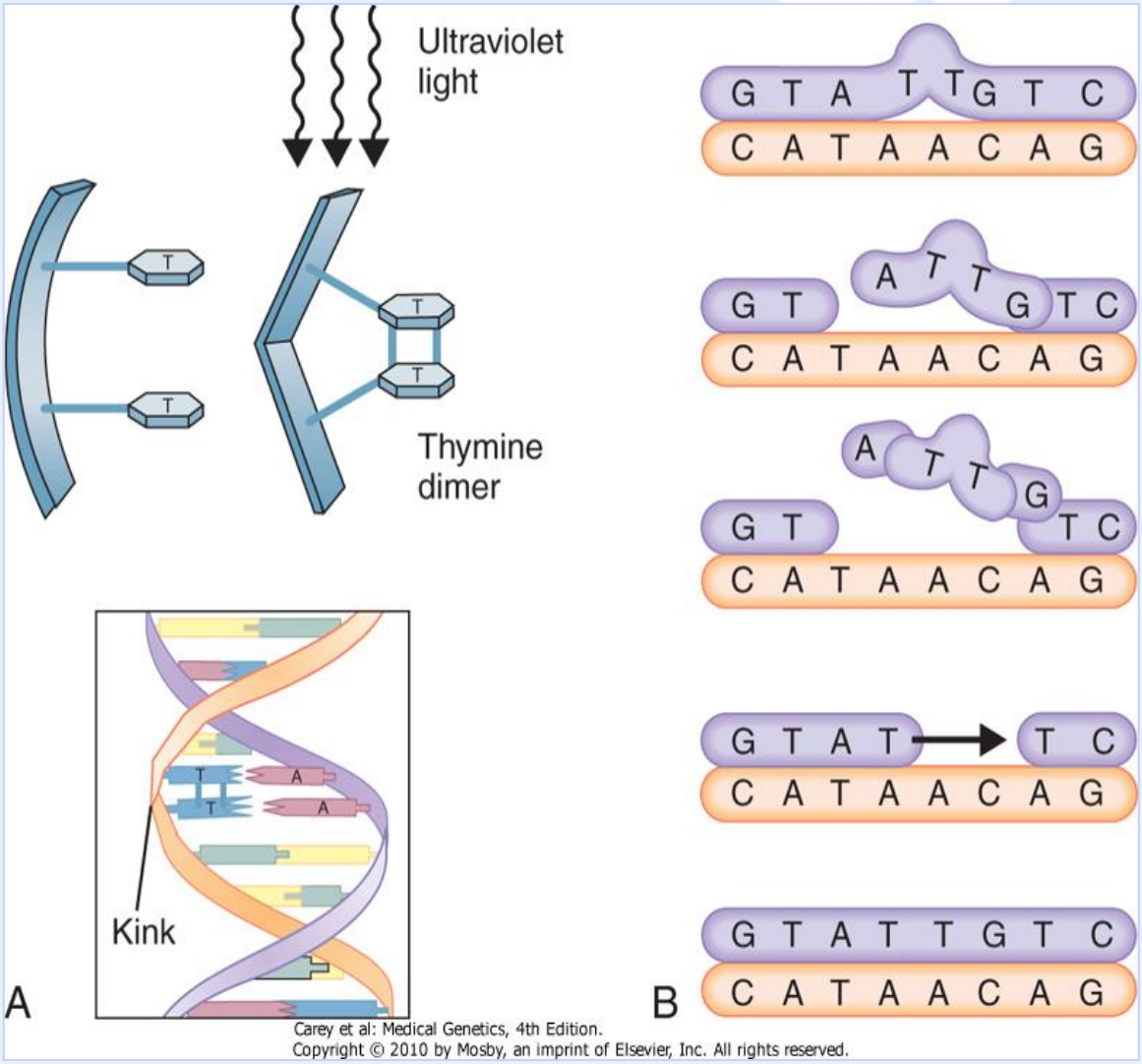
Causes of mutation

- A large number of agents are known to cause **induced mutations**.
- These mutations, which are attributed to known environmental causes, can be contrasted with **spontaneous mutations**, which arise naturally during the process of DNA replication.
- Agents that cause induced mutations are known collectively as **mutagens**. Animal studies have shown that **radiation** is an important class of mutagen
- **Ionizing radiation**, such as that produced by X-rays and nuclear fallout, can eject electrons from atoms, forming electrically charged ions.

Ultraviolet (UV) radiation

- **A, Pyrimidine dimers** originate when covalent bonds form between adjacent pyrimidine (cytosine or thymine) bases. This deforms the DNA, interfering with normal base pairing.

- **B, The defect is repaired** by removal and replacement of the dimer and bases on either side of it, with the complementary DNA strand used as a template



Causes of Mutation

- **Ultraviolet (UV) radiation**, which occurs naturally in sunlight, is an example of **nonionizing radiation.**
- UV radiation causes the formation of covalent bonds between adjacent pyrimidine bases (i.e., cytosine or thymine).
- These **pyrimidine dimers** are unable to pair properly with purines during DNA replication; this results in a base-pair substitution.
- Because **UV radiation** is absorbed by the skin, it **does not reach the germline** but can cause **skin cancer**

The dose of radiation - the amount received by the gonads because it is the effect of radiation on germ cells rather than somatic cells that are important as far as **transmission of mutations to future progeny**

Gonad dose of radiation - the amount received in 30 years *(generation time in humans).*

Approximate average doses of ionizing radiation from various sources to the gonads of the general population		
Source of radiation	**Average dose per year (mSv)**	**Average dose per 30 years (mSv)**
Natural		
Cosmic radiation	0.25	7.5
External γ radiation	1.50	45.0
Internal γ radiation	0.30	9.0
Artificial		
Medical radiology	0.30	9.0
Radioactive fallout	0.01	0.3
Occupational	0.04	1.2
Total	**2.40**	**72.0**

Mutation Rates

- **How often do spontaneous mutations occur?**

  At the nucleotide level, the mutation rate is estimated to be about

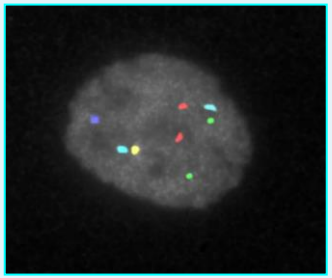
  10^{-9} per base pair per cell division

  (this figure represents mutations that have escaped the process of DNA repair).

- **At the level of the gene**, the mutation rate is quite variable, ranging

  from 10^{-4} to 10^{-7} per locus per cell division.

- There are at least two reasons for this large range of variation: the size of the gene and the susceptibility of certain nucleotide sequences.
- *The **somatostatin gene**, for example, is quite small, containing **1480 bp**. In contrast, the **gene responsible for Duchenne muscular dystrophy** (DMD) spans more than **2 million bp**.*
- Larger genes present larger targets for mutation and usually experience mutation more often than do smaller genes.



Frequency of chromosomal abnormalities

sperm **oocytes**

of healthy people

6 % **50 %**

↘ ↙

embryos

(preimplantation period – I. trimester)

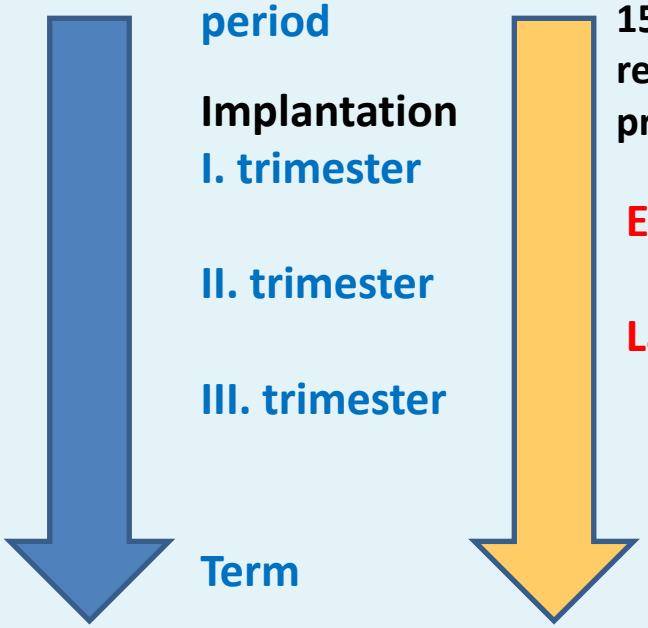
50 - 70 %

↓

newborns

0,5 %

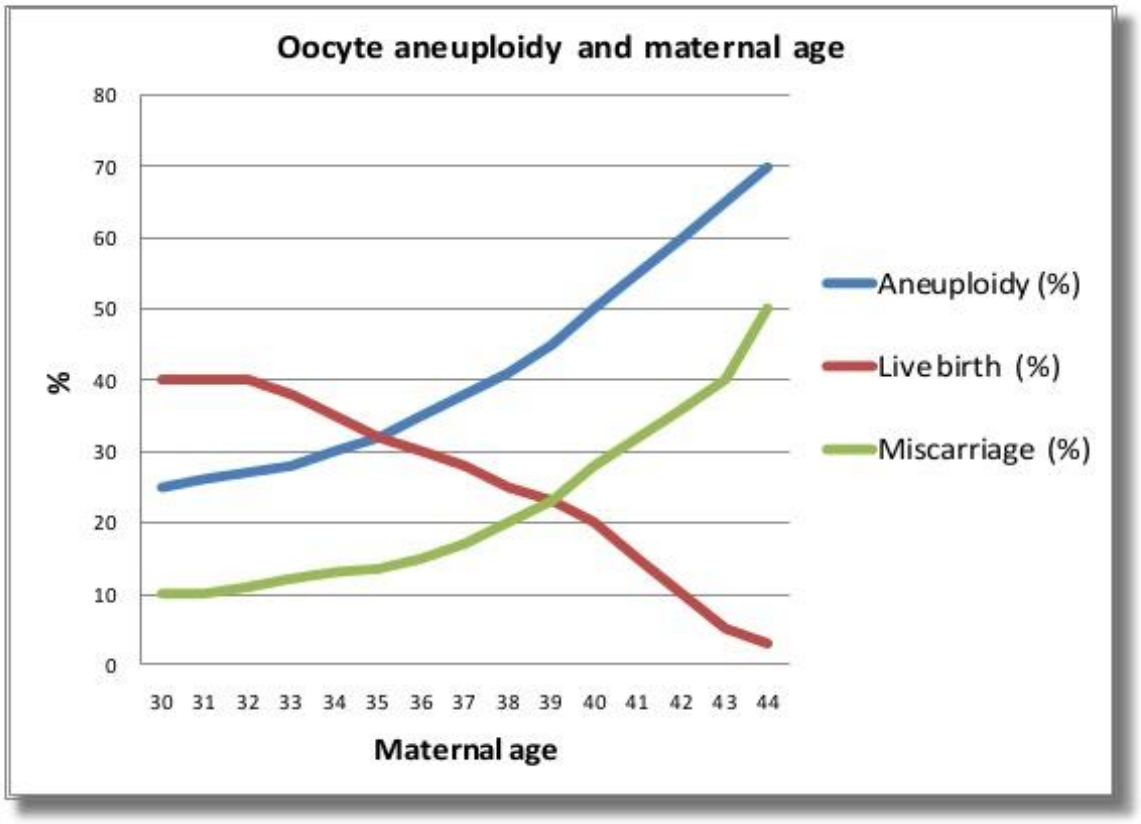
Class of Mutation	Mechanism	Frequency (Approximate)	Examples
Genome mutation	Chromosome missegregation	$2\text{-}4 \times 10^{-2}$/cell division	Aneuploidy
Chromosome mutation	Chromosome rearrangement	6×10^{-4}/cell division	Translocations
Gene mutation	Base pair mutation	10^{-10}/base pair/cell division	Point mutations
		10^{-5}-10^{-6}/locus/generation	



	Spontaneous miscarriages	Chromosomal abnormalities	Inherited birth defects
Preimplantation period	15% of clinically recognized pregnancies	50 – 70 %	
Implantation I. trimester	Early miscarriage	50 – 60 %	
II. trimester			
III. trimester	Late miscarriage	12 %	15%
Term	4 – 5 %	0,5 %	

The frequency of chromosomal abnormalities after birth		
General population	1 : 200	**0,5 %**
Infertile couples (spontaneous miscarriages, stillbirths)	1 : 48	**2 %**
Sterile couples	1 : 10	**10 %**

Chromosomal abnormalities in oocytes and maternal age



US CDC/SART data

Chromosomal abnormalities

Types according to origin:

- **constitutional** – abnormality is present in all cells of the body or in a part of cells (mosaicism)
- **acquired** – results from mutation in one cell in lifespan, then number of cells with mutation is increased by clonal development of orinal cell

Types according to the nature:

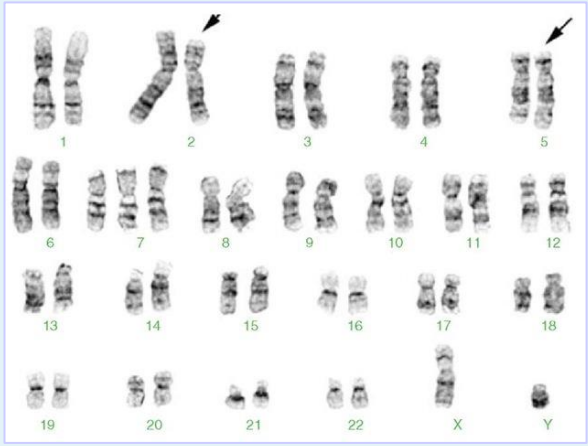
- **numerical** – change of the count of chromosomes *(polyploidy, aneuploidy)*
- **structural** – structural change or rearrangement of chromosome

Types according to inclusiveness of genome:

- **balanced –** structural rearrangement of chromosome, no gain or loss of chromosomal material
- **imbalanced** – loss or gain of chromosomal material

Types according to occurence:

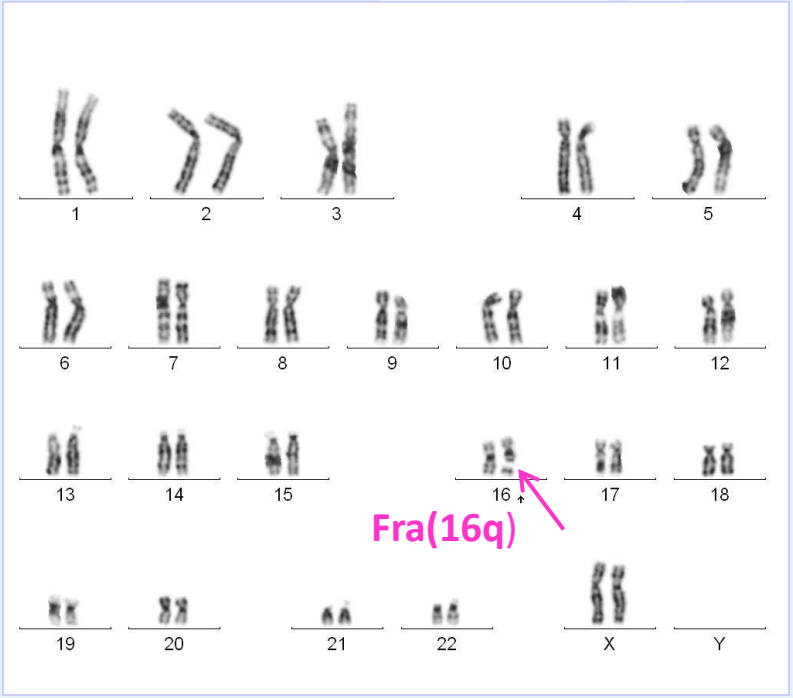
- **only one cell line –** just one cell line in all cells of the body *(47,XX, +21)*
- **mocaicism** – presence of more than 1 cell line *(46,XX/47,XXX, +21 ....)*



Polymorphism of chromosomes

- **Structural variants of chromosomes without phenotype effects**

- Polymorphic regions:
  a. **short arms, bridges and satellites of acrocentric chromosomes** (C- banding, NOR staining)
  b. **1qh ,9qh, 16qh, Yqh** – different size, inversion of heterochomatine in centromeric region (C-banding)
  c. **some inversions** – inv(9)(p12q13), inv(2)(p11.2q13)
  d. **fragile sites –** fra(16q) – normal variant



Numerical chromosomal abnormalities

Number of chromosomes is characteristic for biological spesies
– Homo sapiens: 46 in somatic cells, 23 in gametes

Haploidy – number of chromosomes in gamete (23)

Euploidy – normal number of chromosomes (46) in somatic cells

Polyploidy – multiples of haploid number of chromosomes

- **triploidy (69)**
- **tetraploidy (92)**

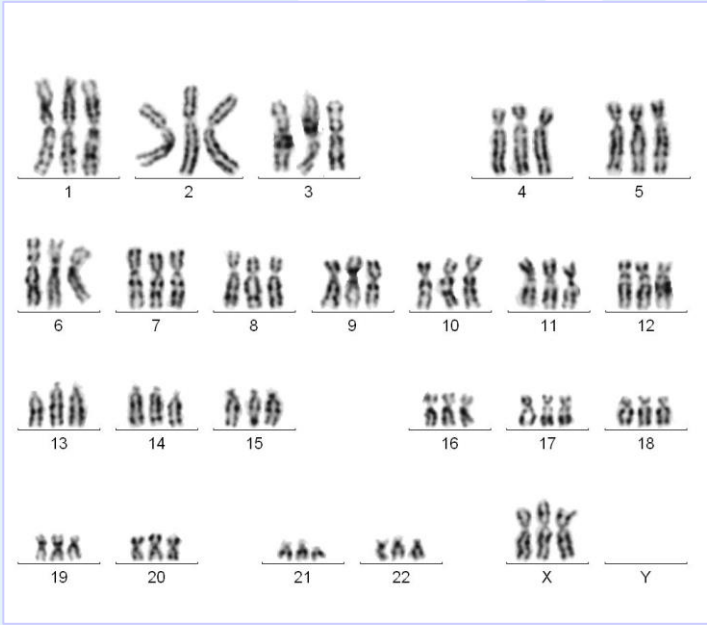
Aneuploidy – loss or gain of one or more chromosomes

- trisomy - Down syndrome 47N, + 21
- monosomy - Turner syndrome 45,X

Numerical abnormalities - Polyploidy

Triploidy: 69,XXX, 69,XXY, 69,XYY

- relatively often in spontaneous miscarriages, but survival beyond mid-pregnancy is rare. Only a few triploid live births have been described and all of them died soon after birth.
- Can be caused by:
  - **failure of meiotic division in ovum or sperm** (retenstion of a polar body or a formation of diploid sperm)
  - **fertilization of an oocyte by two sperm**



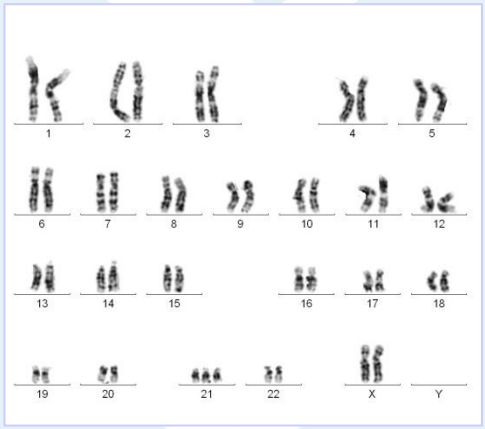
Effect of **„parent-of-origin“** with respect to human genome:

- an additional set of **paternal chromosomes**: the placenta is swollen (partial mola hydatidosa)
- an additional set of **maternal chromosomes** – placenta is small

Tetraploidy: 92,N

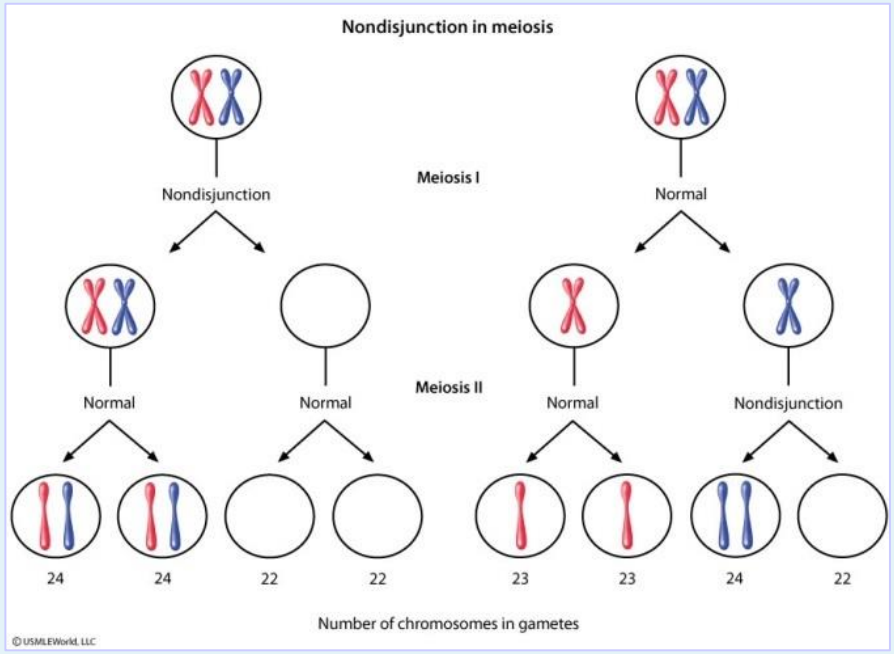
- present in spontaneous miscarriage

Numerical abnormalities - Aneuploidy



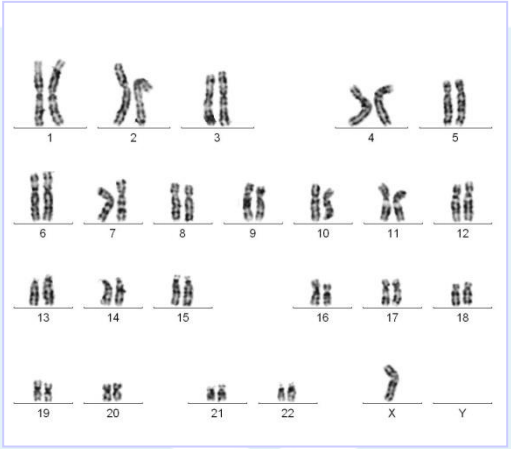
Trisomy – presence of an extra chromosome

- Trisomy **compatible with survival to term**:
  - *Down syndrome: 47, N, +21*
  - *Patau syndrome: 47,N, +13*
  - *Edwards syndrome: 47,N,+18*
- Most autosomal trisomies result in spontaneous miscarriage
- **Gonosomal trisomies** – presence of extra chromosome X or Y: **only mild phenotypic effect**



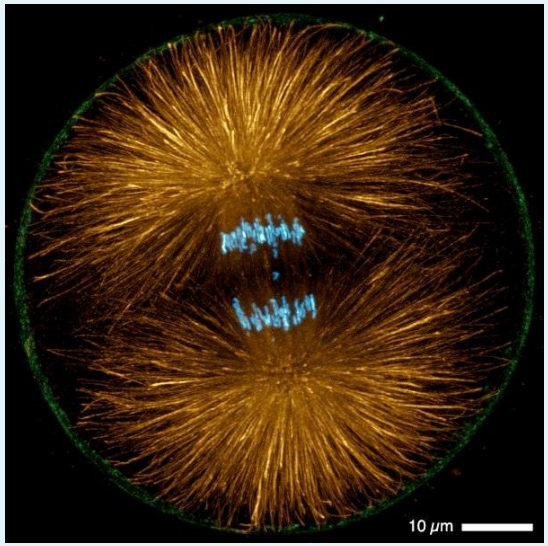
Origin of trisomy:
non-disjunction in meiotic division I or II

Numerical abnormalities - Aneuploidy



Monosomy – absence of a single chromosome

- Autosomal monosomy is almost always incompatible with survival to the term
- Lack of X or Y chromosome: Turner syndrome: 45,X
- Origin: **- non-disjunction in meiosis**
  - anaphase lag – loss of cxhromosome as it moves to the pole of the cell during anaphase



Parental origin of meiotic error leading to aneuploidy

Chromosome abnormality	Paternal (%)	Maternal (%)
Trisomy 13	15	85
Trisomy 18	10	90
Trisomy 21	5	95
45,X	80	20
47,XXX	5	95
47, XXY	45	55
47,XYY	100	0

Structural chromosomal abnormalities

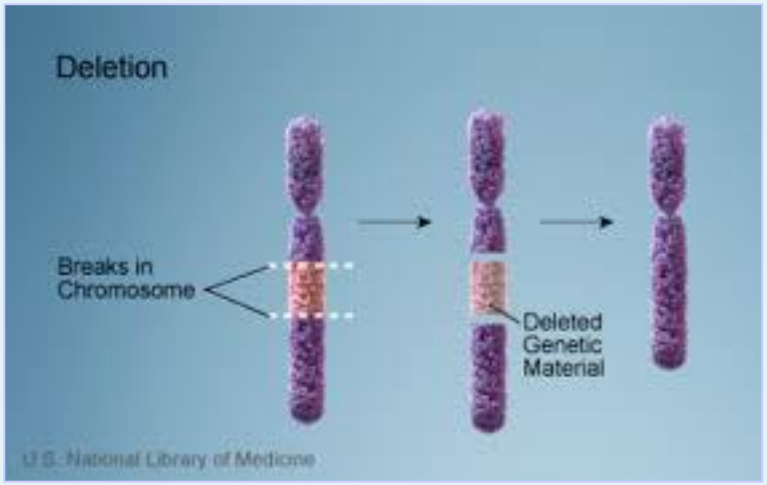
Mechanism of origin: one or more breaks and abnormal rearrangements in the structure of chromosomes

Frequency – **up to 4% - under physiological conditions**

- higher – activity of mutagens *(ionizing radiation, chemicals, viruses)*

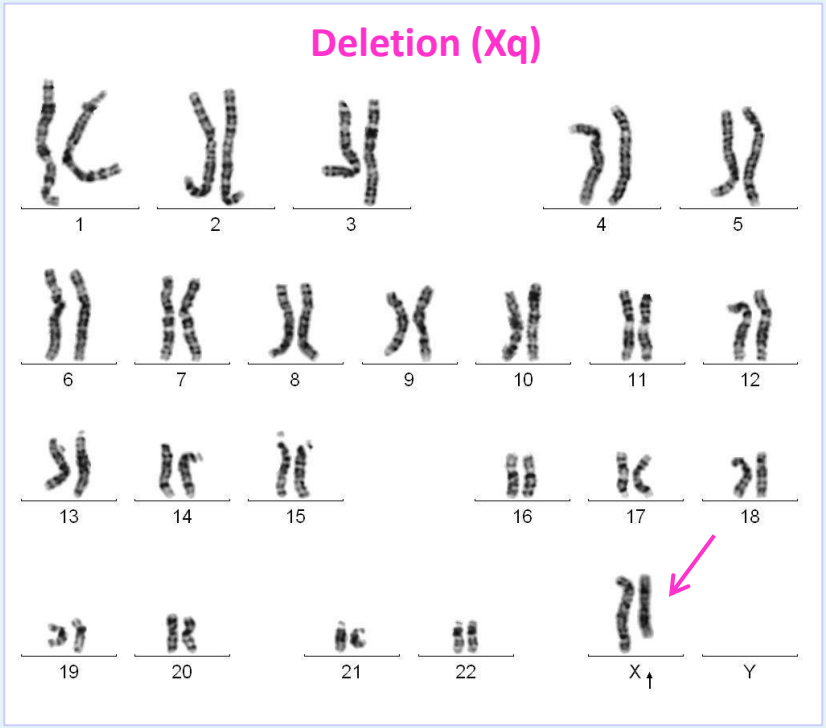
Types in respect to stability in the genome:

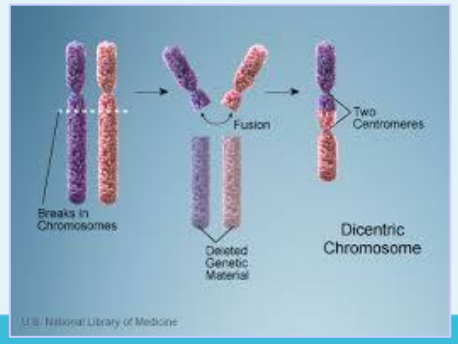
- **stabile** – going through normal cell division
  (deletion, duplication, inversion, insertion, isochromosomes, translocation)
- **non-stabile** – not going through normal cell division
  (dicentric, acentric and ring chromosomes, triradials and multiradials)

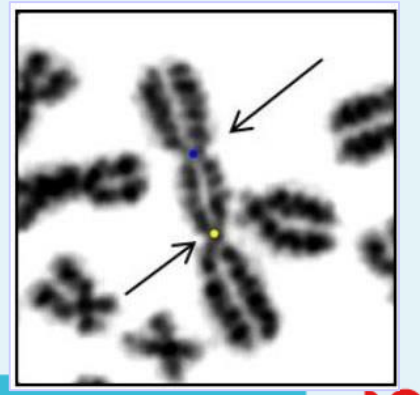


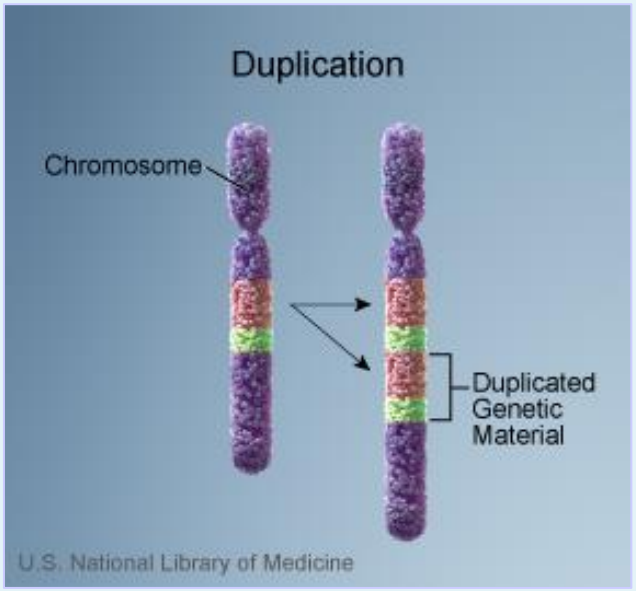
Deletion

- **a loss of a part of chromosome resulting in monosomy for that chromosomal segment**
- Large deletions – incompatible with survival to term
- 2 levels of deletions:
  a. **microscopic** – visible in microscope *(cri du chat syndrome...)*
  b. **submicroscopic microdeletions** – identified on prometaphase chromosomes and by FISH method *(Prader-Willi and Angelman syndromes, Di George syndrome ...)*
- High occurence after radiotherapy – „sticky“ ends of chomosomes resulting in formation of **dicentric chromosome**



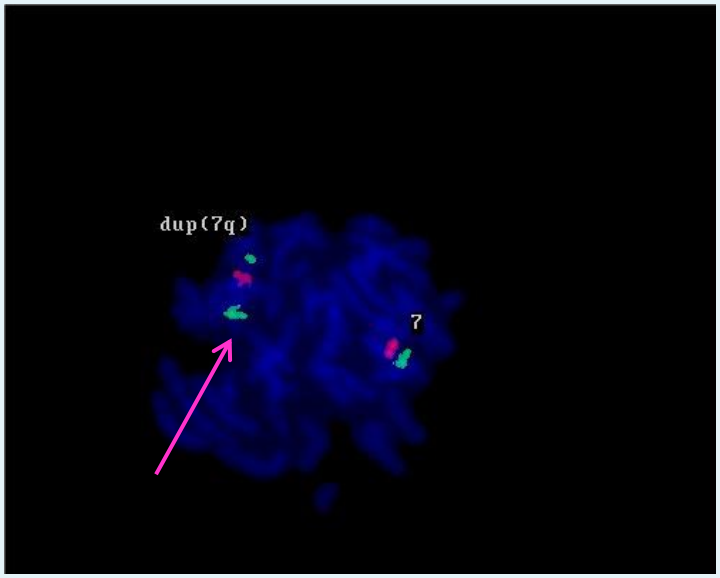


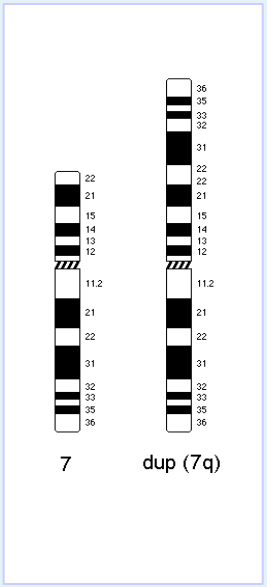




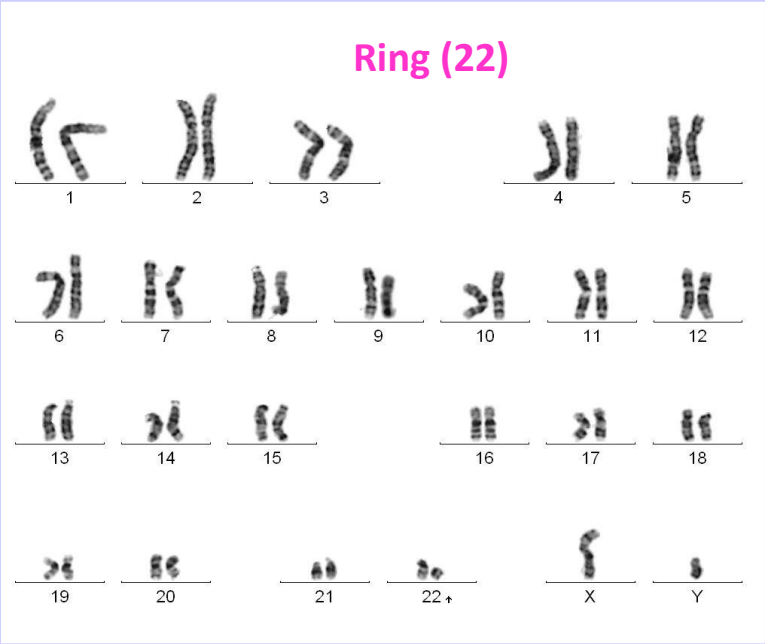
Duplication

- **a duplication of chromosomal segment resulting in partial trisomy**
- The result from unequal crossing-over of the genome where repeat sequences are found
- a duplication is less harmful than partial monosomy





dup (7q)

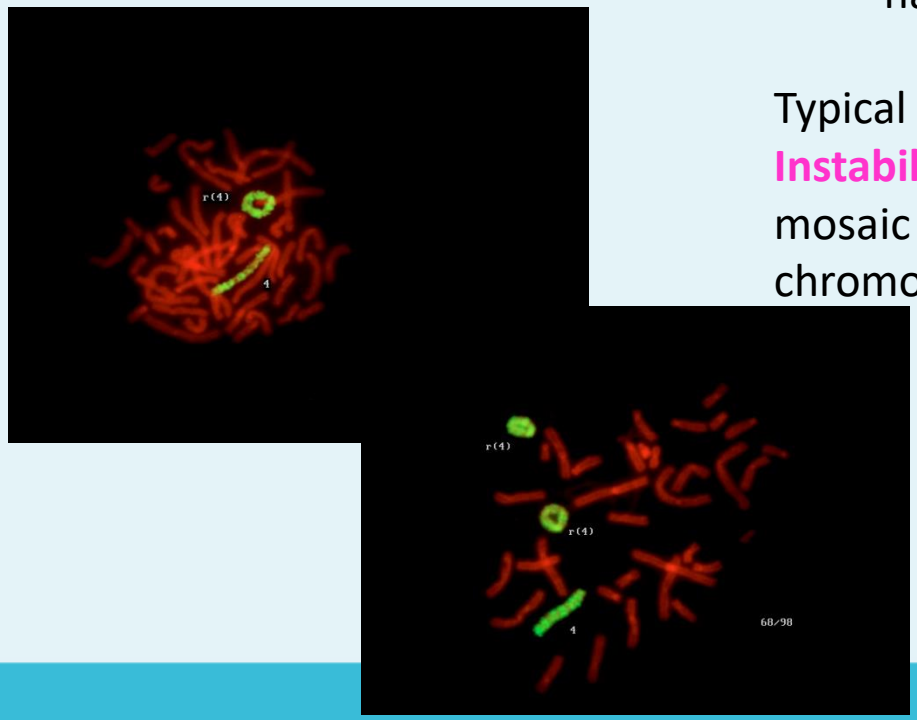


Ring chromosome

2 types of ring chromosome:

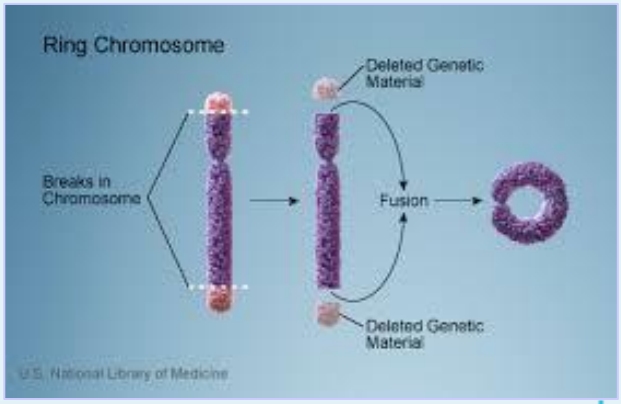
a. **ring with distal deletion** – arise by two breaks on both ends of chromosome and two „sticky“ ends reunite as ring chromosome. Two distal fragments are lost.

b. **ring with asociated chromosomal ends** – without deletion, phenotype is usually less harmful

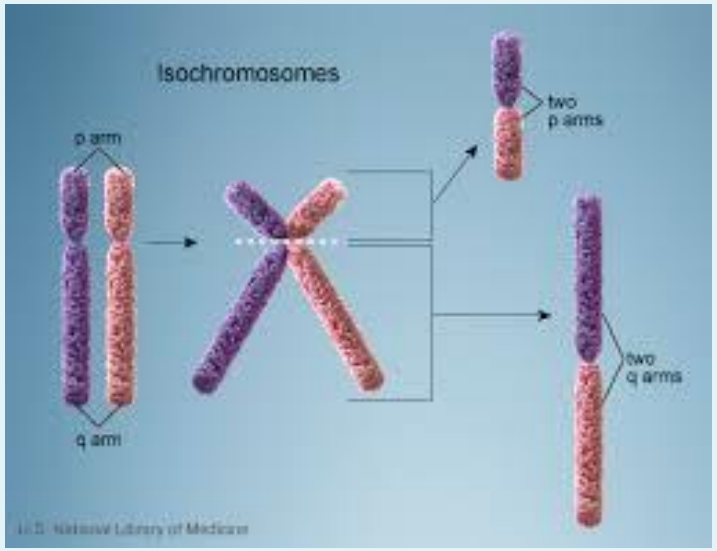


Typical feature of ring chromosome:

Instability during mitotic division of cell – results is mosaic occurence of cell lines with and without ring chromosome

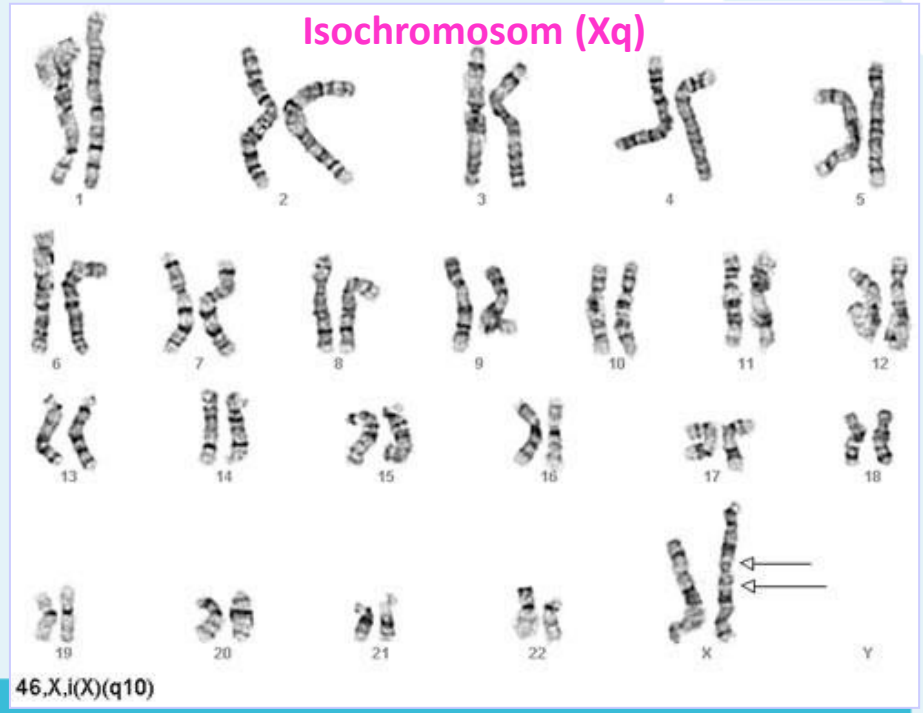


Isochromosome



- **a loss of one arm with a duplication of the other**

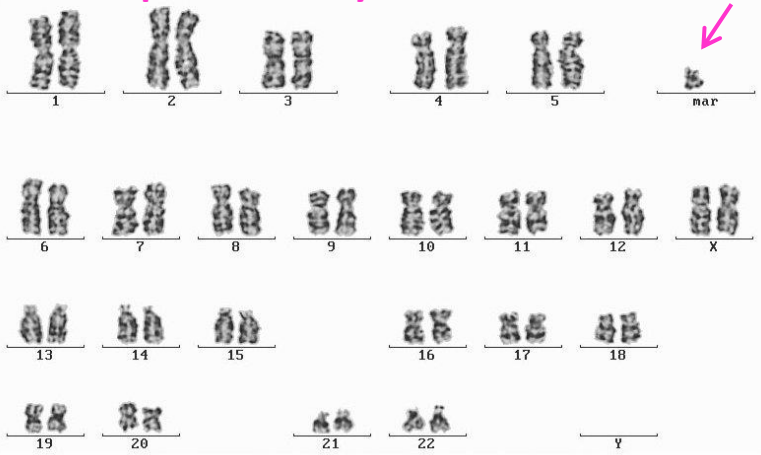
- It results from the transverse division of centromere (not longitudinal)
- i(Xq) – most often occuring ring chromosome in humans (15% of all cases of Turner syndrome)



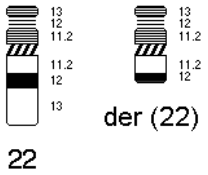
Small supernumerary marker chromosome (SMAC)

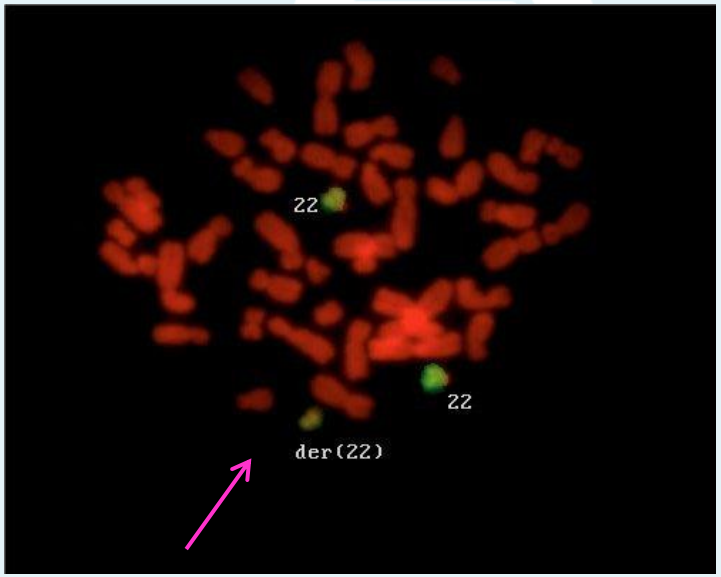
- **a presence of extra small sized chromosome of unknown origin**
- the size is very small and we need FISH technique for identification of its origin
- SMAC is derived from chromosome 15 in 60% of all cases
  (unstable repeated sequences below the centromere), mostly without encoding sequences – no phenoptype effect)
- when encoding genes are present – phenotype effect and mental retardation

Small supernumerary marker chromosome



Derived chromomosome





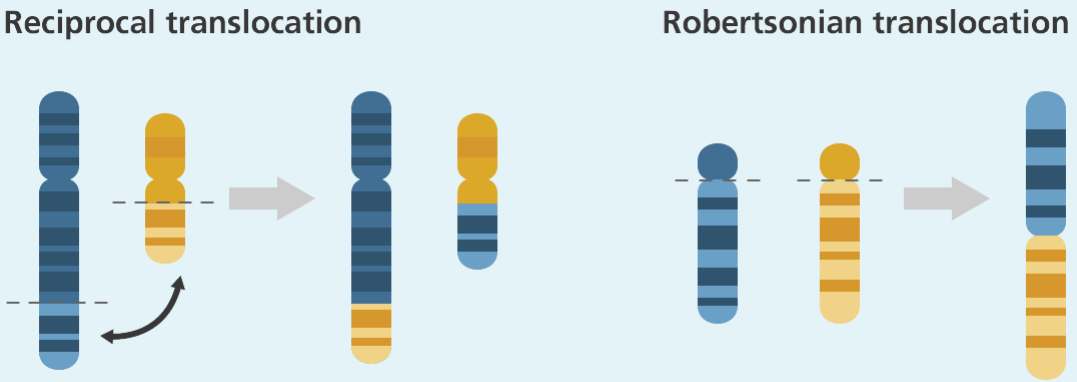
Translocations

Transfer of genetic material from one chromosome to another, chromosome number remains at 46

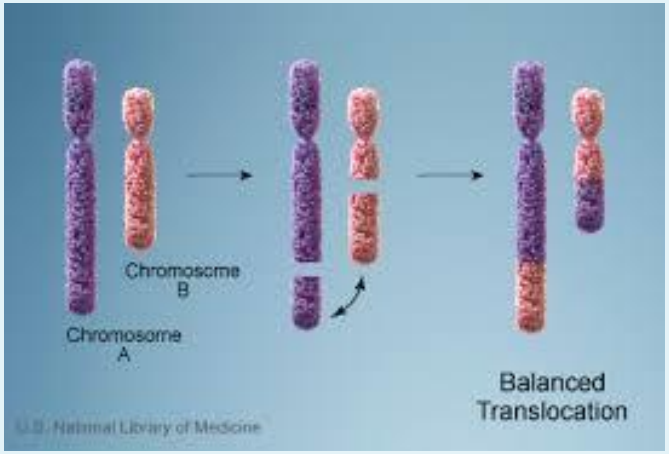
Types of translocations – **reciprocal** – unique for particular family except of t(11;22)

- **Robertsonian**

Overal incidence – **general population 1 : 500**
stillbirths, infertile couples - higher

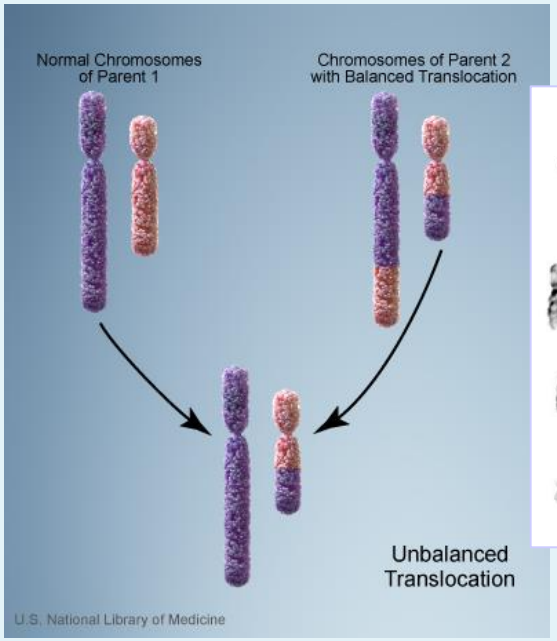


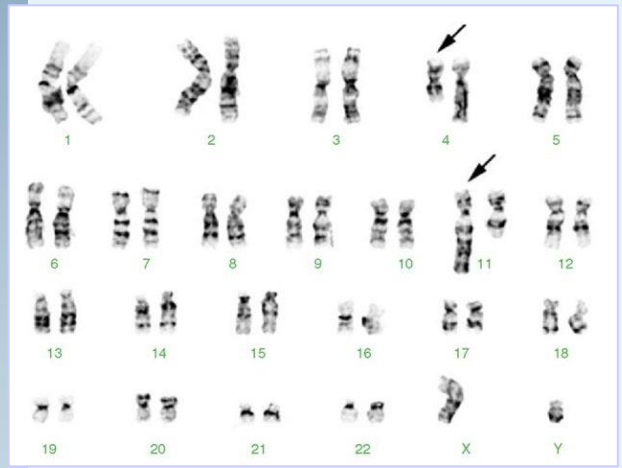
Reciprocal translocations



Two broken off chromosome pieces of non-homologus chromosomes are exchanged

- **when entire genetic material is present** – **balanced translocation,** usually no phenotypic effect (physical or mental). Some children with inherited developmental defects have balanced translocation at microscopic level, but at DNA level there is missing genetic material (aCGH tests) or disrupted important gene by a break.





- **Unbalanced translocation** – incorrect amount of chromosomal material on particular chromosome, clinical effects usually serious
- **Problems occur in gamete formation**

Segregation of reciprocal translocations

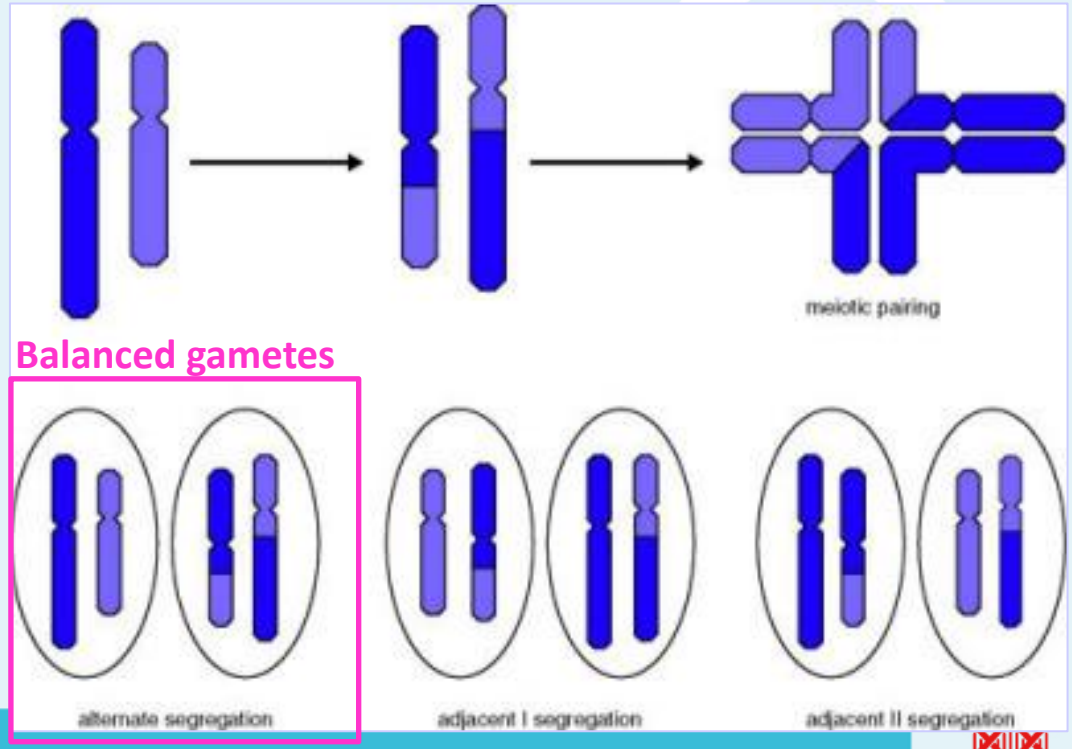
Segregation of translocation – behavior of translocation at meiosis

Problems occur in gamete formation – chromosomes cannot pair normally to form bivalents. We recognize the segregation 2:2 alternate, 2:2 adjacent -1 and 2:2 adjacent-2 and also 3:1 segragation.

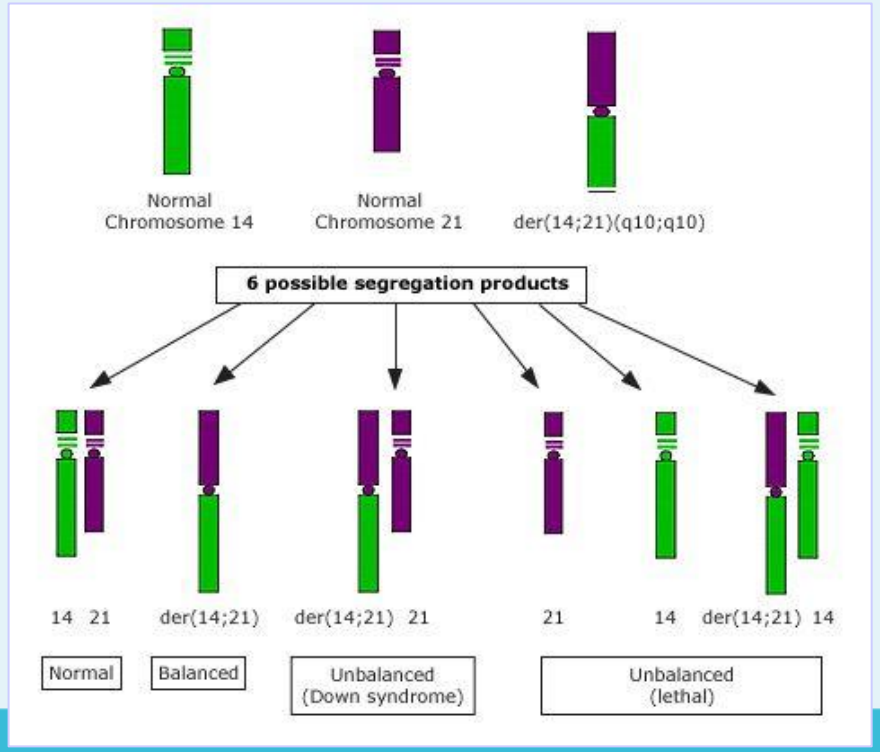
- **generation of significant chromosome imbalance** - *it leads to early pregnancy loss (unsuccessful implantation of embryo, spontaneous miscarriage, birth of infant with multiple abnormalities*
- **infertility of persons with balanced translocation**

Segregation of reciprocal translocation leads to 16 different combinations:

- **2 balanced gametes** – with translocation and without translocation
- **additional 14 imbalanced gametes** with unbalanced translocation



t(13;14)(q10;q10)



Robertsonian translocations

- Results from breakage of two acrocentric chromosomes (13, 14, 15, 21, 22) at or close to their centromere with subsequent fusion of their long arms to form one chromosome. Short arms are lost without any phenotype effect. Number of chromosomes is 45.
- **Individual is clinically normal** = translocation carrier
- Unbalanced products of conception may cause chromosomally abnormal baby, miscarriage, stillbirth, infertility
- Other family member should be offered karyotype examination for carrier status

Risks in translocation for a patient

Reciprocal translocation

- When counseling a carrier of a balanced translocation, it is necessary to consider the particular rearrangement to determine whether it could result in the birth of an abnormal baby
- **Risk for a term of abnormal baby: 1 – 10% for any translocation**, 5% - for a carriers of t(11;22)

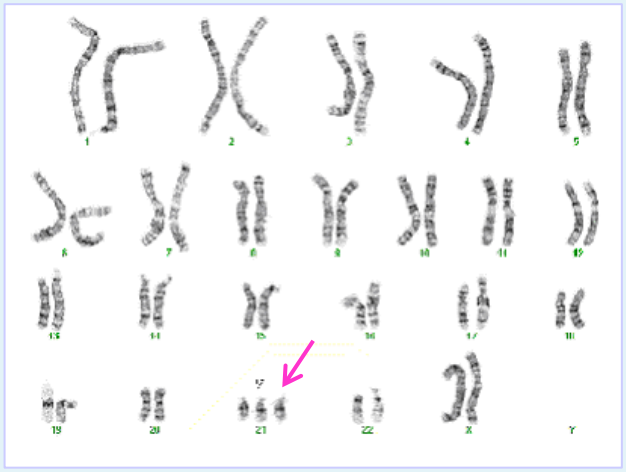
Robertsonian translocation

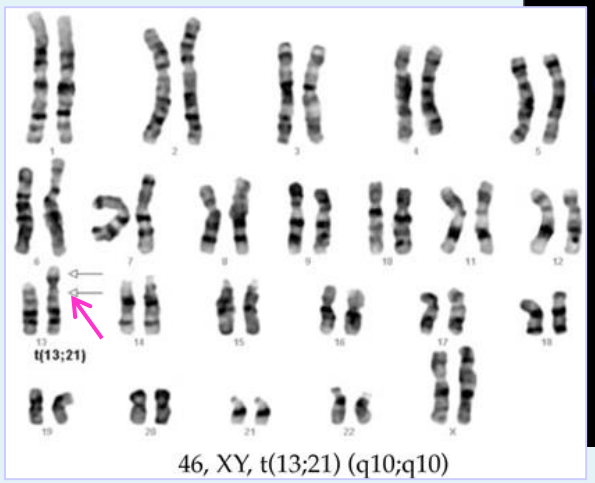
- The risk **for a term a baby with Down syndrome:**
  - when **female is a carrier** of t (13q;21;) or t(14q;21;) – **10%**
  - when **male is a carrier** of t (13q;21;) or t(14q;21;) – **1 - 3%**
  - **for a carrier of t(21q;21;) – 100%**

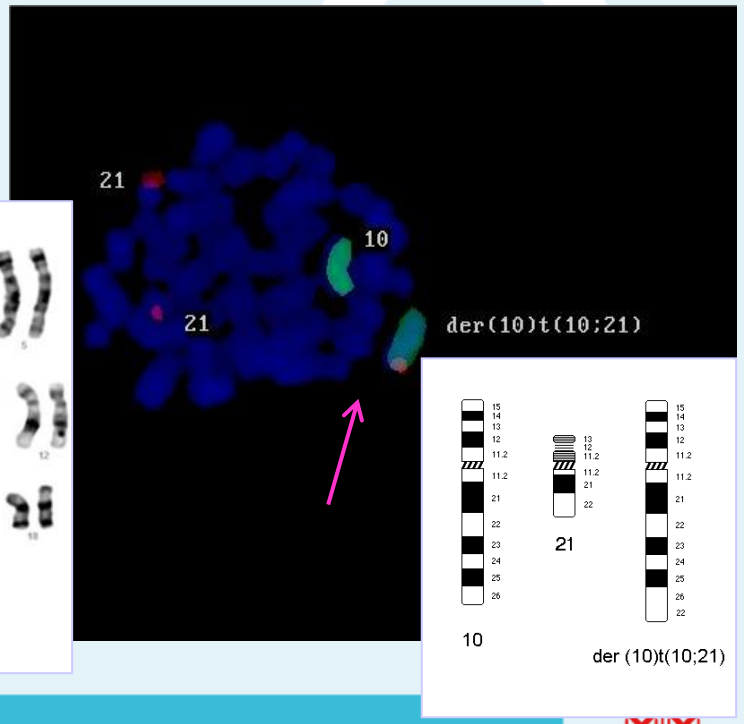
Down syndrome

- **Trisomy 21 (presence of 3 copies of segment 21q in genome:**

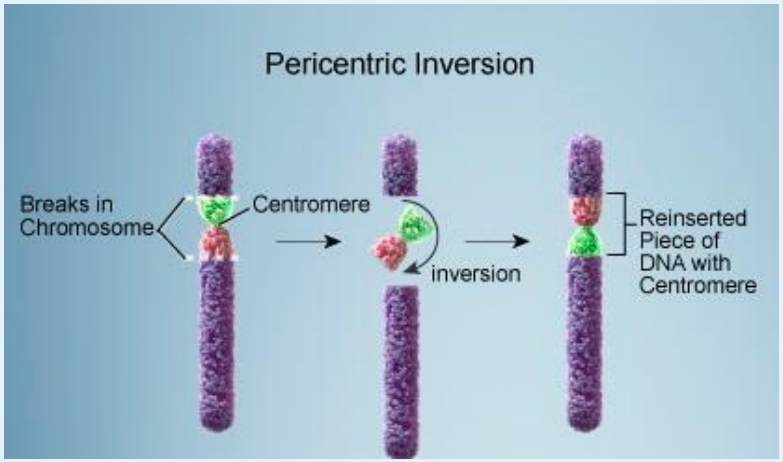
  - **pure trisomy of chromosome 21** – 85% of children with DS
  - **translocated chromosome 21** – Robertsonian translocations, reciprocal translocation between chromosomal segment 21q and other chromosome
  - **mosaic form** – 47,N, +21/46,N – 10% of cases







Inversions

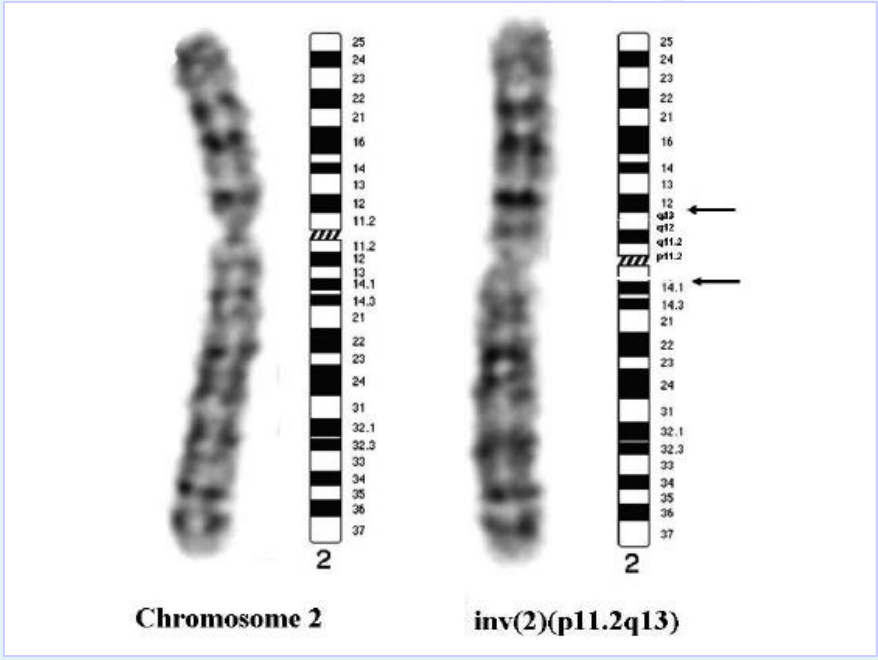


A two-break rearrangement involving a single chromosome in which a segment is inverted (reversed position)

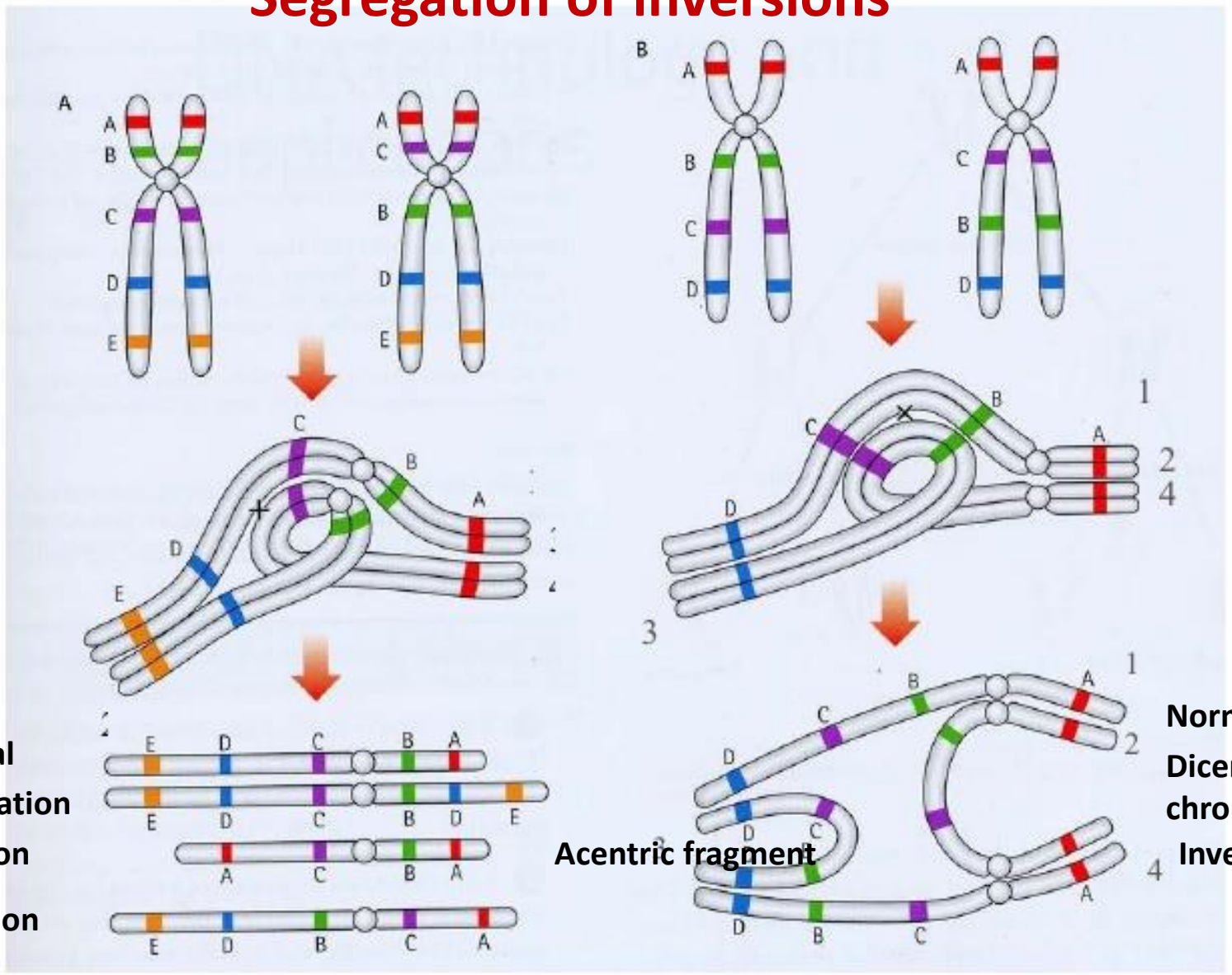
Pericentric inversion – inverted segment contains centromere

- Changed length ratio of p and q arms

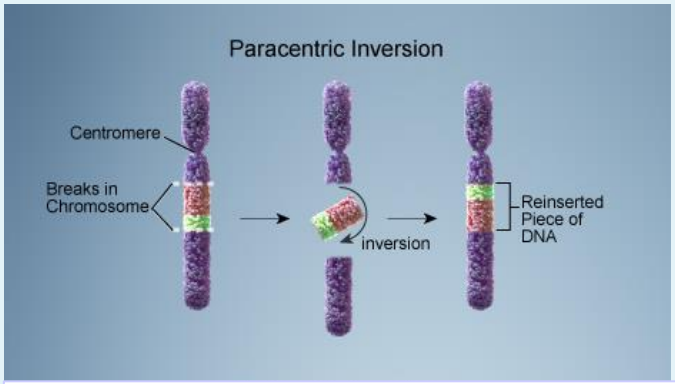
- **Clinical impact on next generation** - production of gametes:
  - normal gamete – no inversion
  - gamete with inversion
  - gamete with partial deletion
  - gamete with partial duplication

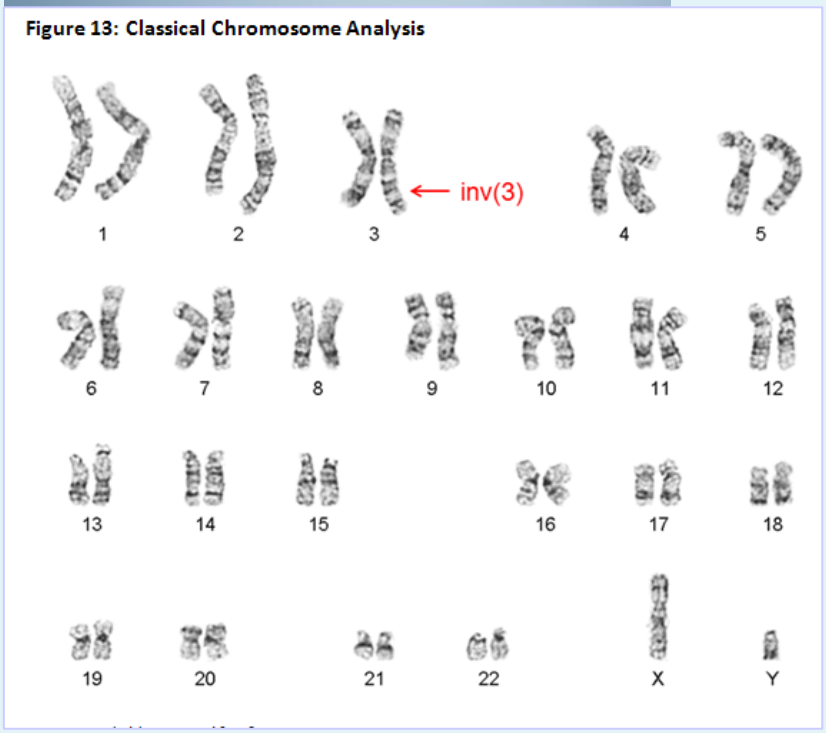


Segregation of inversions



Inversions

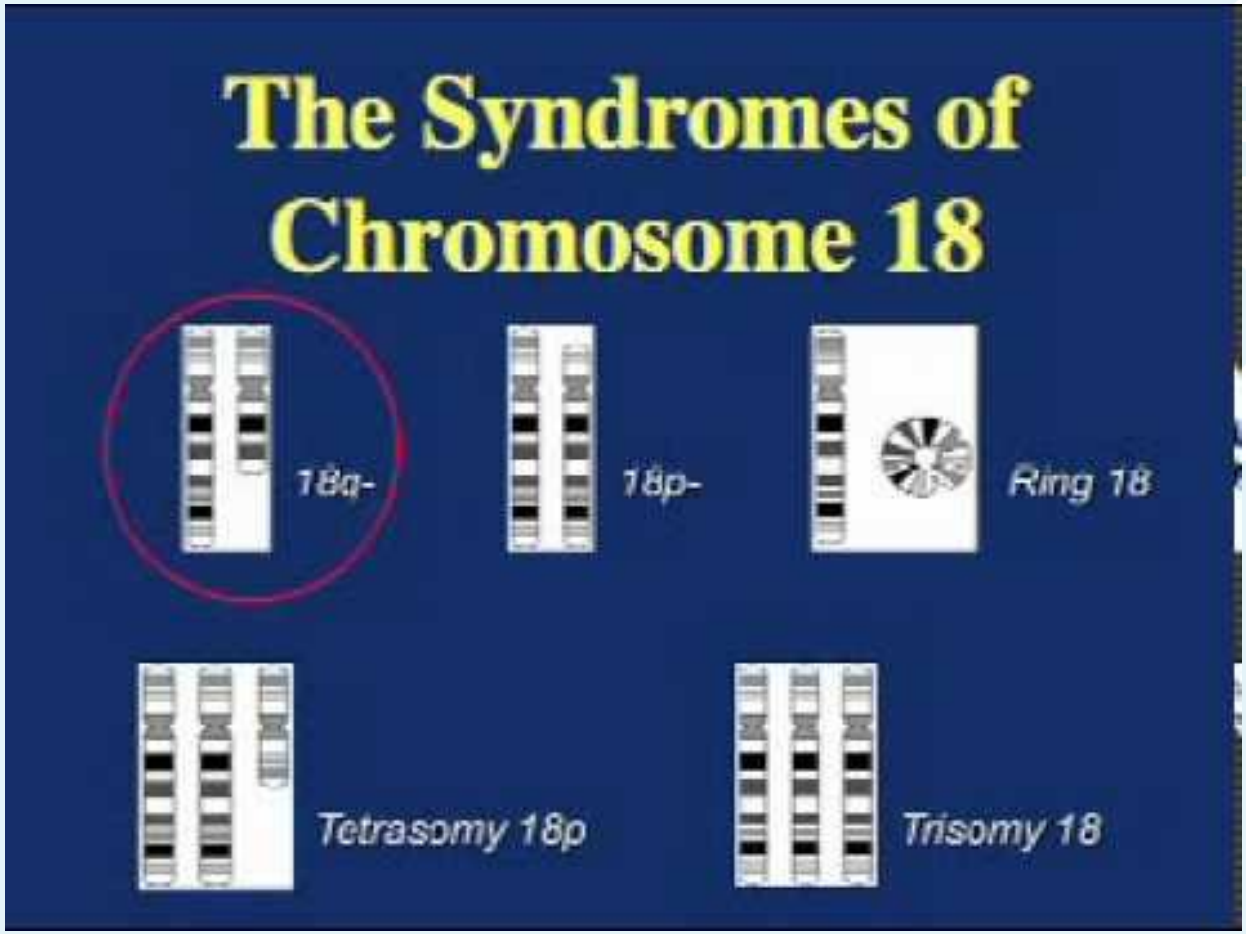


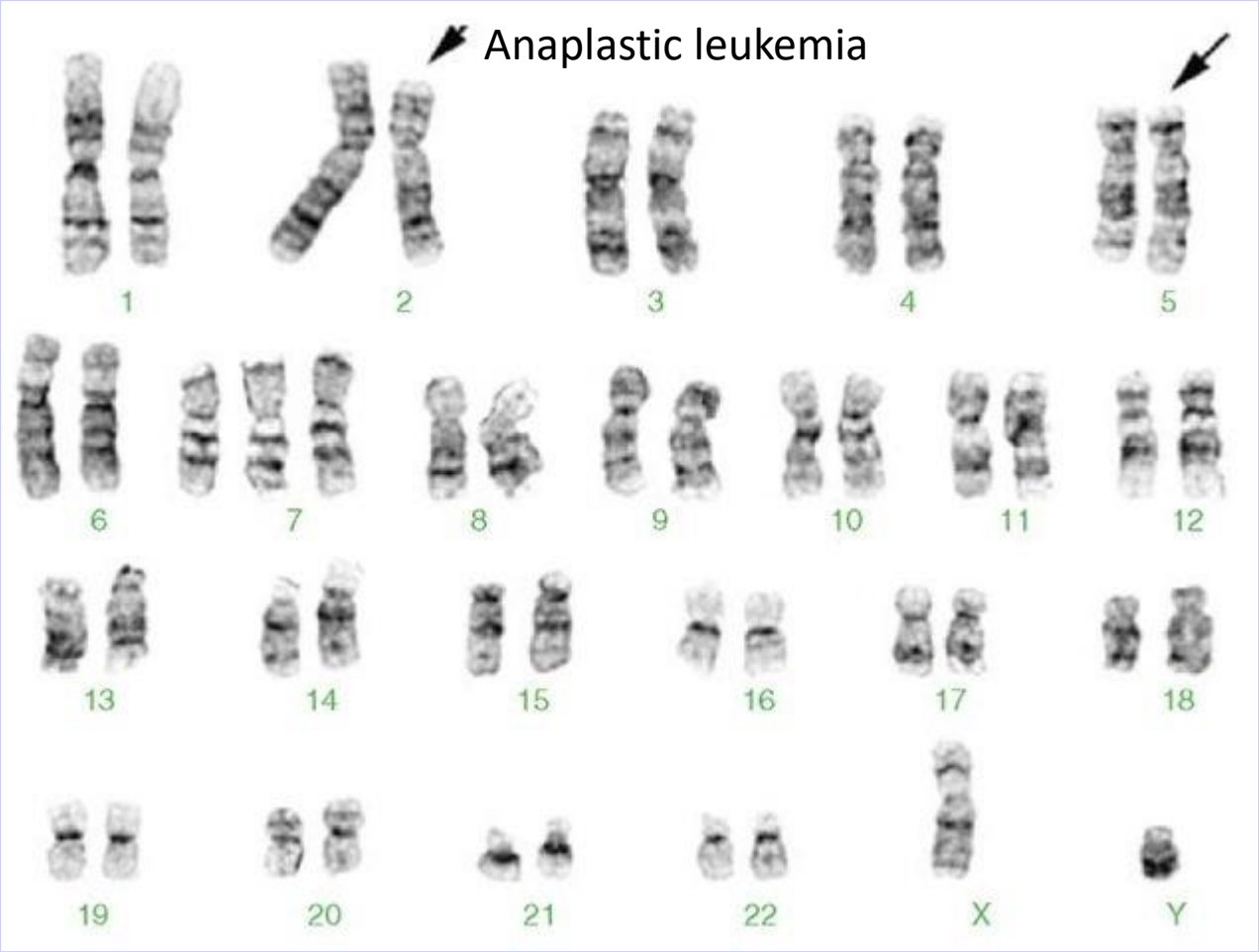


Paracentric inversion – **inverted segment doesn´t contain a centromere**

- Length ratio of p and q arms is not changed

- **Clinical impact on next generation** - production of gametes:
  - normal gamete – no inversion
  - gamete with inversion
  - gamete with dicentric chromosome
  - gamete with acrocentric fragment


The Syndromes of Chromosome 18
18q-
18p-
Ring 18
Tetrasomy 18p
Trisomy 18



Hematological malignancies, tissue tumors – often present many numerical and structural chromosomal abnormalities, progression of disease – more and severe imbalances

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Thank you for your attention

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