Introduction to Clinical Cytogenetics

1/23/03

Joan H.M. Knoll, Ph.D., FACMG, FCCMG
Children's Mercy Hospital
School of Medicine
University of Missouri-Kansas City

What is Cytogenetics?

- study of chromosomes; for clinical cytogenetics, it is applying the study of chromosomes to clinical medicine (diagnosis - postnatal, prenatal, cancer; gene mapping)

What types of tissues can be used to study chromosomes?

- for routine chromosome analysis, cells must have a nucleus, be viable at time of collection and capable of undergoing cell division (cells are grown in culture)

- for certain molecular cytogenetic applications, cell division is not a requirement

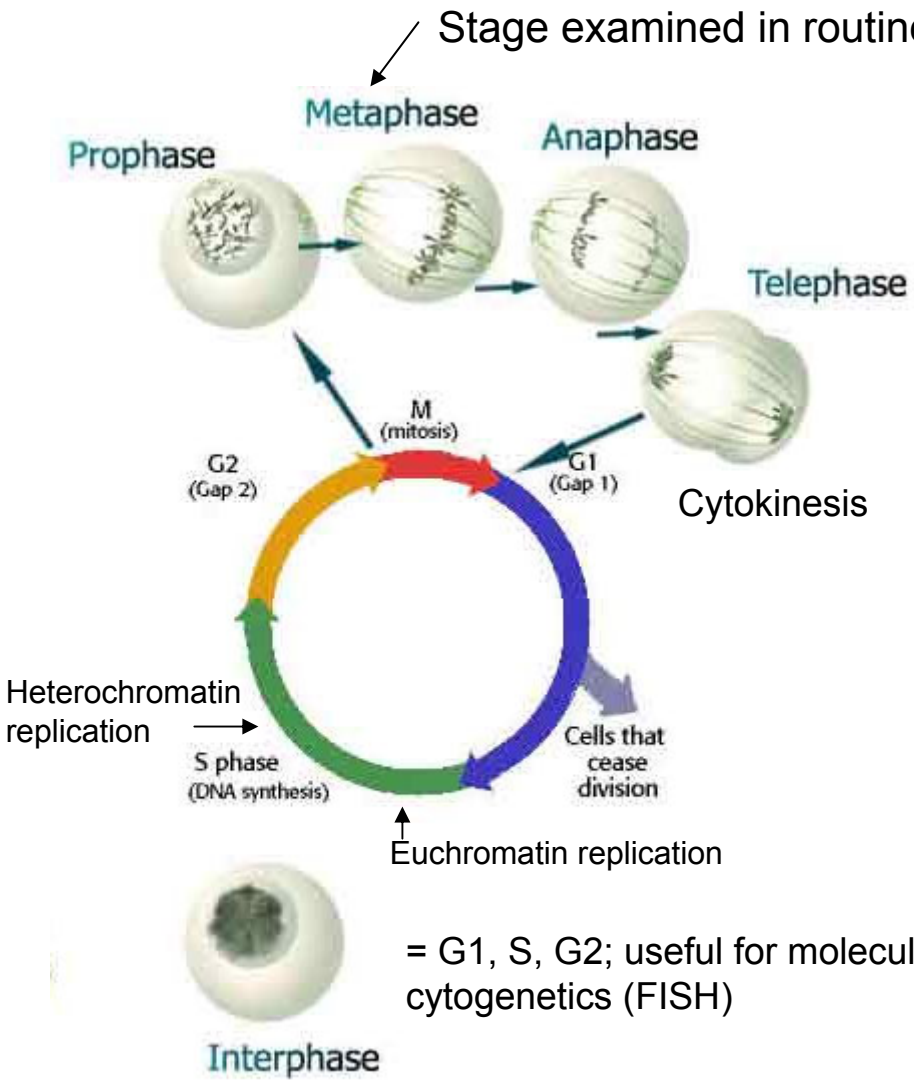
Why perform clinical cytogenetic testing?

- fertility problems – couples with history of infertility or habitual pregnancy loss; females with amenorrhea
- malformed fetus; stillbirths and neonatal deaths where a cytogenetic basis is likely
- pregnancy in women of advanced age
- family history in first degree relatives
- dysmorphology, problems of early growth and development (FTT, DD, Dysmorphic facies, MR, ambiguous genitalia, multiple malformations including cardiac anomalies
- Neoplasia (can be diagnostic or prognostic)

Frequency of Chromosome Abnormalities

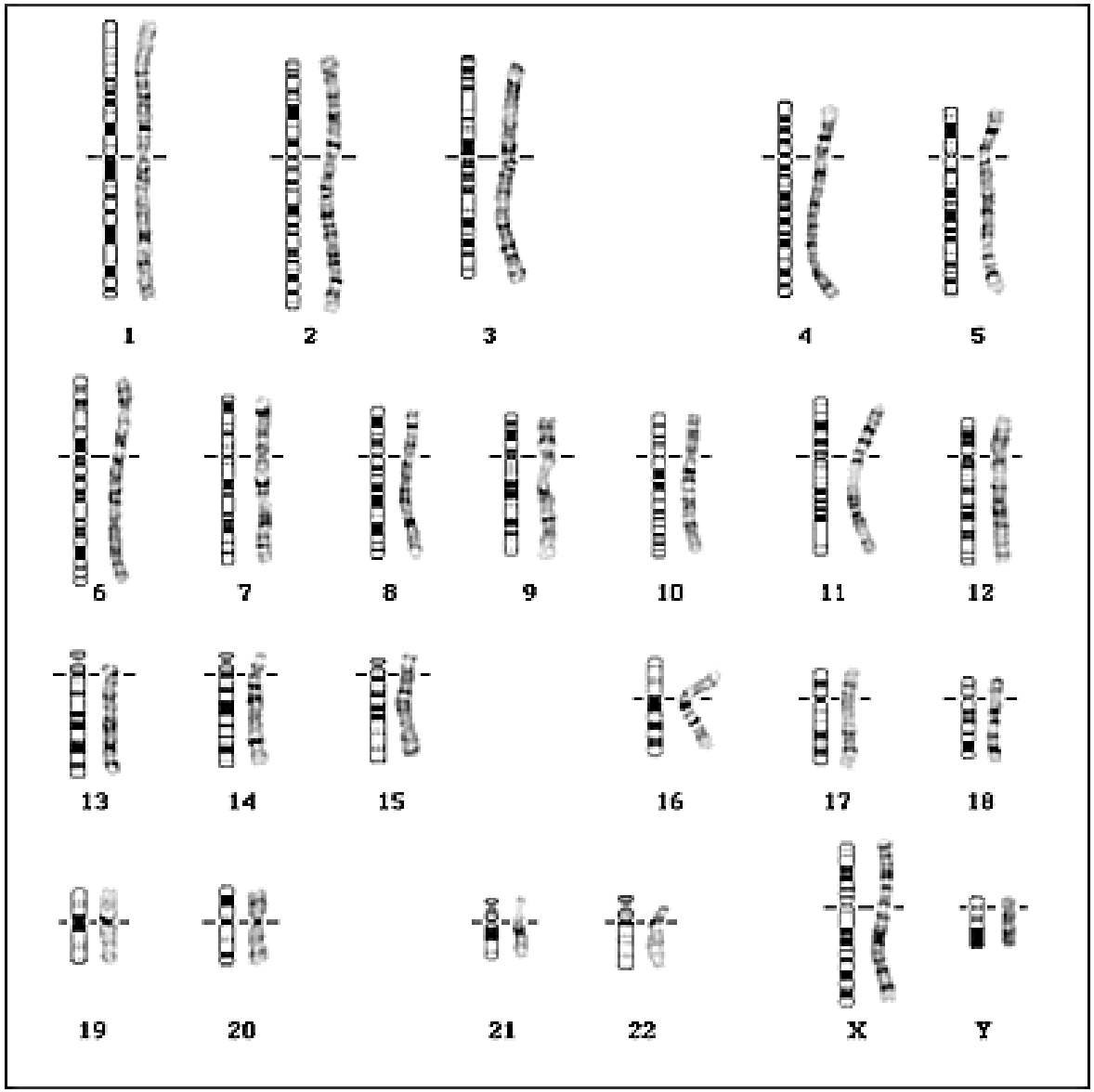
- ½ to 1% of all live births
- 2% of recognized pregnancies in women > 35 y.o.
- 10% of stillbirths
- ½ of recognized spontaneous pregnancy losses
- high in neoplasias (type dependent)

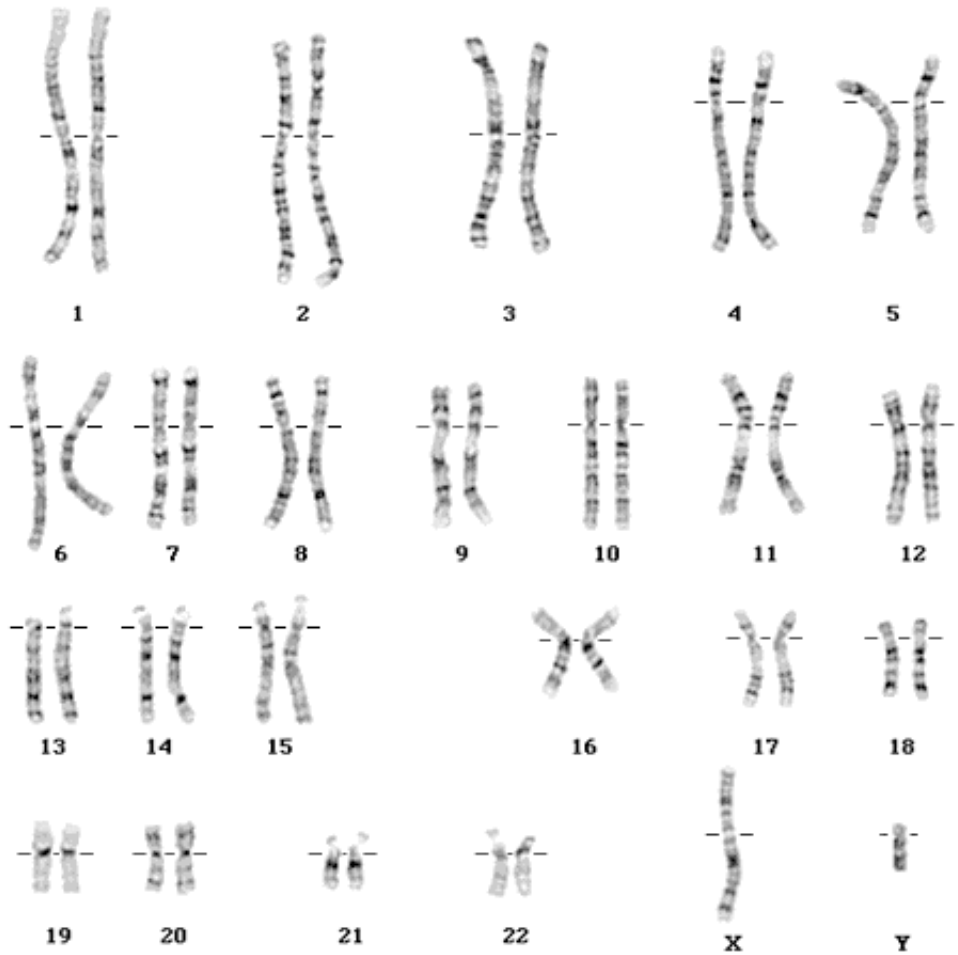
Mitotic Cell Cycle:



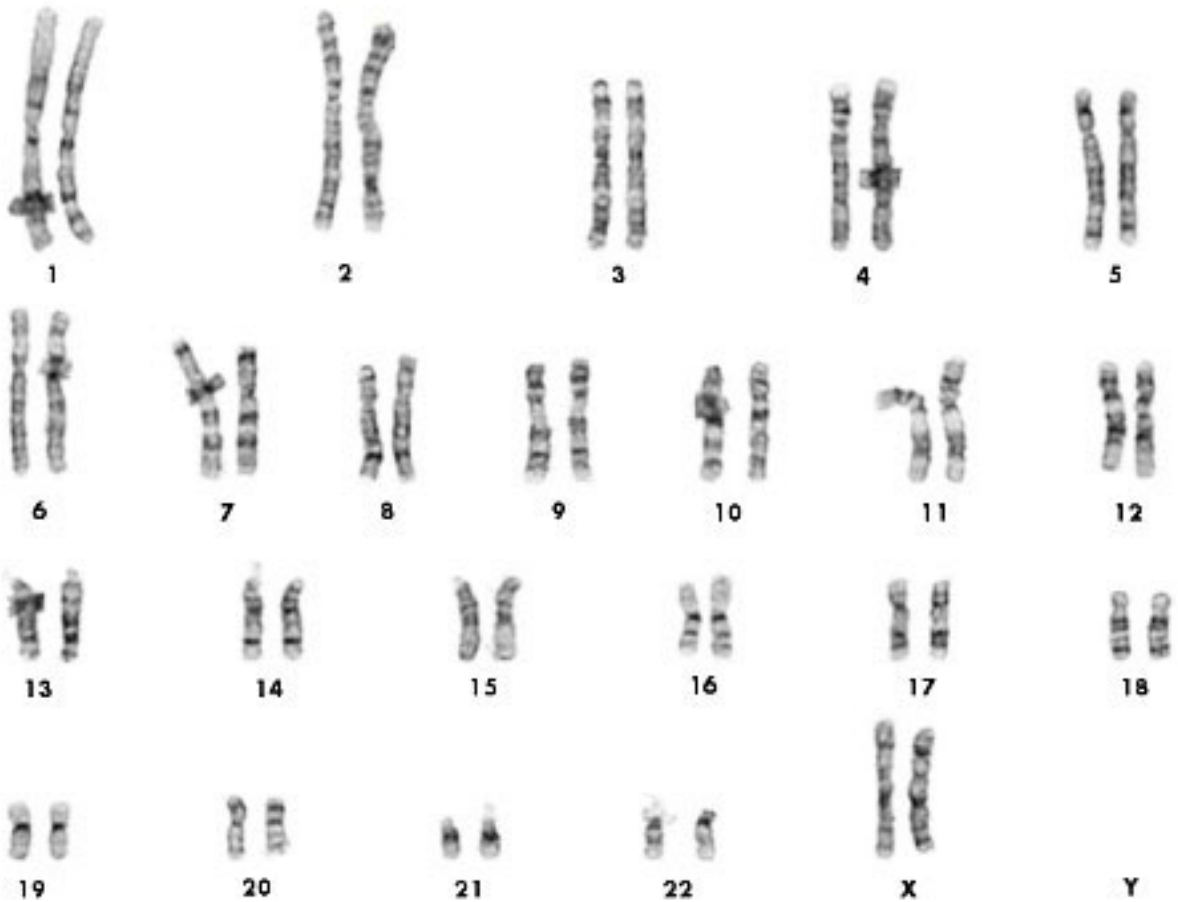
= G1, S, G2; useful for molecular cytogenetics (FISH)

- G1- cell grows and performs normal metabolism; organelles duplicate (~10-12 hours)
- S – DNA replication and chromosome duplication (~6-8 hours)
- G2 – cell grows and prepares for mitosis (~2-4 hours)
- M – mitosis – chromosomes are maximally condensed (~ 1hour)
- transitions between phases mark crucial regulatory checkpoints and are often rate limiting


1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
X
Y

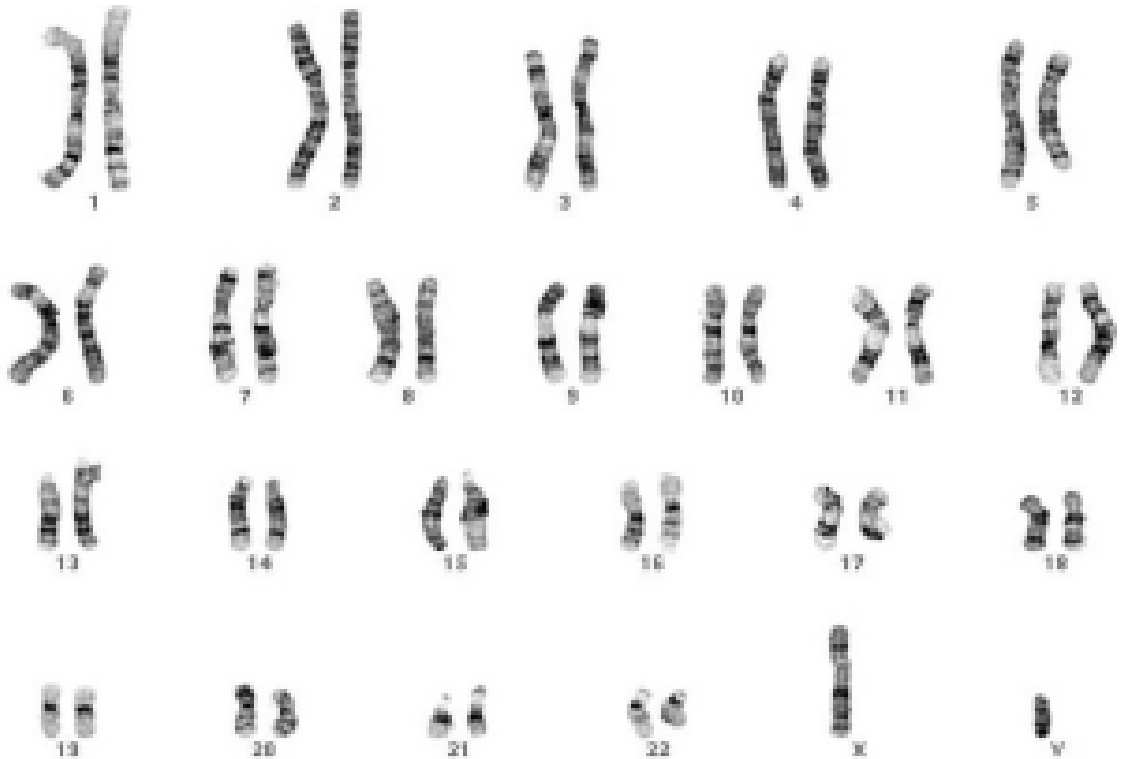

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
X
Y

Normal Female karyotype



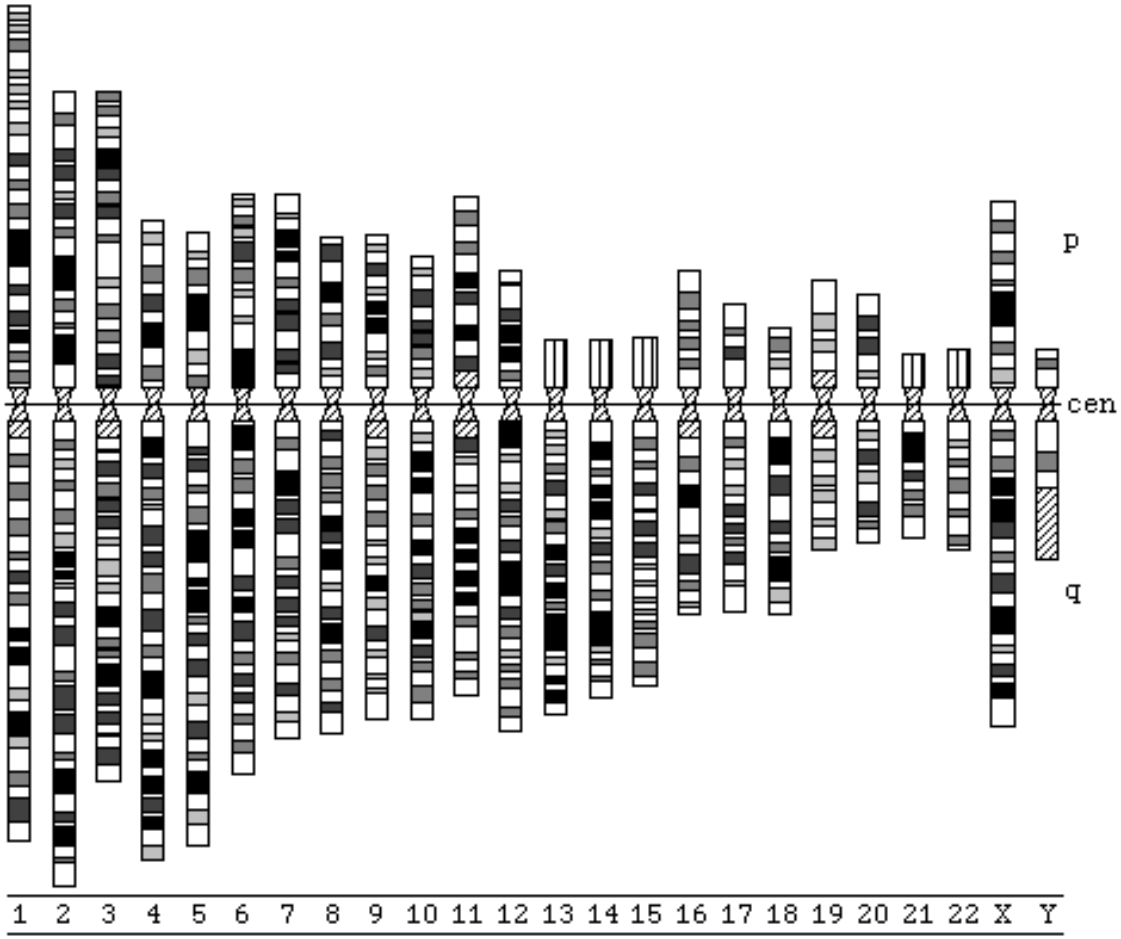
From Children's Mercy Hospital Cytogenetics Laboratory

Normal Male karyotype



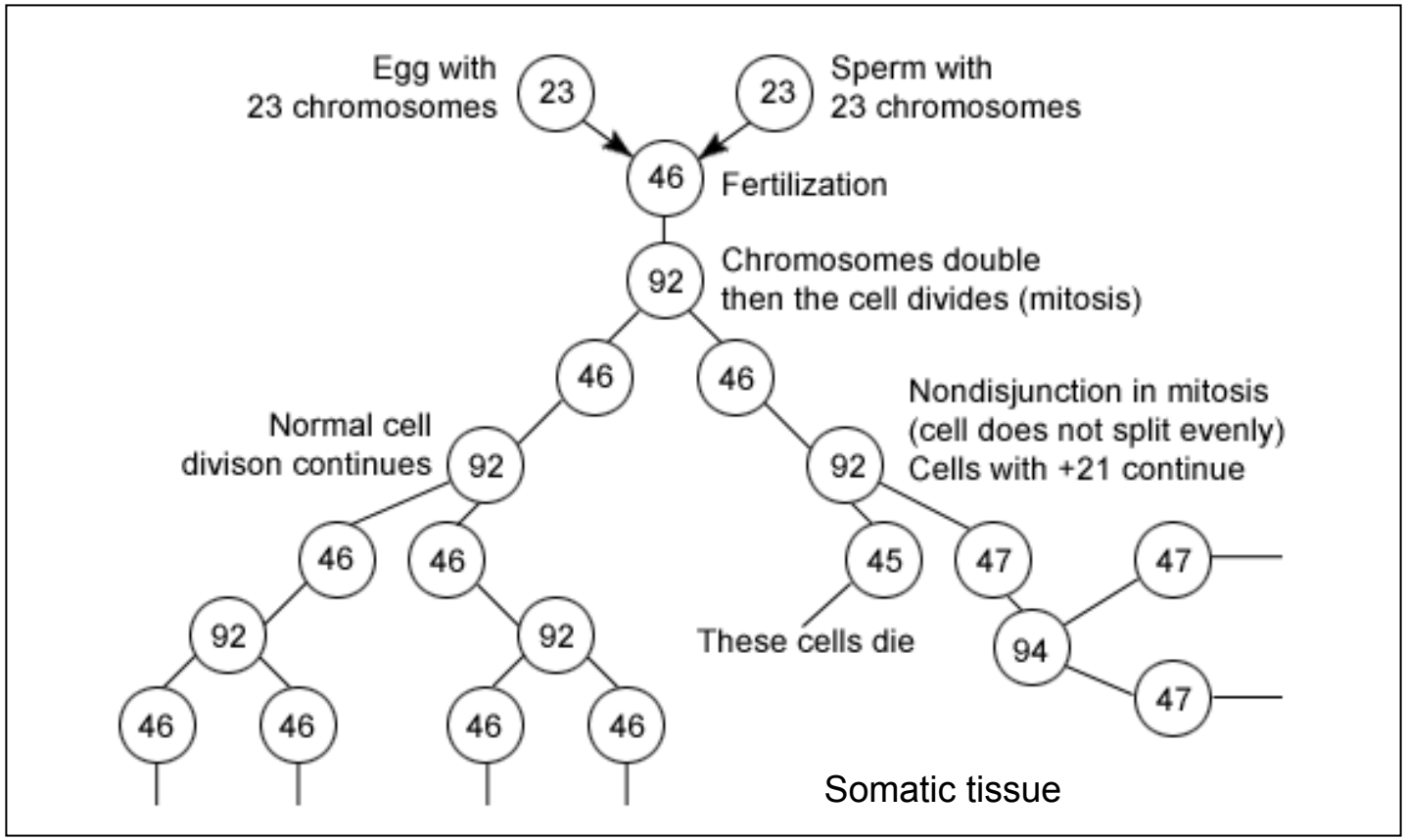
From Children's Mercy Hospital Cytogenetics Laboratory

Ideogram: Human Chromosomes



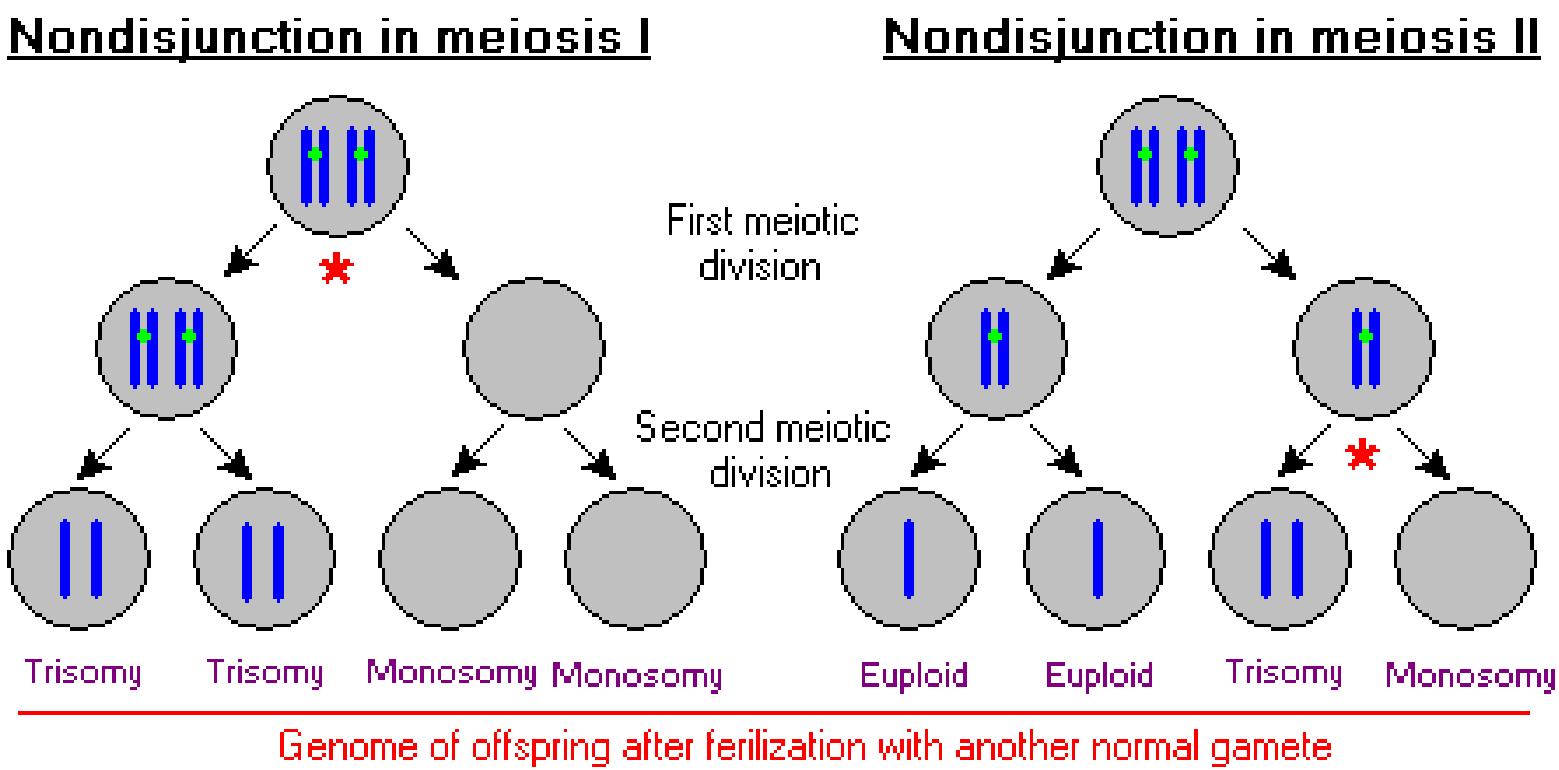
Chromosomal Mosaicism:

One or more cell lines in an individual derived from a single zygote

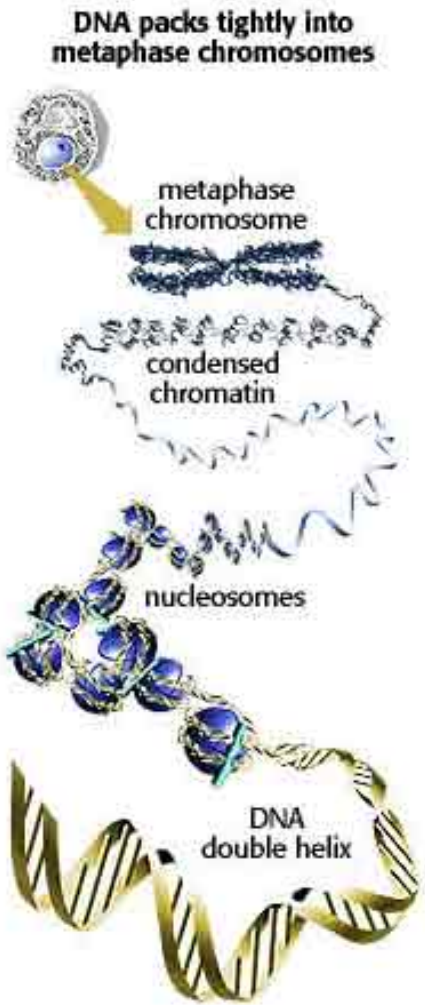


Eg. **47,XX,+21/46,XX or 47,XY,+21/46,XY**

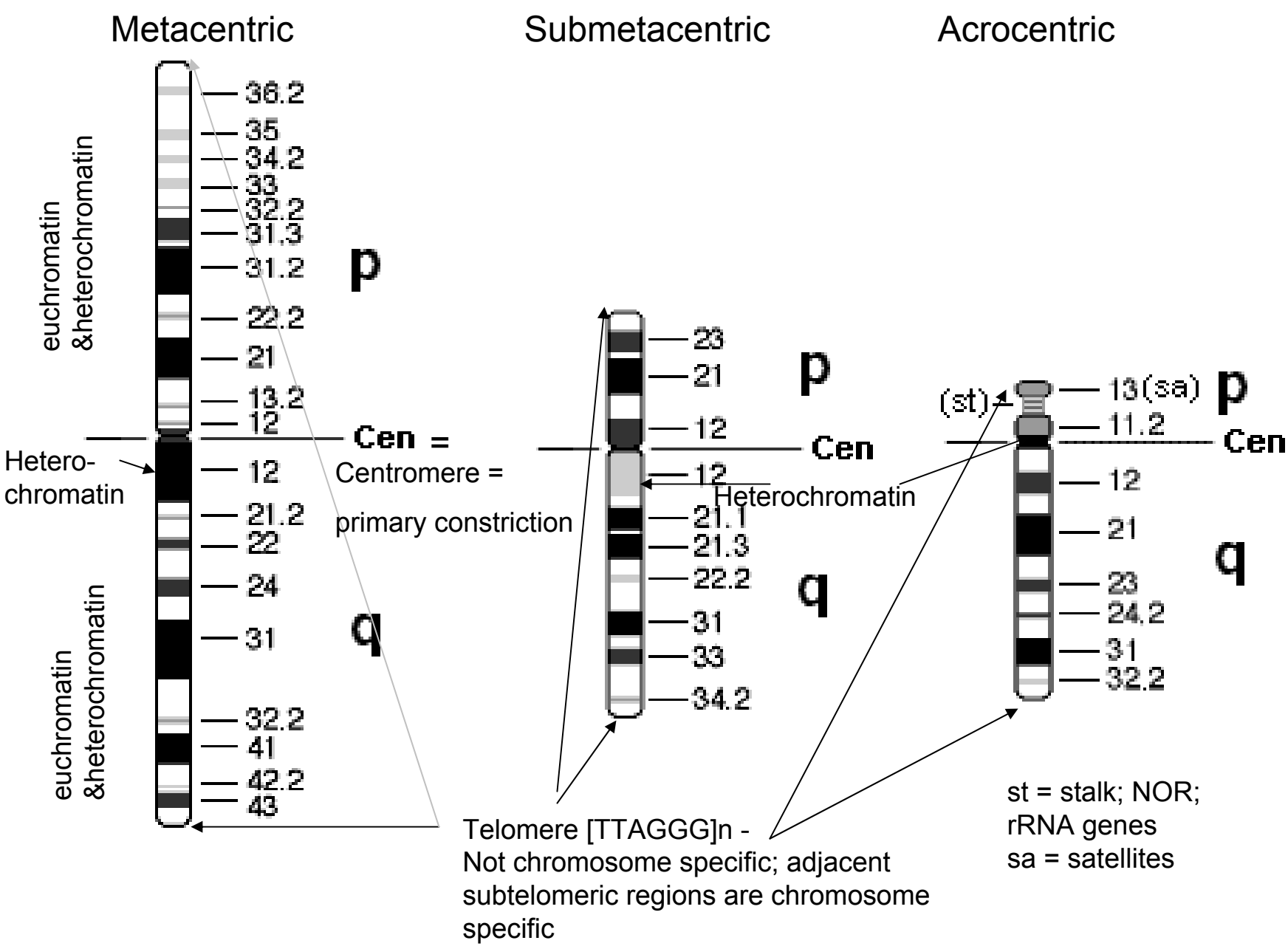
Q: Does 46,XX/46,XY represent a mosaicism?


Nondisjunction in meiosis I
Nondisjunction in meiosis II
First meiotic division
Second meiotic division
Trisomy
Trisomy
Monosomy
Monosomy
Euploid
Euploid
Trisomy
Monosomy
Genome of offspring after ferilization with another normal gamete

Chromosome Structure:



- 3 billion nucleotides per human haploid genome (23 chromosomes)
- chromosome sizes range from 35 million (chrom 22) to ~180 million (chrom 1)
- >2 meters of DNA per diploid nucleus; >10,000 fold compaction
- cell needs to access information in timely and orderly manner
- human genome draft sequence published in Feb'02; completed sequence expected later this year


Metacentric
Submetacentric
Acrocentric
euchromatin &heterochromatin
36.2
35
34.2
33
32.2
31.3
31.2
22.2
21
13.2
12
p
Cen =
Centromere =
primary constriction
Hetero-chromatin
12
21.2
22
24
31
q
euchromatin &heterochromatin
32.2
41
42.2
43
23
21
p
12
Cen
12
Heterochromatin
21.1
21.3
22.2
q
31
33
34.2
(st)
13(sa)
p
11.2
Cen
12
21
q
23
24.2
31
32.2
Telomere [TTAGGG]n -
Not chromosome specific; adjacent subtelomeric regions are chromosome specific
st = stalk; NOR; rRNA genes
sa = satellites

Nomenclature:

of chromosomes with centromeres**,** sex chromosomes**,** abnormalities listed by chromosome number from 1 through 22 and alphabetically by type of abnormality

Egs. 46,XY and 46,XX

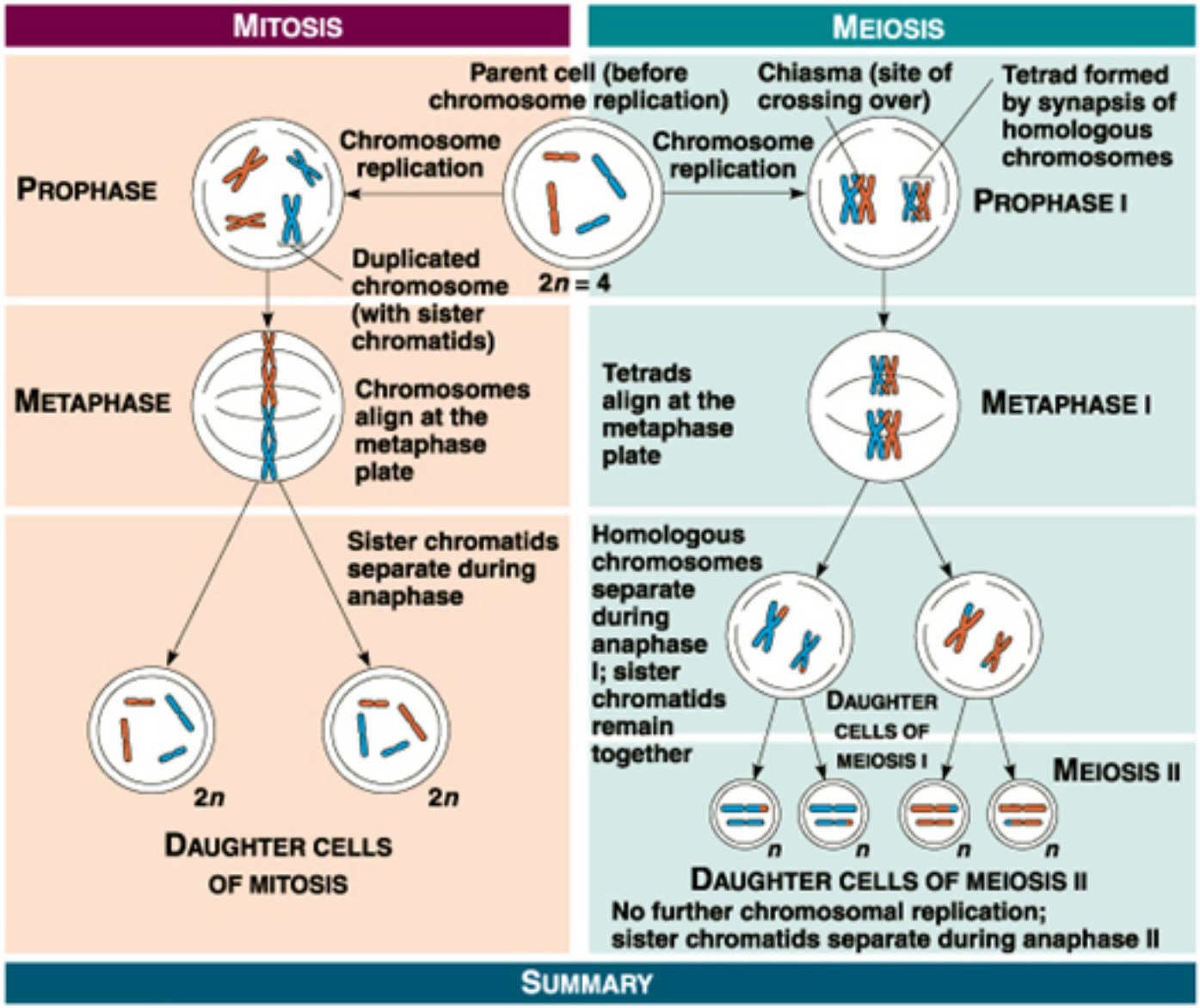
47,XXY and 47,XX,+21 and 45,X

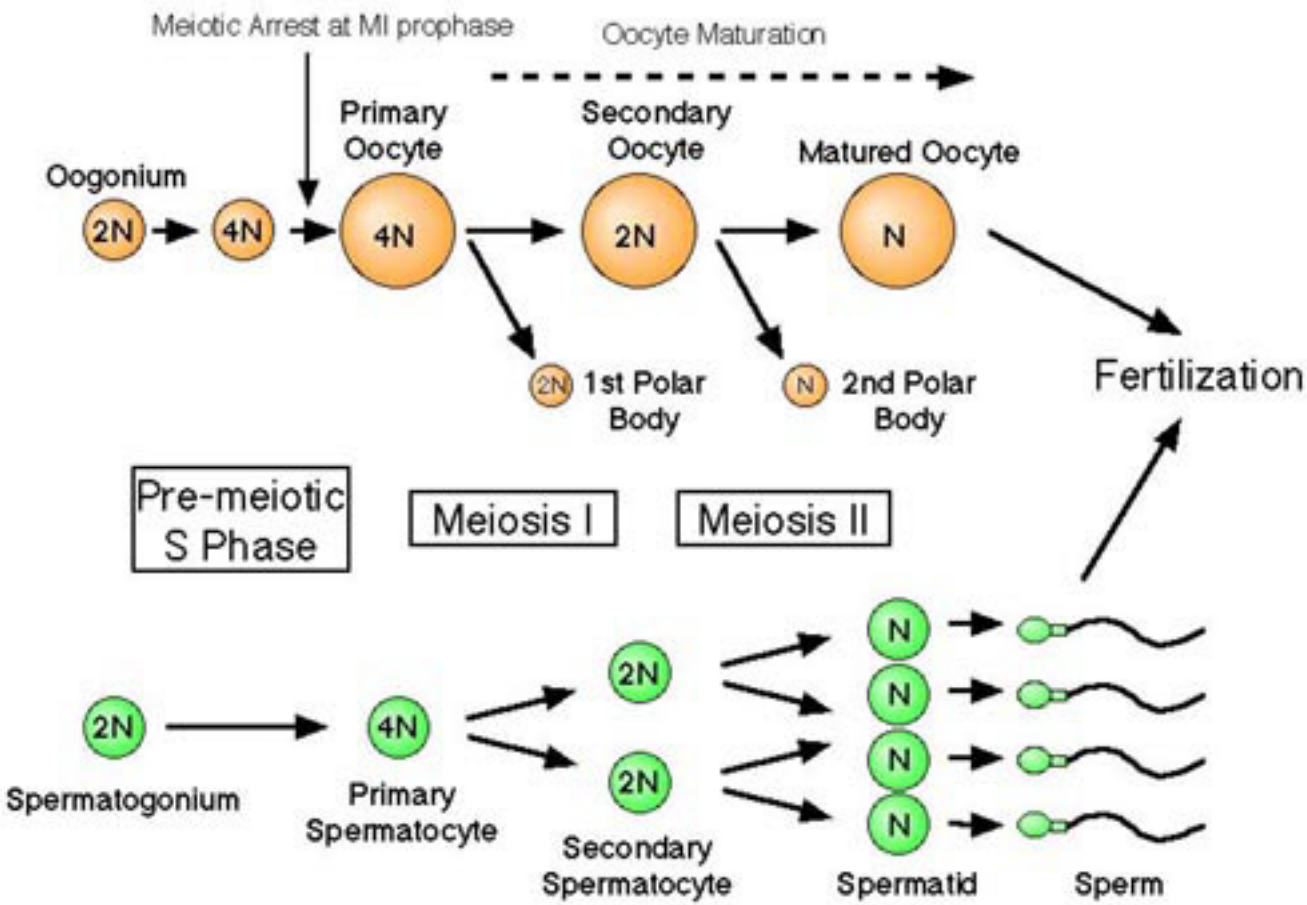
46,XX,del(15)(q11.2q13)

45,XX,t(14;21)(q10;q10)

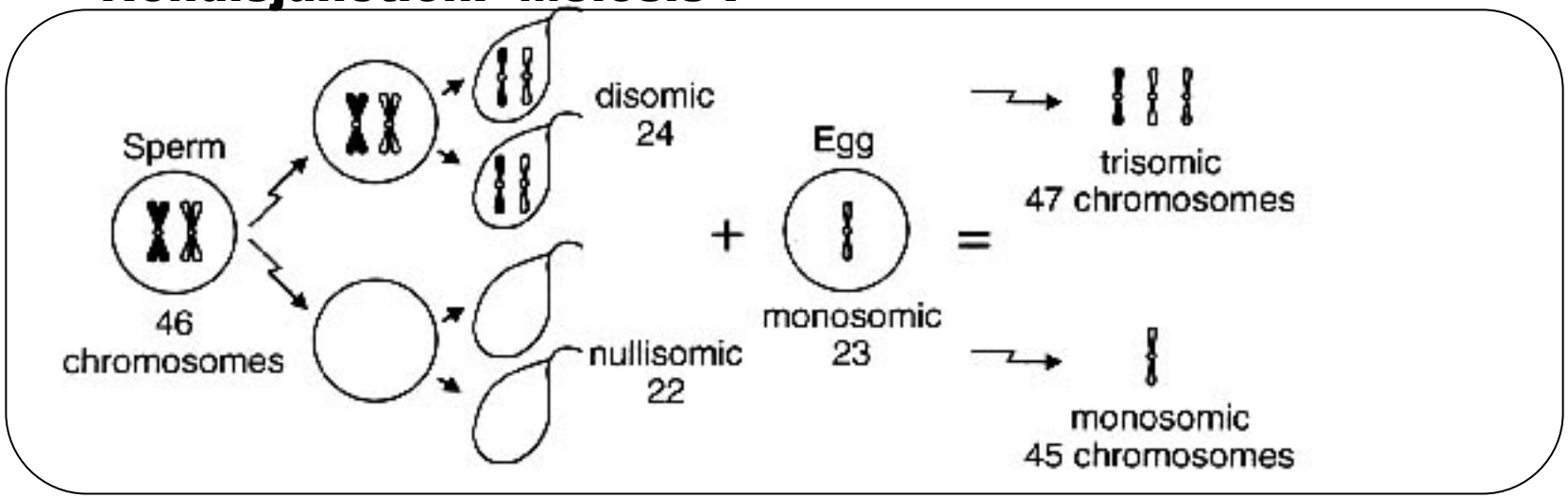
46,XY,t(14;21)(q10;q10),+21

47,XY,t(1;17)(p32;q12),inv(3)(p25),+4,add(6)(q21q23),del(6)(p25)

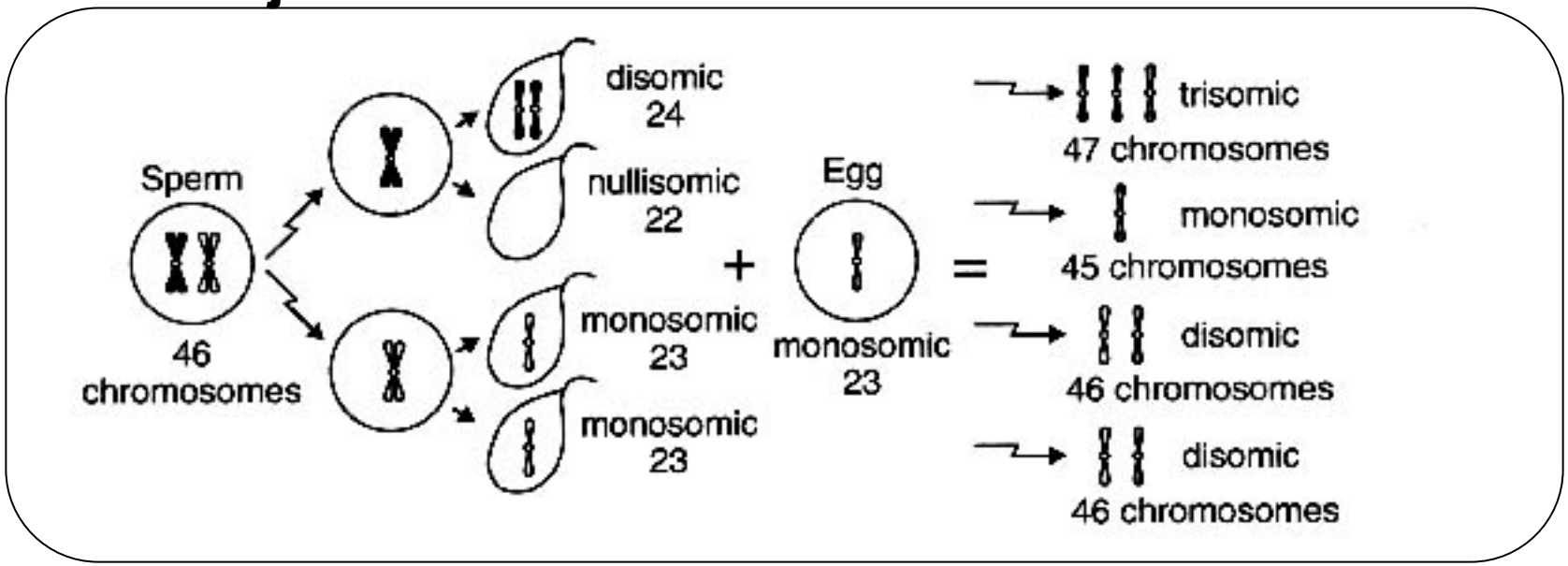

MITOSIS
MEIOSIS
Parent cell (before chromosome replication)
Chiasma (site of crossing over)
Tetrad formed by synapsis of homologous chromosomes
Chromosome replication
Chromosome replication
PROPHASE
PROPHASE I
Duplicated chromosome (with sister chromatids)
2n = 4
METAPHASE
Chromosomes align at the metaphase plate
Tetrads align at the metaphase plate
METAPHASE I
Sister chromatids separate during anaphase
Homologous chromosomes separate during anaphase I; sister chromatids remain together
DAUGHTER CELLS OF MEIOSIS I
MEIOSIS II
2n
2n
n
n
n
n
DAUGHTER CELLS OF MITOSIS
DAUGHTER CELLS OF MEIOSIS II
No further chromosomal replication; sister chromatids separate during anaphase II
SUMMARY


Meiotic Arrest at MI prophase
Oocyte Maturation
Primary Oocyte
Secondary Oocyte
Matured Oocyte
Oogonium
2N
4N
4N
2N
N
2N 1st Polar Body
N 2nd Polar Body
Fertilization
Pre-meiotic S Phase
Meiosis I
Meiosis II
2N
4N
2N
2N
N
N
N
N
Spermatogonium
Primary Spermatocyte
Secondary Spermatocyte
Spermatid
Sperm

Nondisjunction: Meiosis I



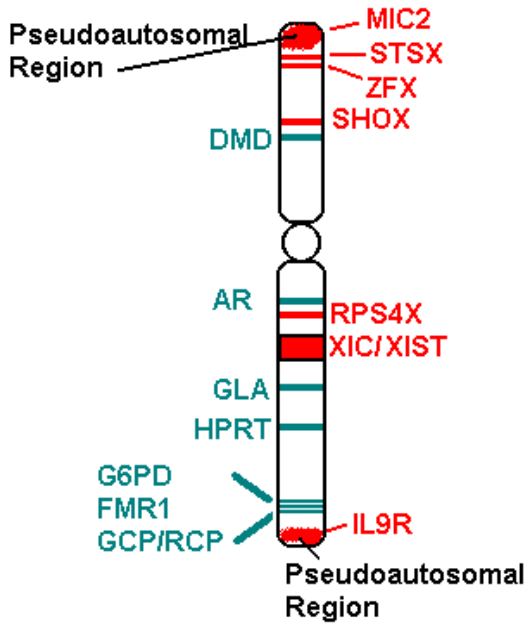
Nondisjunction: Meiosis II



Fertilization **Zygotes**

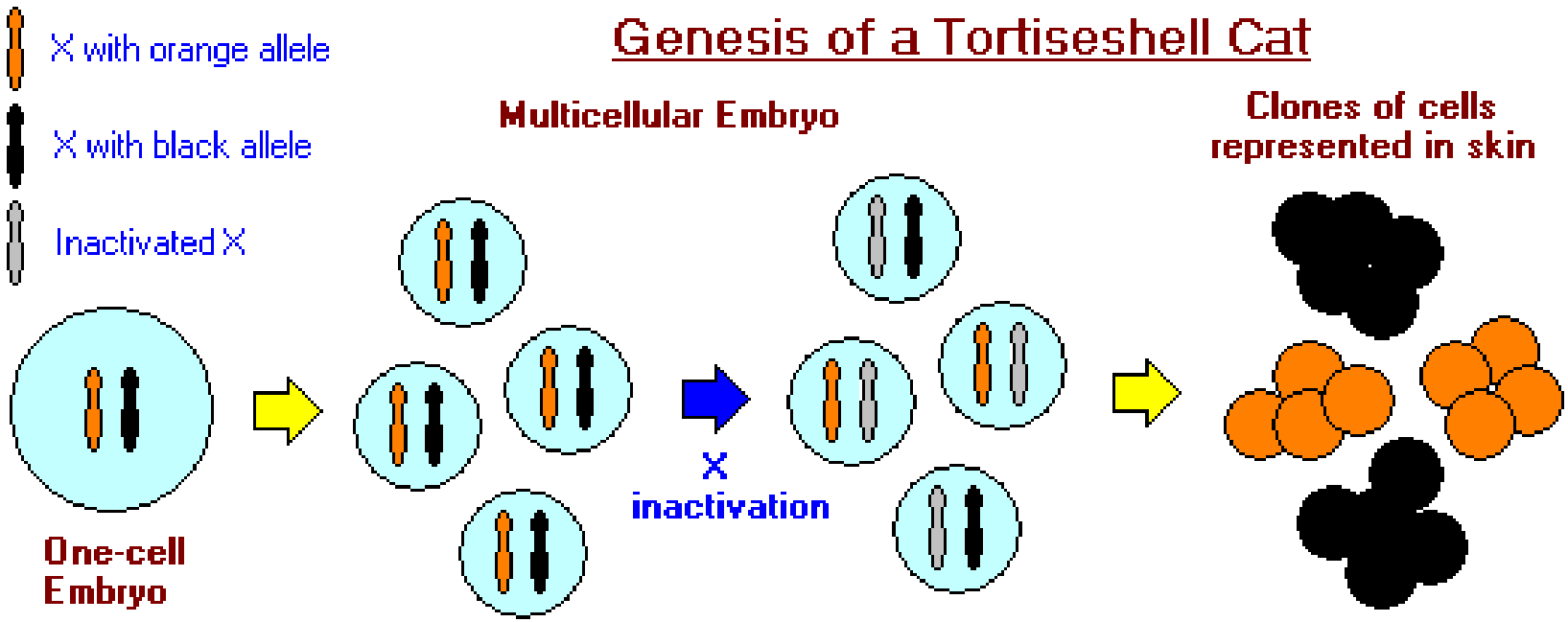
X-Inactivation or Lyonization:

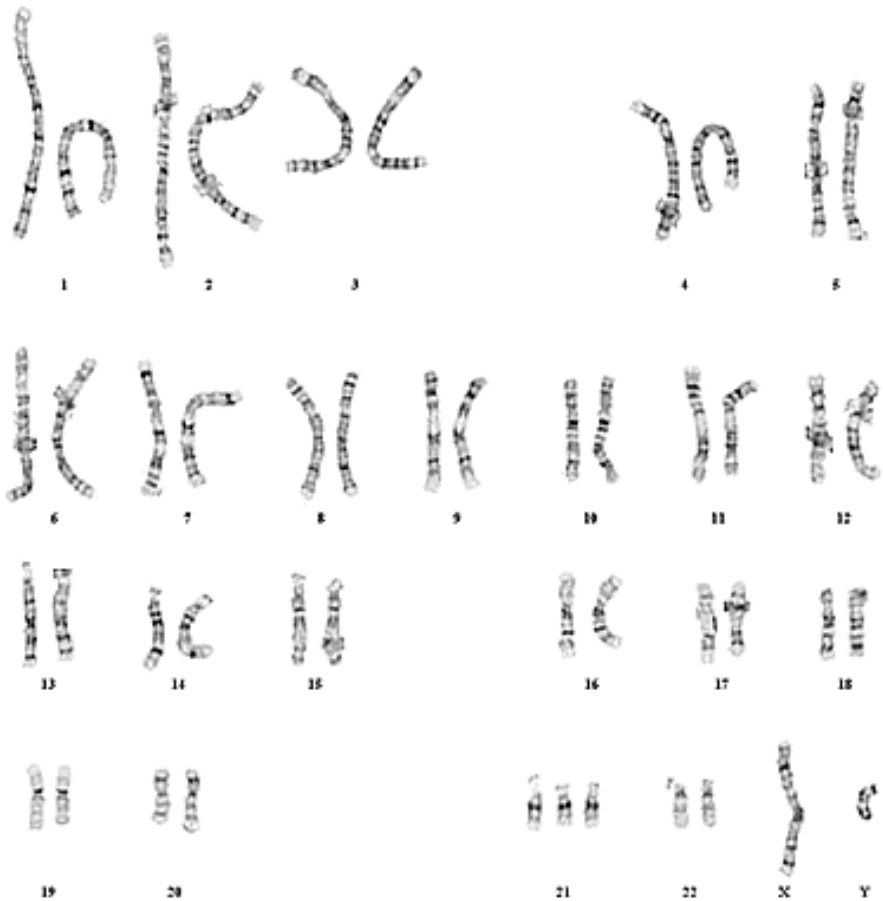
- form of gene dosage compensation between females (XX) and males (XY)
- generally occurs randomly in blastocyst (maternal or paternal)
    - if abnormal X involving t(X;autosome) then abnormal X remains active
    - if abnormal X involves deletion, duplication, etc then abnormal X is inactive
- permanent
- one active X per cell but inactive X does have some genes that remain active
- inactive X is late replicating, forms Barr body and is very compacted
- XIST gene (RNA) initates inactivation and is expressed from inactive X



Egs. of inactive (left) and active (right) genes on 'inactive' X.

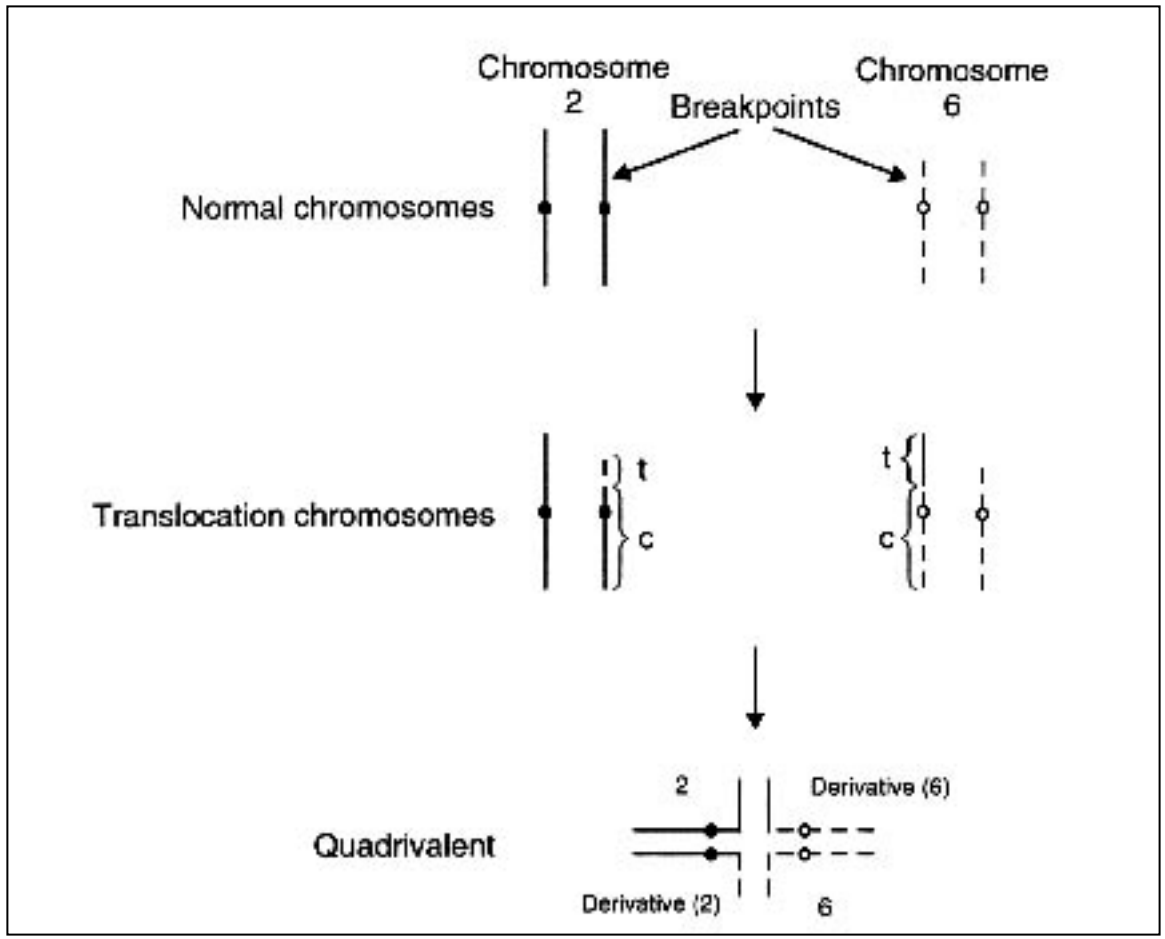
X-Inactivation or Lyonization





47,XY,+21

Meiotic Pairing of a Reciprocal Translocation



Possible Gametes: 2,6 and der(2),der(6) [Alternate segregation – balanced];
2,der(6) and der(2),6 [Adjacent 1 – unbalanced];
2,der(2) and der(6),6 [Adjacent 2 – unbalanced]